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 29, 1985
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. Jerry Hook (Chairperson), Curtis Harper and James Swenberg. Members of the Panel of Experts are: Drs. John Crowley, Kim Hooper, Thomas Jones, Richard Kociba, David Kotelchuck, Frederica Perera, Ian Purchase, Steven Tannenbaum, and Bruce Turnbull. Dr. Jones was unable to attend this meeting.

When available, final NTP Technical Reports for the studies 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 August 14, 1985, in Research Triangle Park, North Carolina. For information, contact Dr. Larry G. Hart, (919) 541-3971; FTS 629-3971.

CONTENTS

C.I. Basic Red 9. Dr. Harper, a principal reviewer for the draft technical report on the toxicology and carcinogenesis studies of C.I. Basic Red 9, agreed with the conclusions that:

> Under the conditions of these feed studies, there was clear evidence of carcinogenicity of C.I. Basic Red 9 monohydrochloride for male and female F344/N rats and for male and female B6C3F$_1$ mice. In male rats, C.I. Basic Red 9 monohydrochloride caused squamous cell carcinomas, trichoepitheliomas and sebaceous adenomas of the skin, subcutaneous fibromas, thyroid gland follicular cell adenomas and follicular cell carcinomas, Zymbal gland carcinomas, and hepatocellular carcinomas. In female rats, C.I. Basic Red 9 monohydrochloride caused subcutaneous fibromas, thyroid gland follicular cell adenomas or carcinomas (combined), and Zymbal gland carcinomas. In male mice, C.I. Basic Red 9 monohydrochloride caused hepatocellular carcinomas. In female mice, C.I. Basic Red 9 monohydrochloride caused hepatocellular carcinomas and adrenal gland pheochromocytomas or malignant pheochromocytomas (combined). Exposure to C.I. Basic Red 9 monohydrochloride also may have been related to increased incidences of bile duct tumors in male rats, mammary gland tumors in female rats, and hematopoietic system tumors in female mice.

He commented on the poor survival, but based on the 13-week studies the doses selected seemed justified. Dr. Harper said that more flexibility in the design of the protocol would allow discontinuing or adjusting dosing when there is an obvious trend of toxicity or decreased survival.

As a second principal reviewer, Dr. Kociba agreed in principle with the conclusions although he questioned the association of increased incidences of bile duct tumors in male rats and of mammary gland tumors in female rats with chemical exposure. With regard to the bile duct tumors, Dr. W. C. Eastin, NTP Chemical Manager, said one lesion was diagnosed as a carcinoma and the other two lesions were more difficult to diagnose. He said references to bile duct tumors would be deleted from the conclusions and the abstract. With regard to mammary gland tumors, Dr. Kociba noted that combining fibroadenomas with adenomas and adenocarcinomas was a departure from NTP guidelines. Dr. E. E. McConnell, NTP, said this was a departure reflecting more recent NTP experiences which indicate occasional occurrence of fibroadenomas and malignant tumors within the same neoplasm, and some evidence that malignant tumors can arise from fibroadenomas. Dr. Kociba stated that thyroid function measurements in the prechronic studies might have helped to select dose levels for the two-year studies.

As a third principal reviewer, Dr. Purchase agreed with the conclusions in male and female rats but thought that categorization of the findings in male and female mice should be some evidence of carcinogenicity. He said the reduction in body weight gain, the considerable compound-induced mortality, and possible compromised health of surviving mice made these conclusions suspect. Dr. Swenberg supported these comments for male mice in view of a high historical rate and varability for liver tumors but agreed with clear evidence of carcinogenicity in female mice. Dr. Harper noted that the liver carcinoma rates in males at both the low dose (40%) and the high dose (54%) were above the highest historical rate of 36%. Dr. J. Huff, NTP, agreed and added that the findings in both male and female mice were supportive.

