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
August 14, 1985
Research Triangle Park, North Carolina

The review meeting began at 8:30 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), Frederica Perera and James Swenberg. Members of the Panel of Experts are: Drs. John Crowley, Kim Hooper, Thomas Jones, Richard Kociba, David Kotelchuck, Franklin Mirer, Ian Purchase, Robert Scala, Steven Tannenbaum, and Bruce Turnbull. Dr. Scala was unable to attend this meeting. These minutes have been reviewed and approved by all members of the Subcommittee and Panel present at the meeting. They were written by Dr. Larry G. Hart, Executive Secretary.

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 December 9, 1985, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, (919) 541-3971; FTS 629-3971.

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n-Butyl Chloride. Dr. J. Roycroft, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of n-butyl chloride by reviewing the experimental designs, results, and proposed conclusions. The conclusions were that:

> Under the conditions of these gavage studies, there was no evidence of carcinogenicity of n-butyl chloride for male and female F344/N rats at doses of 60 or 120 mg/kg, for male B6C3F$_1$ mice at doses of 250, 500, or 1,000 mg/kg, or for female B6C3F$_1$ mice at doses of 250 or 500 mg/kg. Chemical-induced toxicity in high dose rats (primarily females) reduced the sensitivity of the study for determining carcinogenicity.

Dr. Crowley, a principal reviewer for the draft technical report, agreed with the conclusions for male and female rats but suggested that the findings in male and female mice indicated an inadequate study of carcinogenicity. He said, that the first study was terminated after one year and the variability between the control groups for the two studies lends support for an inadequate study. Dr. Turnbull and Dr. Kotelchuck indicated some concurrence for the mice studies being inadequate. Dr. Kotelchuck questioned the combining of the control groups. Dr. J. Huff, NIEHS, reported that in only one site (liver tumors in female mice) were there statistical differences between the two control groups. Thus, it was proper to combine control groups for data comparisons.

As a second principal reviewer, Dr. Kociba agreed with the conclusions. He said the rationale for dose selection should have included some additional parameters other than body weight depression and observations of clinical convulsions. In the absence of comparative absorption and metabolism data, inhalation exposure or skin application might have been a more appropriate route than corn oil gavage. Dr. Roycroft said the overt toxicity observed in the chronic study was not predictable from the prechronic studies in that there were minimal effects on body weight and convulsive episodes only in two high dose female mice. As a third principal reviewer, Dr. Jones agreed with the conclusions as stated.

In further discussion on the appropriateness of combining the two concurrent control groups in the studies on mice, Dr. E. McConnell, NIEHS, said that this procedure was also followed for the oral asbestos studies. The current studies on n-butyl chloride were done in the same laboratory with similar environmental factors, and the animals were genetically the same. Dr. Swenberg proposed adding a footnote explaining that combining control groups is done infrequently and why this combining was considered appropriate for n-butyl chloride.

Dr. Hook said the Panel needed to decide whether these were adequate studies before they could rule on the conclusions as written. Dr. Swenberg moved that this be considered an adequate study for at least one dose level per sex per species. Dr. Kociba seconded the motion, and it was approved by five affirmative votes (Dr. Hook, Dr. Jones, Dr. Kociba, Dr. Kotelchuck, and Dr. Swenberg) to four negative votes (Dr. Crowley, Dr. Hooper, Dr. Perera, and Dr. Turnbull) with one abstention (Dr. Purchase). As Chairperson, Dr. Hook cast the tie-breaking vote to approve the motion.

Dr. Kociba then moved that the conclusions as written for rats and mice of both sexes, no evidence of carcinogenicity, be accepted, including the last sentence, i.e., "Chemical-induced toxicity in high dose rats (primarily females) reduced

the sensitivity of the study for determining carcinogenicity."[88] Dr. Turnbull seconded the motion, and it was approved by six affirmative votes (Dr. Hooper, Dr. Jones, Dr. Kociba, Dr. Kotelchuck, Dr. Perera and Dr. Turnbull) and one negative vote (Dr. Swenberg) with two abstentions (Dr. Crowley and Dr. Purchase). Dr. Mirer and Dr. Tannenbaum were not present for the review of n-butyl chloride.

Chlorendic Acid. Dr. J. French, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of chlorendic acid by reviewing the experimental designs, results, and proposed conclusions. The conclusions were that:

> Under the conditions of these feed studies, there was clear evidence of carcinogenicity for chlorendic acid in male F344/N rats as shown by increased incidences of neoplastic nodules of the liver and acinar cell adenomas of the pancreas. Increased incidences of alveolar/bronchiolar adenomas and preputial gland carcinomas may also have been related to the administration of chlorendic acid. There was clear evidence of carcinogenicity for chlorendic acid in female F344/N rats as shown by increased incidences of neoplastic nodules and of carcinomas of the liver. There was clear evidence of carcinogenicity for chlorendic acid in male B6C3F$_1$ mice as shown by increased incidences of hepatocellular adenomas and of hepatocellular carcinomas. There was no evidence of carcinogenicity for chlorendic acid in female B6C3F$_1$ mice given chlorendic acid in the diet at concentrations of 620 or 1,250 ppm for 103 weeks.

Dr. Purchase, a principal reviewer for the draft technical report, agreed with the conclusions for male and female mice but proposed that the conclusions in male and female rats be changed to some evidence of carcinogenicity. This was suggested because male rats had increased incidence of only benign tumors in the liver and pancreas while the incidence of malignant tumors in the liver decreased with increasing dose. In female rats, he opined that the increased incidence of liver carcinomas was offset by the top dose being in excess of the maximum tolerated dose (MTD). Dr. French, stated that the conclusions in male and female rats were supported by overwhelming incidences of neoplastic nodules of the liver especially in males and a significant increase in carcinomas in females. For female mice, Dr. Purchase said the use of life table analysis for lung adenomas was not appropriate as these are not life-threatening lesions. He thought the description of genotoxicity data to be too scanty for the general reader.

As a second principal reviewer, Dr. Kotelchuck agreed with the conclusions for male and female rats and male mice but thought the conclusion for female mice should be changed to equivocal evidence of carcinogenicity because there was a marginal increase in alveolar/bronchiolar adenomas and carcinomas (combined). He said the trend tests and pairwise comparisons for these tumors were statistically significant, and although the concurrent control incidences were low, the high-dose incidence was about 75 percent greater than the historical control average incidence.

As a third principal reviewer, Dr. Kociba agreed with the conclusions for male and female mice and female rats, while he supported Dr. Purchase's rationale for changing the conclusion in male rats to some evidence of carcinogenicity or preferably, to some evidence of benign tumor induction. He noted that both dose levels selected for the chronic study in mice induced hepatic necrosis. Dr. Swenberg commented on the increased emphasis on reporting metastases of liver tumors to the lungs in mice and urged that this be more standardized, since the number of sections examined clearly affects the results.

In further discussion on the strength of evidence for liver tumors in rats, Dr. Perera stated that substantially increased incidences of benign neoplasms

support the conclusions as written. Dr. Hooper added that although the increases in benign liver tumors in female rats were less striking than in males, the significant increases in carcinomas strengthened support for the stated conclusions. Dr. Hook commented that the definitions for the levels of evidence are our working guidelines, and the Panel should attempt to follow these definitions in reaching their conclusions.

Dr. Purchase moved that the conclusions as written for male mice, clear evidence of carcinogenicity, and female mice, no evidence of carcinogenicity, be accepted. Dr. Swenberg seconded the motion and it was approved unanimously with nine affirmative votes. Dr. Kotelchuck moved that the conclusions as written for female rats, clear evidence of carcinogenicity, be accepted. Dr. Hooper seconded the motion and it was approved by eight affirmative votes to one negative vote (Dr. Purchase). Dr. Purchase moved that the conclusion for male rats be changed to some evidence of carcinogenicity. Dr. Kociba seconded the motion and it was defeated by seven negative votes (Dr. Crowley, Dr. Hooper, Dr. Jones, Dr. Kotelchuck, Dr. Perera, Dr. Swenberg and Dr. Turnbull) to two affirmative votes (Dr. Kociba and Dr. Purchase). Dr. Hooper then moved that the conclusions as written for male rats, clear evidence of carcinogenicity, be accepted. Dr. Kotelchuck seconded the motion and it was approved by seven affirmative votes to two negative votes (Dr. Kociba and Dr. Purchase). Dr. Mirer and Dr. Tannenbaum were not present for any of the votes.