Dr. Purchase questioned the positive findings reported in the mutagenicity studies noting the marginally positive increase for mutations in Salmonella and for sister chromatid exchanges (SCEs) in Chinese hamster ovary cells along with what he felt to be an inadequate experimental design and/or reporting in the latter system. He said the guidelines of the United Kingdom Environmental Mutagen Society recommended a doubling of the sister chromatid exchange incidence as being necessary for a positive effect. Dr. E. Zeiger, NIEHS, replied that regardless of the method of statistical analysis used there was a strong positive response in Salmonella while in Chinese hamster ovary cells, the chemical was studied up to a concentration showing toxicity and there were dose-related increases in SCEs that were greater than 30 percent above background for two of the three doses. Dr. Tannenbaum said the differences measured were statistically significant. Dr. Hook suggested that the chemical manager expand the discussion on the interpretation of the mutagenicity data.

Dr. Harper moved that the conclusion of clear evidence of carcinogenicity for male and female rats and female mice be accepted as written. Dr. Perera seconded the motion and it was approved unanimously. Dr. Harper then moved that the conclusion of clear evidence of carcinogenicity for male mice be accepted as written. Dr. Hooper seconded the motion and it was approved with six affirmative votes; there were four negative votes (Dr. Kotelchuck, Dr. Purchase, Dr. Swenberg, and Dr. Tannenbaum).

C.I. Disperse Blue 1. The conclusions for the draft technical report on the toxicology and carcinogenesis studies of C.I. Disperse Blue 1 were that:

> Under the conditions of these feed studies of C.I. Disperse Blue 1, there was clear evidence of carcinogenicity for male and female F344/N rats as shown by the increased occurrence of transitional cell papillomas and carcinomas, of leiomyomas and leiomyosarcomas,and of squamous cell papillomas and carcinomas of the urinary bladder. Urinary bladder calculi were observed in the groups of rats in which urinary bladder neoplasms were increased. Positive associations existed between the presence of calculi and transitional cell neoplasms in male and female rats, leiomyomas or leiomyosarcomas (combined) in female rats, and squamous cell neoplasms in male rats. A marginally increased occurrence of pancreatic islet cell adenomas or carcinomas (combined) was observed in male rats exposed to C.I. Disperse Blue 1. There was equivocal evidence of carcinogenicity of C.I. Disperse Blue 1 in male and female $B6C3F_1$ mice as shown by a marginally increased incidence of hepatocellular adenomas or carcinomas (combined) in low dose and high dose male mice, a marginally increased occurrence of alveolar/bronchiolar adenomas or carcinomas (combined) in high dose male mice, and an increased occurrence of hepatocellular adenomas in low dose female mice.

Dr. Kociba, a principal reviewer, agreed with most of the conclusions in rats, but thought that interpretation of the data on pancreatic tumors in male rats should be based on use of historical control incidence data. Dr. E. Rauckman, NTP Chemical Manager, replied that the Program gives more weight to concurrent controls rather than historical control values, and there was a good dose-response if allowance was made for reduced survival at the high dose. Dr. Kociba stated that the conclusions in mice should be reevaluated after factoring in historical control incidences of lung and liver tumors and early mortality in male concurrent control mice. He said the doses selected for the two-year studies in both species were higher than warranted based on the type and magnitude of toxicity observed in the 13-week studies.

As a second principal reviewer, Dr. Crowley agreed with the conclusions on the rat studies but thought the data in mice were at most equivocal evidence of carcinogenicity, and that consideration should be given to an assessment of no evidence of carcinogenicity. As a third principal reviewer, Dr. Kotelchuck agreed with the conclusions noting that they were all appropriate even if the high dose animals were excluded.

Most of the discussion dealt with the levels of evidence from the experiments in mice. Dr. Rauckman said the level of evidence chosen in male mice was based on concurrent controls along with a reasonable dose-response if reduced survival at the high dose is considered. In females, the low dose incidence was higher than ever observed Program -wide in controls. Dr. Kociba emphasized that concurrent control values for liver (both sexes) and lung lesions (males) were low while historical control values are variable thus making it difficult to attribute causality to the chemical treatment. Both Dr. Kotelchuck and Dr. Hooper supported greater weight being given to concurrent control values. Dr. J. Haseman, NIEHS, noted that the increases in liver tumors were seen in both sexes at the low dose. Dr. Huff commented that the chemical is mutagenic, and in other long-term studies, various anthraquinone derivatives have been shown to induce lung

and liver tumors.