Chlorinated Paraffins (C_{23}, 40% Cl). Dr. J. Bucher, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of chlorinated paraffins (C_{23}) by reviewing the experimental designs, results and proposed conclusions. The conclusions were that:

> Under the conditions of these studies, there was no evidence of carcinogenicity of chlorinated paraffins (C_{23}, 40% chlorine) for male F344/N rats. There was equivocal evidence of carcinogenicity of chlorinated paraffins (C_{23}, 40% chlorine) for female F344/N rats as shown by an increased incidence of adrenal gland medullary pheochromocytomas. There was clear evidence of carcinogenicity of chlorinated paraffins (C_{23}, 40% chlorine) for male B6C3F$_1$ mice as shown by an increase in the incidence of malignant lymphomas. There was equivocal evidence of carcinogenicity of chlorinated paraffins (C_{23}, 40% chlorine) for female B6C3F$_1$ mice as shown by a marginal increase in the incidence of hepatocellular neoplasms.

Dr. Hooper, a principal reviewer for the draft technical report, agreed with the conclusions. He commented that although high viscosity of the dosing vehicle may have prevented administration of maximally tolerated doses, the linear increase in liver weight indicated achievement of a biologically effective dose, at least in rats. However, the decreased survival in female mice due to a utero-ovarian infection may have limited the sensitivity of the study. More comparisons of the findings between this study and that with chlorinated paraffins (C_{12}, 58% Cl) would be useful, especially with regard to liver toxicity and carcinogenicity in rats.

As a second principal reviewer, Dr. Tannenbaum agreed with the conclusions. He said that if serum enzyme changes were an indication of liver toxicity then discussion was warranted as to whether the MTD may have been exceeded. Dr. Bucher agreed that increases in serum enzyme levels reflected liver damage in male rats but noted that there were no effects on weight gain or survival, and, in male rats, no chemically related tumors. With regard to chemical characterization, Dr. Tannenbaum stated that capillary gas chromatography for a mixture profile would have been preferable for both the chlorinated paraffins (C_{23}, 40%) and (C_{12}, 58% Cl). Dr. T. Goehl, NIEHS, said earlier analytical studies indicated the compounds do not chromatograph reproducibly, and tend to dehydrohalogenate when heated. Dr. Tannenbaum replied that recent technology allows analysis of thermolabile compounds using the capillary columns.

As a third principal reviewer, Dr. Kotelchuck agreed with the conclusions but suggested inclusion of more discussion in the text about why the marginal increases in male rats of pancreatic islet cell adenomas and neoplastic liver nodules were not considered chemically related. He observed that the striking difference in incidence and patterns of neoplastic lesions between these studies with the longer-chain (C_{23}) and the shorter-chain (C_{12}) chlorinated paraffins suggested the need for further studies, especially in examining differential metabolism in mammalian species.

In response to the reviewer's comments, Dr. Bucher said suggested comparisons of the results between the C_{23} and C_{12} compounds would be included in each report.

In other discussion, Dr. Mirer reported that these substances are used in two to five percent concentration in some cutting fluids in machining operations. He

said there is a substantial literature about excess cancer among workers exposed to machining and cutting fluids although no good evidence pointing at specific constituents of the fluids. He said more mention should be given to significant non-tumor pathology.

There was further discussion as to whether or not the conclusion in male mice should remain clear evidence of carcinogenicity or be changed to some evidence of carcinogenicity. Dr. Swenberg noted that malignant lymphoma is one of the more variable tumors and has a viral etiology in many cases. Dr. Purchase commented that statistically significant trends were obtained only if the lymphocytic and histiocytic tumor types were combined. Dr. McConnell said this was done routinely. Dr. Hooper said support for the original conclusion derived from a clearly significant trend test, significant pairwise comparison at the high dose, and the fact that both low-dose and high-dose incidences of the tumors are above the historical control range.

Dr. Hooper moved that the conclusions as written for both rats and mice be accepted. Dr. Kotelchuck seconded the motion and it carried by five affirmative votes (Dr. Crowley, Dr. Hooper, Dr. Kotelchuck, Dr. Mirer and Dr. Perera) to four negative votes (Dr. Jones, Dr. Kociba, Dr. Swenberg and Dr. Tannenbaum) with two abstentions (Dr. Purchase and Dr. Turnbull). Due to the closeness of the vote, Dr. Hook asked that separate votes be taken. Dr. Hooper moved that the conclusions for male rats, no evidence of carcinogenicity, and female rats, equivocal evidence of carcinogenicity, be accepted as written. Dr. Kotelchuck seconded the motion and it was approved by ten affirmative votes with one abstention (Dr. Purchase). Dr. Hooper then moved that the conclusion for female mice, equivocal evidence of carcinogenicity, be accepted as written. Dr. Kotelchuck seconded the motion and it was approved by ten affirmative votes with one abstention (Dr. Purchase). Dr. Hooper moved that the conclusion for male mice, clear evidence of carcinogenicity, be accepted as written. Dr. Perera seconded the motion and it was approved by five affirmative votes (Dr. Crowley, Dr. Hooper, Dr. Kotelchuck, Dr. Mirer and Dr. Perera) to four negative votes (Dr. Jones, Dr. Kociba, Dr. Swenberg and Dr. Tannenbaum) with two abstentions (Dr. Purchase and Dr. Turnbull).

Chlorinated Paraffins (C_{12}, 58% Cl). Dr. J. Bucher, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of chlorinated paraffins (C_{12}) by reviewing the experimental designs, results, and proposed conclusions. The conclusions were that:

> Under the conditions of these 2-year gavage studies, there was clear evidence of carcinogenicity of chlorinated paraffins (C_{12}, 58% chlorine) in F344/N rats based on increased incidences of hepatocellular neoplasms (primarily neoplastic nodules) in male and female rats, of adenomas or adenocarcinomas (combined) of the kidney tubular cells in male rats, and of follicular cell adenomas or carcinomas (combined) of the thyroid gland in female rats. Mononuclear cell leukemia in dosed male rats may have been related to administration of chlorinated paraffins (C_{12}, 58% chlorine). There was clear evidence of carcinogenicity of chlorinated paraffins (C_{12}, 58% chlorine) in B6C3F$_1$ mice as shown by increased incidences of hepatocellular adenomas and adenomas or carcinomas (combined) in dosed male and female mice and increased incidences of adenomas and adenomas or carcinomas (combined) of the thyroid gland follicular cells in dosed female mice.

Dr. Kotelchuck, a principal reviewer for the draft technical report, agreed with the conclusions. He suggested that there be discussion as well as consideration of further stud-ies examining the differential metabolism and patterns of carcinogenicity for the C_{12} chlorinated paraffins as compared with the C_{23} chlorinated paraffins.

As a second principal reviewer, Dr. Tannenbaum agreed with the conclusions. He also thought there was overt toxicity in both sexes and almost all dose groups. He questioned why there was no examination of serum enzyme levels in view of the liver toxicity. As a third principal reviewer, Dr. Hooper agreed with and elaborated in detail on the findings supporting these conclusions. He commented on the poor survival in rats but did not feel this jeopardized the validity of the findings.

Dr. Swenberg proposed adding a statement in the Abstract that the maximum tolerated dose (MTD) may have been exceeded in rats. Dr. Kociba said the doses in rats were excessive with the considerable toxicity making interpretation of the carcinogenesis results difficult; better doses might have been achieved if more parameters, such as serum enzyme levels, had been added to the prechronic studies. For this reason, he thought the data in rats supported some evidence of carcinogenicity. Dr. Bucher commented that most of the mortality in dosed male rats occurred after eighty weeks while overall survival in dosed female rats was reasonable compared with controls. Dr. J. Huff, NIEHS, added that of 26 male rats in the two dose groups with mononuclear cell leukemia (MNCL), 2 died before the end of the study. Dr. E. McConnell, NIEHS, opined that based on experience with some of the solvents, the kidney lesions and attendant decreased survival would not have been predicted from the 90-day studies. Dr. Perera asked that increases in MNCL in female rats, pancreatic acinar cell neoplasms in male rats, and alveolar/bronchiolar carcinomas in mice be mentioned in the Abstract.

Dr. Kotelchuck moved that the conclusions as written for both rats and mice, clear evidence of carcinogenicity, be accepted. Dr. Hooper seconded the motion

and the conclusions were approved by nine affirmative votes; there was one negative vote (Dr. Kociba) and one abstention (Dr. Purchase).
Following the vote, there ensued discussion as to the definition of MTD. Dr. McConnell said the NTP adhered to the definition in the Report of the Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation. On that basis, the consensus of the Panel was that the MTD may have been exceeded in male and female rats. Dr. Hook said a statement to that effect should be added to the Abstract.