Dr. Hooper moved that the conclusion of clear evidence of carcinogenicity in male and female rats be accepted as written. Dr. Swenberg seconded the motion and it was approved unanimously. Dr. Hooper moved that the conclusion of equivocal evidence of carcinogenicity in male mice be accepted as written. Dr. Kotelchuck seconded that motion and it was approved with six affirmative votes; there were four negative votes (Dr. Crowley, Dr. Kociba, Dr. Purchase, and Dr. Swenberg). Dr. Hooper moved that the conclusion for female mice be no evidence of carcinogenicity. Dr. Swenberg seconded the motion and it was approved with seven affirmative votes; there were two negative votes (Dr. Harper and Dr. Kotelchuck) and one abstention (Dr. Turnbull).

HC Red No. 3. Dr. Kotelchuck, a principal reviewer for the draft technical report on the toxicology and carcinogenesis studies of HC Red No. 3, agreed in principle with the conclusions that:

> Under the conditions of these two-year gavage studies of HC Red No. 3, there was no evidence of carcinogenicity for male F344/N rats; these animals may have been able to tolerate a higher dose. There was equivocal evidence of carcinogenicity for female F344/N rats as shown by the increased incidence of fibroadenomas of the mammary gland in the low dose (250 mg/kg) group. There was equivocal evidence of carcinogenicity for male B6C3F$_1$ mice as indicated by an increased incidence of hepatocellular adenomas or carcinomas (combined) in the high dose group. The male mice may have been able to tolerate higher doses of HC Red No. 3. Poor survival coupled with negative findings rendered the study in female B6C3F$_1$ mice an inadequate study of carcinogenicity.

He stated that the prechronic results indicated that the top dose used in the two year studies was well below a dose that could have been easily tolerated in both sexes of both species and not just in male animals. Thus, the conclusions should reflect this.

As a second principal reviewer, Dr. Tannenbaum said he agreed with use of the gavage route over dermal exposure but asked that the discussion indicate that metabolism by the two routes could be quite different, for example, nitrophenylenediamine dyes are extensively metabolized in the gastrointestinal tract. He was pleased to note that nitrosamines were analyzed but said more information was needed on methods of analysis, levels found, and possible biological effects of these contaminants. Dr. J. Mennear, NTP Chemical Manager, said the discussion would be expanded on route-specific metabolism and on the nitrosamines.

As a third principal reviewer, Dr. Hooper disagreed with the evidence categories in male and female rats and male mice because these animals could have tolerated higher doses as shown by no effects on body weight or survival. Further, in recently completed NTP studies of the structurally related dyes, HC Blue 1 and HC Blue 2, much higher doses were well tolerated. Dr. Hooper proposed that there be two categories, one referring to the strength of evidence, the second referring to the adequacy of the study design. For example, the conclusions in male mice could be that this was an inadequate study of carcinogenicity producing equivocal evidence of a carcinogenic effect. At the least, the study design was inadequate because low doses were used for all four sex/species groups. Dr. McConnell replied that an increase in the number of categories or qualifiers to the existing ones is not appropriate. And Dr. Huff noted there were already qualifiers for the stated conclusions on male rats and mice that higher doses may have been tolerated. Dr. Hooper moved that the conclusions as written be accepted with a conditional modifying statement: "The sensitivity of this study for detecting a carcinogenic effect may have been limited by poor survival (female mice), or by administration of less than a maximum tolerated dose (rats and male mice)." Dr. Turnbull seconded the motion.

In subsequent discussion, Dr. Kociba and Dr. Purchase questioned the interpretation of equivocal evidence of carcinogenicity based on the data for mammary

tumors in female rats. Despite the high incidence in the low dose group, this was a common and variable tumor and the incidence in the high dose group was lower than in the concurrent control group, and, thus, there was not a biological basis for even a marginal effect of treatment. Dr. Haseman noted that the low dose rate, however, was well above the historical range for gavage controls.