Decabromodiphenyl Oxide. Dr. H. B. Matthews, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of decabromodiphenyl oxide by reviewing the experimental designs, results, and proposed conclusions. The conclusions were that:

> Under the conditions of these feed studies of decabromodiphenyl oxide, there was some evidence of carcinogenicity for male and female F344/N rats as shown by increased incidences of neoplastic nodules of the liver in low dose (25,000 ppm) males and high dose (50,000 ppm) groups of each sex. There was equivocal evidence of carcinogenicity for male $B6C3_1$ mice as shown by increased incidences of hepatocellular adenomas or carcinomas (combined) in the low dose group and of thyroid gland follicular cell adenomas or carcinomas (combined) in both dosed groups. There was no evidence of carcinogenicity for female $B6C3F_1$ mice. Several nonneoplastic lesions were observed at increased incidences, the most notable being increased thyroid gland follicular cell hyperplasia in male mice.

Dr. Mirer, a principal reviewer for the draft technical report, agreed with the conclusions. He considered the chemical disposition study to be a significant contribution to the technical report and suggested the description of the findings should be in the results section rather than only in an appendix. The results could be important in interpretation of studies involving doses by other routes or biological monitoring data. Further, Dr. Mirer said statistical tests would be desirable where there is increased non-tumor pathology, whether or not the lesions are correlated with neoplasia.

As a second principal reviewer, Dr. Swenberg agreed with the conclusions. He said the decreased survival in control male mice was very striking and could be highlighted in the abstract as well as the text. A summary paragraph should be included that states the implications of the pharmacokinetic data in respect to the doses used in the study.

As a third principal reviewer, Dr. Turnbull also agreed with the conclusions. He noted that the increased incidence of leukemias in dosed male rats was not considered biologically significant due to a high incidence in the concurrent controls and lack of significant increase in females. Yet, in the females the incidence was almost significant. Dr. McConnell, NIEHS, reported that the incidence rates for leukemia in Fischer rats have been increasing over the last couple of years primarily, he thought, because of better diagnosis, particularly in the early stages, rather than a true increase in the incidence. Thus, concurrent control rates would be most appropriate for comparisons.

As a fourth principal reviewer, Dr. Hooper agreed with the conclusions in female rats and male and female mice. He said the conclusions in male rats should be upgraded to clear evidence of carcinogenicity based on the substantial dose related increases in benign liver tumors (neoplastic nodules). Dr. Matthews said the conclusion reflected, in part, the lack of substantive increases in hepatocellular carcinomas. Dr. Kociba contended that the categorization for rats was too strong in that only a small percentage of neoplastic nodules progress to malignant tumors. Rather, a category such as "some evidence of benign tumor induction" would be more appropriate. Dr. Perera said that

until changed we should adhere to the wording as given in the Note to the Reader, and on that basis she agreed with Dr. Hooper. Dr. Hooper commented that the design would have been improved if only a single lot of the 99% pure chemical had been used. The use of four lots of varying purity coupled with very low (2%) absorption might have affected the experimental outcome, particularly if the active agents were present as impurities in only one of the less pure batches. Dr. Matthews acknowledged the low absorption but said they had confirmed that the absorbed chemical was decabromodiphenyl oxide and not impurities. Further, only two lots were used in the long-term studies and they would be identified in the report.

There was considerable discussion about the strength of evidence for carcinogenicity in male mice. Dr. Kociba stated that poor survival in concurrent controls pointed to use of historical rates as appropriate. Since, the rates of hepatocellular adenomas and carcinomas (combined) for both low and high dose groups were within the historical control range, he felt the correct conclusion was no evidence of carcinogenicity. Dr. J. E. Huff, NIEHS, noted that the low and high dose rates were greater (36% and 44%) than the mean historical rate (30%); thus equivocal evidence of carcinogenicity was proper. Dr. Perera commented that the stated genetic non-uniformity of the mice was another reason that concurrent controls should be used. Dr. Purchase said he could not accept equivocal evidence of carcinogenicity as there was a lack of statistical significance with both liver and thyroid neoplasms. Dr. Huff noted that there was a statistically significant increase in liver neoplasia for low dose male mice, and Dr. G. Boorman, NIEHS, said the conclusion was influenced by the high incidence of uncommon lesions, thyroid follicular cell hyperplasias. Dr. Swenberg was of the opinion that the conclusion was correct in that the liver and thyroid findings were neither clearly positive nor clearly negative. Dr. Tannenbaum asked for more consistency in deciding when to use historical controls. Dr. Swenberg commented that when you have a tumor whose incidence rates are highly variable historical control values for comparisons are useful.

Dr. Hooper moved that the conclusion for female rats, some evidence of carcinogenicity, be accepted as written. Dr. Turnbull seconded the motion and it was approved by ten affirmative votes with one abstention (Dr. Kociba). Dr. Hooper moved that the conclusion for female mice, no evidence of carcinogenicity, be accepted as written. Dr. Turnbull seconded the motion and it was approved by ten affirmative votes with one abstention (Dr. Kociba). Dr. Hooper moved that the conclusion for male mice, equivocal evidence of carcinogenicity, be accepted as written. Dr. Turnbull seconded the motion and it was approved by six affirmative votes (Dr. Hooper, Dr. Kotelchuck, Dr. Mirer, Dr. Perera, Dr. Swenberg, and Dr. Turnbull) with three negative votes (Dr. Crowley, Dr. Purchase and Dr. Tannenbaum) with one abstention (Dr. Kociba). Dr. Hooper moved that the conclusion for male rats be changed to clear evidence of carcinogenicity. Dr. Perera seconded the motion and it was defeated by six negative votes (Dr. Jones, Dr. Kotelchuck, Dr. Purchase, Dr. Swenberg, Dr. Tannenbaum and Dr. Turnbull) to four affirmative votes (Dr. Crowley, Dr. Hooper, Dr. Mirer and Dr. Perera) with one abstention (Dr. Kociba). Dr. Hooper then moved that the conclusion for male rats, some evidence of carcinogenicity, be accepted as written. The motion was seconded and it was approved by eight affirmative votes to two negative votes (Dr. Crowley and Dr. Mirer) with one abstention (Dr. Kociba).

Ephedrine Sulfate. Dr. R. Irwin, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of ephedrine sulfate by reviewing the experimental designs, results, and proposed conclusions. The conclusions were that:

> Under the conditions of these studies, there was no evidence of carcinogenicity in F344/N rats or B6C3F$_1$ mice of either sex receiving 125 or 250 ppm ephedrine sulfate in the diet for 2 years.

Dr. Kociba, a principal reviewer for the draft technical report, agreed with the conclusions. He said the rationale needed to be expanded for not considering adrenal phenochromocytomas in rats to be chemically-related. He considered the endometrial mucosal cyst gland formation to be treatment-related and as such it could be mentioned in the Abstract.

As a second principal reviewer, Dr. Perera agreed with the conclusions. However, she questioned whether maximum tolerated doses (MTDs) had been achieved. She suggested that there be mention in the Abstract of two prior studies of nitrosoephedrine, a potential reaction product of ephedrine and nitrite in the stomach, and of ephedrine administered with sodium nitrite. These studies were positive for carcinogenicity and presuppose further studies of ephedrine with nitrite because of relevance to human exposure. Dr. J. Huff, NIEHS, noted that the Program mentions in the Abstract only studies performed or supported by the NTP, and references other studies more appropriately in the Introduction or the Discussion Section. This policy previously had been endorsed by the Panel.

Dr. Kociba moved that the technical report on the toxicology and carcinogenesis studies of ephedrine sulfate be accepted with modifications as discussed. Dr. Swenberg seconded the motion and the technical report was approved unanimously by the Panel.

Marine Diesel Fuel and JP-5 Navy Fuel. Dr. M. Dieter, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of marine diesel fuel and JP-5 Navy fuel by reviewing the experimental designs, results, and proposed conclusions. The conclusions were that:

> Under the conditions of these 2-year dermal studies, marine diesel fuel at doses of 250 and 500 mg/kg resulted in dose-related increased incidences of squamous cell neoplasms of the skin (primarily carcinomas), providing equivocal evidence of carcinogenicity in male and female B6C3F$_1$ mice. The sensitivity for detecting systemic carcinogenicity in female mice dosed with marine diesel fuel was reduced by poor survival. Under the conditions of these 2-year dermal studies, JP-5 navy fuel at doses of 250 and 500 mg/kg provided no evidence of carcinogenicity in male and female B6C3F$_1$ mice.

Dr. Tannenbaum, a principal reviewer for the draft technical report, agreed with the conclusions. He considered the study flawed primarily for two reasons. First, the high degree of ulceration, especially in diesel fuel treated mice, leading to early termination of high-dose groups made difficult the interpretation of the results. Also, the design did not allow for development of data on whether the fuels were tumor promoters. Second, Dr. Tannenbaum opined that studies conducted on chemically poorly defined materials, i.e., complex mixtures, were fraught with difficulties. For instance, one does not know whether the materials evaluated were representative, or which ingredient caused the toxic response.