Dr. Hook said he would accept a motion for an amendment to Dr. Hooper's previous motion to change the conclusion in female rats. Dr. Kociba moved that the original motion be amended to propose that the conclusion read: "there was no evidence of carcinogenicity for male and female rats." The conclusion would include the modifying statement for all four experiments, namely: "The sensitivity of this study for detecting a carcinogenic effect may have been limited by poor survival (female mice), or by administration of less than a maximum tolerated dose (rats and male mice)." Dr. Purchase seconded the amended motion and it was approved by six affirmative votes; there were four negative votes (Dr. Harper, Dr. Hooper, Dr. Kotelchuck and Dr. Perera). Dr. Hook asked for a vote on Dr. Hooper's motion, including Dr. Kociba's amendment. The motion was approved unanimously.

Methylene Chloride (Dichloromethane). The conclusions for the draft technical report on the toxicology and carcinogenesis studies of methylene chloride were that:

> Under the conditions of these inhalation studies, there was some evidence of carcinogenicity of dichloromethane for male F344/N rats as shown by an increased incidence of neoplasms of the mammary gland. There was clear evidence of carcinogenicity of dichloromethane for female F344/N rats as shown by increased incidences of neoplasms of the mammary gland. There was clear evidence of carcinogenicity of dichloromethane for male and female B6C3F$_1$ mice, as shown by increased incidences of alveolar/bronchiolar neoplasms and of hepatocellular neoplasms.

Dr. Swenberg, a principal reviewer, agreed with the conclusions for three of the four studies. He did not agree with the conclusions for female rats because the increase in neoplasia was for benign mammary gland fibroadenomas. He said the significant and dose-related increases in these tumors along with the fact that this same type of tumor has been induced in two other studies supported a conclusion of some evidence of carcinogenicity for female rats. Dr. Swenberg said statements on causal relationships between leukemia and survival in female rats and between liver and lung tumors and survival in mice should be better supported. Dr. Hooper also asked for clarification as to whether the high incidence of leukemia in rats may have caused increased mortality. Dr. J. Mennear, NTP Chemical Manager, presented data that supported causal relationships. For example, in female rats dying before the termination of the study, 22 of 35 high dose animals had leukemia versus nine out of 20 controls.

As a second principal reviewer, Dr. Hooper agreed with the conclusions. He asked whether the NTP had looked for a dose-related increase in multiplicity of mammary fibroadenomas in rats. Such an examination might influence the strength of the evidence for carcinogenicity, especially in male rats. He suggested inclusion of a table summarizing the experimental conditions and tumor findings for the various reported long term methylene chloride studies. Dr. Hooper asked whether the testicular atrophy in male mice and the ovarian/uterine atrophy in female mice could be attributed to direct or indirect effects of the chemical. Dr. E. McConnell, NTP, replied that these effects were believed to be secondary to neoplasia.

As a third principal reviewer, Dr. Turnbull agreed with the conclusions as written. He asked for a statement in the methods section as to whether the pathologic slides were read in a coded or blind fashion.

The discussion focused primarily on whether the appropriate descriptor for the conclusions in female rats was clear evidence of carcinogenicity, as written, or some evidence of carcinogenicity*. The key issues centered on: (1) the relative weight given to concurrent control data versus historical (laboratory and Program) control data; (2) the interpretation of "a substantially increased incidence of benign neoplasms"; and (3) the issue of whether or not a conclusion of clear evidence of carcinogenicity based on benign neoplasms was appropriate. With regard to (1) and (2), Dr. Kociba, Dr. Purchase and Dr. Swenberg argued that historical rates should be emphasized since the concurrent rate was lower (10%) than the mean Program-wide rates (28%). Thus, Dr. Swenberg maintained

*As defined in the Note to the Reader on page 2 of each NTP Technical Report - Attached.

that the rate in the high dose group for fibroadenomas (22/50; 44%) was less than a doubling of the historical average, and not a "substantial" increase. Dr. J. Haseman, NIEHS, said the historical data base came primarily from feed studies and there was not a good data base of chamber controls from inhalation studies. Dr. J. Huff , NIEHS, stated that the concurrent control data are given more weight by the NTP while historical control data are there for balance. He noted that the rates for fibroadenomas at the test laboratory (Battelle Northwest Laboratories) for two previous inhalation studies were 14 and 18% (average of 16%). With regard to (3), Dr. Hook and Dr. Kotelchuck commented that the definitions of the categories for strength of evidence were what the Panel had used since June 1983. Dr. Kociba spoke for being able to factor in qualitative considerations such as tumor types and their commonality, and absence of malignancy. Dr. McConnell said the NTP's stance mirrored that of the International Agency for Research on Cancer that: "If a substance is found to induce only benign tumors in experimental animals, it should nevertheless be suspected of being a carcinogen..." Dr. Huff summarized the NTP reasoning for the conclusion. Using concurrent controls, there was a significant positive trend, a dose-related effect in which the incidence in the high dose animals was significantly higher than in the controls, the effects were observed in both sexes, and these findings were supported by studies in the literature.

Dr. Kotelchuck moved that the conclusion of clear evidence of carcinogenicity in female rats be accepted with the addition of the word "benign" in front of "neoplasms". Dr. Perera seconded the motion and it was approved with six affirmative votes; there were two negative votes (Dr. Crowley and Dr. Swenberg) and two abstentions by reason of company affiliation (Dr. Kociba and Dr. Purchase). Dr. Hooper then moved that the conclusion of some evidence of carcinogenicity in male rats be accepted also with inclusion of the word "benign" in front of "neoplasms." Dr. Perera seconded the motion and it was approved by seven affirmative votes. There was one negative vote (Dr. Crowley) and two abstentions (Dr. Kociba and Dr. Purchase). Dr. Swenberg moved that the conclusions of clear evidence of carcinogenicity in male and female mice be accepted as written. Dr. Hooper seconded the motion and it was approved by eight affirmative votes. There were two abstentions (Dr. Kociba and Dr. Purchase).

NOTE TO THE READER

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for testing in the NTP Carcinogenesis Program are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's carcinogenic potential. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical has the potential for hazard to humans. The determination of the risk to humans from chemicals found to be carcinogenic in animals requires a wider analysis which extends beyond the purview of this study.

Five categories of interpretative conclusions were adopted in June 1983 for use in the Technical Reports series to specifically emphasize consistency and the concept of actual evidence of carcinogenicity. For each definitive study result (male rats, female rats, male mice, female mice), one of the following quintet will be selected to describe the findings. These categories refer to the strength of the experimental evidence and not to either potency or mechanism.

- **Clear Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing a chemically related increased incidence of malignant neoplasms, studies that exhibit a substantially increased incidence of benign neoplasms, or studies that exhibit an increased incidence of a combination of malignant and benign neoplasms where each increases with dose.
- **Some Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing a chemically related increased incidence of benign neoplasms, studies that exhibit marginal increases in neoplasms of several organs/tissues, or studies that exhibit a slight increase in uncommon malignant or benign neoplasms.
- **Equivocal Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing a chemically related marginal increase of neoplasms.
- **No Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms.
- **Inadequate Study of Carcinogenicity** demonstrates that because of major qualitative or quantitative limitations, the studies cannot be interpreted as valid for showing either the presence or absence of a carcinogenic effect.

Additionally, the following concepts (as patterned from the International Agency for Research on Cancer Monographs) have been adopted by the NTP to give further clarification of these issues:

> The term *chemical carcinogenesis* generally means the induction by chemicals of neoplasms not usually observed, the earlier induction by chemicals of neoplasms that are commonly observed, or the induction by chemicals of more neoplasms than are generally found. Different mechanisms may be involved in these situations. Etymologically, the term *carcinogenesis* means induction of cancer, that is, of malignant neoplasms; however, the commonly accepted meaning is the induction of various types of neoplasms or of a combination of malignant and benign neoplasms. In the Technical Reports, the words *tumor* and *neoplasm* are used interchangeably.

This study was initiated by the National Cancer Institute's Carcinogenesis Testing Program, now part of the National Institute of Environmental Health Sciences, National Toxicology Program. The study described in this Technical Report has been conducted under NTP health and safety requirements and/or guidelines for toxicity studies. Individual toxicology testing contractors are required to demonstrate corporate health and safety programs in compliance with NTP chemical health and safety requirements and to meet or exceed all applicable Federal, state, and local health and safety regulations.