As a second principal reviewer, Dr. Perera agreed with the conclusions for diesel fuel in female mice and for Navy fuel in male and female mice. She proposed that the conclusion for diesel fuel in male mice be changed to some evidence of carcinogenicity based on a significant positive trend for squamous cell papillomas or squamous cell carcinomas at the site of application, and at the site of application combined with the inguinal skin. There was also a positive trend for hepatocellular adenomas or carcinomas and a significant increase of these tumors in the high dose group. This was supported by a positive trend in female mice for hepatocellular carcinomas and a similar significant increase in the high-dose group. Dr. Dieter said the liver tumors were not emphasized due to the overlap with the historical control range. Dr. Perera said the reduced survival rates should be mentioned in the Abstract.

As a third principal reviewer, Dr. Crowley stated he agreed with the conclusions for Navy fuel in male and female mice but did not agree with the conclusions for diesel fuel treated mice. He suggested a change to no evidence of carcinogenicity based on use of the incidental tumor test which shows no difference in male mice between control and high dose animals due to the small numbers of tumors involved. There was no statistical analysis presented for female mice. Dr. Dieter said the tumor rate for diesel fuel exposed females was too low for statistical analysis in the text. Because of the low rates an analysis was presented combining benign and malignant tumors (seven of nine were carcinomas) and tumors from both site of application and the site of chemical migration. The conclusion of equivocal evidence of carcinogenicity was based on there being a statistically significant trend by the life table test in both male and female mice. Dr. J. Huff, NIEHS, mentioned that the background rates for these neoplasias were quite low.

In other discussion, Dr. Purchase said there should have been analysis done for polycyclic aromatic hydrocarbons which are known active carcinogens in mineral oils. Dr. Dieter replied that this might be done retrospectively and could help explain why there were tumor responses with the diesel fuel and not the jet fuel. Dr. Purchase commented on the frequent and extensive skin alterations observed in treated mice and, therefore, criticized the protocol and conduct of the studies on scientific and humanitarian grounds. He asked whether the animals that had tumors also had ulcers. If so, the study should more properly be described as a study wherein repeated trauma was applied to damaged skin. Dr. Purchase said that repeated trauma on its own can lend to neoplasms of the skin and, hence, the presence of ulcers is a confounding factor which invalidates a conclusion of a carcinogenic effect. Dr. Dieter said there would be an analysis done and added to the report on the relationship between ulcers and tumors. He reported that there were significant numbers of animals in the parallel Navy fuel studies with dermatitis and ulceration yet there were no skin tumors so correlation did not exist. Dr. Hook pointed out that the NTP states the results obtained are specific for the conditions of that study. Dr. Mirer commented that there is a body of data primarily in male rats showing kidney toxicity and tumors arising from exposure to petroleum hydrocarbons. He asked why the fuels were not studied in rats. Dr. Dieter replied that an earlier gavage study in rats had been terminated as the gavage route was considered inappropriate. Further, there is an ongoing study with Navy fuel JP-5 by the inhalation route in rats.

Dr. Tannenbaum moved that the conclusions as written be accepted, equivocal evidence of carcinogenicity for male and female mice treated with marine diesel fuel, and no evidence of carcinogenicity for male and female mice treated with JP-5 Navy fuel. Dr. Crowley seconded the motion. In the ensuing discussion, there seemed to be a consensus that the incidental tumor test rather than the life table test was the most appropriate statistical test. In the diesel fuel studies, there were no statistically significant differences among groups with use of the incidental tumor test, and, as such, Dr. Crowley stated that the conclusion should be no evidence of carcinogenicity. Dr. G. Boorman, NIEHS, responded that the conclusion of equivocal evidence of carcinogenicity was based in part on the fact that seven of the nine tumors were squamous cell carcinomas, tumors that occur only rarely in control mice. The motion was approved by nine affirmative votes to two negative votes (Dr. Crowley and Dr. Purchase). Dr. Hook asked that narrative be added to reflect the uncertain composition of the mixtures, and, that data be added showing the degree of correlation in the same animal, if any, between ulceration and tumor formation. Both would have been helpful to the Panel if included in the draft technical report.

Tetrachloroethylene (Perchloroethylene). Dr. J. Mennear, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of tetrachloroethylene by reviewing the experimental designs, results, and proposed conclusions. The conclusions were that:

> Under the conditions of these inhalation studies, there was some evidence of carcinogenicity of tetrachloroethylene in F344/N rats as shown by increased incidences of mononuclear cell leukemia in males and females and rare renal tubular cell neoplasms in males. There was clear evidence of carcinogenicity in B6C3F$_1$ mice as shown by increased incidences of both hepatocellular adenomas and carcinomas in males and of hepatocellular carcinomas in females.