Although every effort is made to prepare the Technical Reports as accurately as possible, mistakes may occur. Readers are requested to identify any mistakes so that corrective action may be taken. Further, anyone who is aware of related ongoing or published studies not mentioned in this report is encouraged to make this information known to the NTP. Comments and questions about the National Toxicology Program Technical Reports on Toxicology and Carcinogenesis Studies should be directed to Dr. J.E. Huff, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709 (919-541-3780).

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge (and while supplies last) from the NTP Public Information Office, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709.

o-Phenylphenol. Dr. Tannenbaum, a principal reviewer for the draft technical report on the toxicology and carcinogenesis studies of o-phenylphenol, agreed with the conclusions that:

> Under the conditions of these dermal application studies, there was no evidence of carcinogenicity in male or female Swiss CD-1 mice administered o-phenylphenol alone or as a promoter following initiation with DMBA. o-Phenylphenol, however, caused nonneoplastic lesions at the site of application which included ulceration, inflammation, and hyperkeratosis.

He wondered if the current categories for strength of evidence of carcinogenicity encompassed findings in studies of chemicals as promoters.

As a second principal reviewer, Dr. Crowley agreed with the conclusions. However, he questioned the use of the recently derived statistical test by Korn and Liu to assess significance of possible synergistic or antagonistic effects of DMBA and o-phenylphenol on tumor induction. Given the negative results, the test could have been deleted. Dr. Haseman agreed that in this particular study the interaction test had little impact on overall interpretation of the data and could well be deleted. He said the NTP was evaluating the relative merits of several statistical procedures proposed for assessing synergistic effects for tumor incidence data.

As a third principal reviewer, Dr. Swenberg also agreed with the conclusions. In that the chemical was poorly absorbed from the skin, he questioned the examination of so many tissues histopathologically. Dr. McConnell replied that was routine for all studies, including those by the dermal route, at the time this study was designed. Now, the design for histopathology would be tailored to whether or not appreciable absorption was known or expected. Dr. Huff stated that for dermal studies staff are considering limiting the amount of pathology to the skin site of application, to known target organs, and to observations and trends from gross pathology.

In further discussion, Dr. Purchase said the methodology as written did not allow the reviewer to determine whether the different types of responses to skin carcinogens could have been measured, such as shortening of time-to-tumor, increases in number of animals with tumors, and increases in multiplicity of tumors. Dr. W. Kluwe, NIEHS, replied that current design incorporates tumor mapping, counting numbers, and more reliance on microscopic evaluation which should provide for better interpretation of the data than was possible in this study. Dr. Kociba noted the development of ulceration at the site of chemical application in many of the animals, and asked whether, from the standpoint of animal health more could be done to minimize the development and duration of these lesions. Dr. Huff indicated this was certainly a primary consideration for all current studies.

Dr. Tannenbaum moved that the technical report on the toxicology and carcinogenesis studies of o-phenylphenol be accepted with modifications as requested. Dr. Swenberg seconded the motion and the report was approved by nine affirmative votes. There was one abstention by reason of company affiliation (Dr. Kociba).

4-Vinylcyclohexene. The conclusions for the draft technical report on the toxicology and carcinogenesis studies of 4-vinylcyclohexene were that:

> Under the conditions used, the study of 4-vinylcyclohexene in male rats was considered an inadequate study of carcinogenicity because of extensive and early mortality at both doses tested and the lack of conclusive evidence of a carcinogenic effect. There was equivocal evidence of carcinogenicity of 4-vinylcyclohexene in female rats, as shown by the marginally increased incidence of adenomas or squamous cell carcinomas (combined) of the clitoral gland in low dose female rats. The high dose female rat group, in which no tumors occurred at an increased incidence, experienced severe and early mortality that limited its use in this evaluation. There was equivocal evidence of carcinogenicity in male mice, as demonstrated by marginally increased incidences of malignant lymphomas and of alveolar/bronchiolar adenomas or carcinomas (combined) of the lung in high dose male mice; the sensitivity of this study for detecting possible carcinogenic effects may also have been limited by the poor survival of the high dose group. There was clear evidence of carcinogenicity of 4-vinylcyclohexene in female mice, as shown by increased incidences of ovarian neoplasms at both doses. In addition, the increased incidence of adrenal gland adenomas in high dose female mice may have been related to the administration of 4-vinylcyclohexene.

Dr. Purchase, a principal reviewer, agreed with the conclusion in male rats but not with those for female rats or male and female mice. With respect to female rats and male mice, he said high and early mortality compromised the studies, both from the standpoint of insufficient numbers of animals for proper statistical analysis and from the compromised health of the animals. Thus, Dr. Purchase preferred an interpretation of inadequate study of carcinogenicity for female rats and male mice. He proposed a conclusion for female mice of some evidence of carcinogenicity based on increased incidences of ovarian neoplasms at both doses; high mortality at the high dose may have had some confounding influence. Dr. J. Collins, NTP Chemical Manager, replied that the studies in female rats and male and female mice were considered appropriate for interpretation of carcinogenicity based on adequate survival in the low dose groups. In low dose mice, survival after two years was equal to or greater than in the concurrent vehicle control group. He stated that the substantial increases in ovarian tumors in female mice, at the highest incidences ever seen in any single NCI or NTP study, supported a categorization of clear evidence of carcinogenicity.

As a second principal reviewer, Dr. Turnbull said a more detailed rationale should be given as to why the gavage rather than inhalation route was used. He had general comments on cage placement and on whether there are more gavage errors in high dose animals than in low dose or control groups. Dr. Collins said the gavage route was chosen primarily because there was a lack of good inhalation facilities at the time the studies were initiated, and because gavage studies were more common at that time.

As a third principal reviewer, Dr. Perera said the study in female rats should be considered an inadequate study of carcinogenicity. Her major concern was

that some of the positive findings in rats would not be considered biologically significant or would be minimized because of the poor survival. The results should be noted and emphasized. especially statistically significant increases in skin tumors in high dose male rats, and increased incidences of benign and malignant tumors of the clitoral gland and anterior pituitary along with appearance of a rare transitional cell carcinoma of the urinary bladder in low dose female rats. Dr. Collins said that because this was considered an inadequate study there was only modest discussion of tumors in male rats. However, if reexamination determined that the skin tumors were detected on visual examination, more discussion could be warranted. Dr. Hooper noted that the significantly increased incidence of skin tumors in male rats at the high dose should support a designation of equivocal evidence of carcinogenicity. Dr. W. Kluwe, NIEHS, replied that when the study is considered inadequate, a level of evidence is not given. Dr. Perera said the possibility of false negative results as a result of low survival should be discussed along with the already discussed problem of possible false positives.

In further discussion, Dr. Swenberg stated that the NTP should have terminated the study during the first year after the early mortality in high dose animals; the causes of death should have been more closely monitored and then a new study should have been started with lower doses. Dr. Kotelchuck also felt that the poor survival in female rats made this an inadequate study.

Dr. Hooper moved that the studies in both male and female rats be considered inadequate studies of carcinogenicity. Dr. Perera seconded the motion. She asked that increases in the various tumor types be mentioned. The Panel agreed to mention the increases in tumors in the abstract but not in the conclusion. The amended motion was approved unanimously. Dr. Purchase then moved that the conclusion for male mice be inadequate study of carcinogenicity. Dr. Kociba seconded the motion and it was approved unanimously by the Panel. Dr. Purchase moved that the conclusion for female mice be some evidence of carcinogenicity. Dr. Kotelchuck seconded the motion. In discussion preceding a vote, Dr. Haseman pointed out that in the low dose group there was a sizable increase in the incidence of ovarian tumors with neither mortality nor body weight loss. Dr. Swenberg said the study was also acceptable in the high dose group since more than 50% of the animals were alive at 78 weeks. In addition, these are uncommon tumors and both malignant and benign tumors were induced.

Dr. Kotelchuck suggested that the lack of a dose-response relationship weighed against the strongest category of evidence. The motion for some evidence of carcinogenicity in female mice was defeated by seven negative votes to three affirmative votes (Dr. Kociba, Dr. Kotelchuck, and Dr. Purchase). Dr. Hooper moved that the conclusions be accepted as written (i.e., clear evidence of carcinogenicity in female mice). Dr. Swenberg seconded the motion and it was approved by seven affirmative votes; there were two negative votes (Dr. Kociba and Dr. Kotelchuck) and one abstention (Dr. Purchase).