Dr. Swenberg, a principal reviewer for the draft technical report, agreed with the conclusions. He stated that the report should clearly note that the interpretation of mononuclear cell leukemia (MNCL) was based on the standard method of data evaluation supported by the dose-response effect on tumor latency and the staging evaluation, and point out that this is a preliminary attempt to develop staging criteria for MNCL. Dr. Swenberg said the discussion should be expanded to examine possible mechanisms of carcinogenesis, pointing out that the mutagenicity studies were negative and that tetrachloroethylene caused tissue toxicity at the same site as neoplasia in two of the three tissues: mouse liver and rat kidney. Further, he recommended that studies be considered to determine the potential immunotoxicity of tetrachloroethylene.

As a second principal reviewer, Dr. Mirer agreed with the conclusions in mice but thought the conclusions in male and female rats should be changed to clear evidence of carcinogenicity. He said the neoplasms (MNCLS) were malignant, present in increased incidence, and the increases appeared by all the usual criteria to be chemically related. Dr. Hooper supported the interpretation of clear evidence of carcinogenicity for male rats based on the statistical values, similar findings in female rats, and the 8% incidence of a rare tumor, gliomas of the brain, in high-dose male rats. Dr. Swenberg disagreed and stated that brain tumors are not as rare as previously believed and the high control incidences of MNCLs in this and in the concurrently run methylene chloride study argued against changing the conclusion. Dr. Mirer asked that greater emphasis be placed in the summary on the doses associated with the appearance of non-tumor pathology. He commented that the fact of testing tetrachloroethylene at the existing OSHA human exposure limit (100 ppm) in mice and finding a substantial effect at that level should be noted.

As a third principal reviewer, Dr. Turnbull agreed with the conclusions as written. He asked whether the data from the control group for the inhalation study on methylene chloride, reviewed and approved previously by the Panel, could be considered as a second concurrent control group to increase the power of the statistical tests. (The studies on methylene chloride were run concurrently with those on tetrachloroethylene at the same laboratory). As a fourth principal reviewer, Dr. Jones also agreed with the conclusions.

Dr. Thomas Robinson, Vulcan Chemicals, representing the Halogenated Solvents Industry Alliance (HSIA) gave a presentation which proposed that the NTP conclusion in rats of some evidence of carcinogenicity was not supported by the data. In their opinion, the appropriate conclusion was equivocal evidence of car-

cinogenicity based on lack of early mortality from MNCL in treated compared with control groups, and the confounding high incidence in untreated controls. Secondly, Dr. Robinson said the current study was the first to base conclusions, at least in part, on the staging of MNCL in F344 rats. The HSIA considered the staging method not well established.

Dr. Mennear responded that the conclusions in rats were not based solely upon staging of the leukemias but rather on the significantly increased incidences of MNCL in treated animals. Further, examination of causes of early mortality showed a dose-related increase in the incidence of death considered due to MNCL.

Dr. Swenberg moved that the conclusions as written, some evidence of carcinogenicity in rats and clear evidence of carcinogenicity in mice, be accepted. Dr Jones seconded the motion and it was defeated by five negative votes (Dr. Crowley, Dr. Hooper, Dr. Kotelchuck, Dr. Mirer, and Dr. Perera) to four affirmative votes (Dr. Jones, Dr. Swenberg, Dr. Tannenbaum and Dr. Turnbull) with two abstentions (Dr. Kociba and Dr. Purchase). Dr. Hooper then moved that the conclusions in mice be accepted as written. Dr. Perera seconded the motion and it was approved by nine affirmative votes with two abstentions (Dr. Kociba and Dr. Purchase). Dr. Hooper moved that the conclusions in female rats, some evidence of carcinogenicity, be accepted as written. Dr. Crowley seconded the motion and it was approved by eight affirmative votes; there was one negative vote (Dr. Kotelchuck) and two abstentions (Dr. Kociba and Dr. Purchase). Dr. Mirer moved that the conclusion in male rats be changed to clear evidence of carcinogenicity. Dr. Perera seconded the motion and it was approved by five affirmative votes (Dr. Crowley, Dr. Hooper, Dr. Kotelchuck, Dr. Mirer and Dr. Perera) to four negative votes (Dr. Jones, Dr. Swenberg, Dr. Tannenbaum and Dr. Turnbull) with two abstentions (Dr. Kociba and Dr. Purchase).