Board of Scientific Counselors
National Toxicology Program

Summary Minutes
from

Peer Reviews of Draft Technical Reports of Long-Term
Toxicology and Carcinogenesis Studies and Short-Term Toxicity Studies
by the Technical Reports Review Subcommittee
and Panel of Experts

on

March 13, 1989

Research Triangle Park, North Carolina

The review meeting began at 8:30 a.m. on March 13 in the Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the Subcommittee are: Drs. Robert Scala (Chairperson), Michael Gallo and Frederica Perera. Members of the Panel of Experts are: Drs. John Ashby, Robert Garman, Lois Gold, Curtis Klaassen, William Lijinsky, Barbara McKnight, Franklin Mirer, Paul Newberne and James Popp. Dr. Mirer was unable to attend this meeting. These minutes have been reviewed and approved by all members of the Subcommittee and Panel present. They were written by Dr. Larry G. Hart, Executive Secretary.

When available, a limited number of final NTP Technical Reports for the studies may be obtained free of charge from the NTP Public Information Office, MD 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 June 27, 1989, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, (919) 541-3971; FTS 629-3971.

CONTENTS

SUMMARY MINUTES-PEER REVIEW PANEL MEETING-3/13/89

Benzofuran. Dr. R.D. Irwin, NIEHS, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of benzofuran 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 benzofuran for male F344/N rats receiving doses of 30 or 60 mg/kg per day. There was some evidence of carcinogenic activity of benzofuran for female F344/N rats, based on increased incidences of tubular cell adenocarcinomas of the kidney. There was clear evidence of carcinogenic activity for male and female B6C3F1 mice, based on increased incidences of neoplasms of the liver, lung, and forestomach.

Dr. Irwin reported an unusually high incidence of neurilemomas in control and chemically exposed rats of both sexes. Dr. M.P. Jokinen, NIEHS, described the origins, anatomical characteristics, and patterns of occurrence of these neoplasms of peripheral nerve sheaths. Dr. Irwin said there was no apparent explanation for the high incidence of these uncommon neoplasms.

Dr. Newberne, a principal reviewer, agreed with the conclusions. He opined that the poor survival of male rats might have obscured possible neoplastic effects in the kidneys. He noted there were two separate lots of chemical used and inquired as to how they were phased into the studies. Dr. Irwin said that the one lot was used for the 14-day and 13-week studies while all animals in the chronic studies received the second lot. Dr. J. Huff, NIEHS, indicated both lots were of equal purity.

Dr. McKnight, the second principal reviewer, agreed with the conclusions; she added, because renal tubular cell adenomas are so rare in female rats and because the study showed a clear dose-related trend she could support changing the level in female rats to clear evidence of carcinogenic activity. Dr. Irwin said there was not unanimity among the staff on the level of evidence but the consenus was that the incidence was not significant enough for clear evidence. Dr. McKnight asked about the 10 male mice that died early in the study as a result of an overdose and wondered if these should have been included in the statistical analyses. Dr. Irwin commented that the mice died in the 20th or 21st weeks and were really not yet at risk so it was considered appropriate to censor them.

Dr. Gold, the third principal reviewer, agreed with the conclusions. She asked for additional information on the relationship between renal hyperplasia and neoplasia in these rat experiments, and, specifically, the incidence of hyperplasia and the severity of nephropathy in the individual female rats with tubular cell adenocarcinomas compared to female rats with kidney tumors. Dr. Irwin said that none of the rats that had kidney tumors had tubular cell hyperplasia. Dr. Huff mentioned that for certain tumors one may not find all stages of the biologic continuum; and, in some cases malignant neoplasia may arise in situ.

Dr. Newberne moved that the Technical Report on benzofuran be accepted with the revisions discussed and the conclusions as written for male rats, no

evidence of carcinogenic activity, for female rats, some evidence of carcinogenic activity, and for male and female mice, clear evidence of carcinogenic activity. Dr. Gold seconded the motion, which was accepted unanimously with 10 votes.

N,N-Dimethylaniline. Dr. K.M. Abdo, NIEHS, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of N,N-dimethylaniline 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 N,N-dimethylaniline for male F344/N rats, as indicated by the increased incidences of sarcomas or osteosarcomas (combined) of the spleen. There was no evidence of carcinogenic activity of N,N-dimethylaniline for female F344/N rats given 3 or 30 mg/kg body weight by gavage for 2 years. There was no evidence of carcinogenic activity of N,N-dimethylaniline for male B6C3F1 mice given 15 or 30 mg/kg body weight by gavage for 2 years. There was equivocal evidence of carcinogenic activity of N,N-dimethylaniline for female B6C3F1 mice, as indicated by an increased incidence of squamous cell papillomas of the forestomach. Both rats and mice could have tolerated doses higher than those used in these studies.
>
> There were decreased incidences of mononuclear cell leukemia in dosed male and high dose female rats. Increased incidences of compound-related splenic fibrosis, hemosiderosis, and fatty metamorphosis were observed in male rats.

Dr. Perera, a principal reviewer, agreed with the conclusions. She asked that a comment be added to the last sentence in the conclusions, that being - "Both rats and mice could have tolerated doses higher than those used in these studies.", which would state that the sensitivity of the studies for detecting the presence of carcinogenic responses was likely reduced. Dr. Abdo said such a phrase would be added.

Dr. Garman, the second principal reviewer, agreed with the conclusions. He considered the doses selected for the two-year studies to be adequate but wondered why the dose range was two-fold for mice but 10-fold for rats. Dr. Abdo indicated the wider exposure range for rats was an attempt to administer a dose low enough so that hemosiderosis would not be produced, and this was successful.

Dr. Popp commented that the lesions reported were very typical of the aniline class of compounds and stated that the chemical neoplasia comparison table in the discussion was a good idea.

Dr. Perera moved that the Technical Report on N,N-dimethylaniline be accepted with the conclusions as written for male rats, some evidence of carcinogenic activity, for female rats and male mice, no evidence of carcinogenic activity, and for female mice, equivocal evidence of carcinogenic activity. Dr. Garman seconded the motion, which was accepted unanimously with 10 votes.

alpha-Methylbenzyl Alcohol. Dr. M.P. Dieter, NIEHS, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of alpha-methylbenzyl alcohol 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 alpha-methylbenzyl alcohol for male F344/N rats, as shown by increased incidences of renal tubular cell adenomas and adenomas or adenocarcinomas (combined), and no evidence of carcinogenic activity for female F344/N rats administered 375 or 750 mg/kg. Renal toxicity characterized by severe nephropathy and related secondary lesions was observed in the dosed rats, and excessive mortality occurred during the last quarter of the studies. Poor survival reduced the sensitivity of the studies for detecting the presence of a carcinogenic response in both chemically exposed groups of male rats and in the high dose group of female rats. There was no evidence of carcinogenic activity of alpha-methylbenzyl alcohol for male or female B6C3F1 mice administered 375 or 750 mg/kg for 2 years.

Dr. McKnight, a principal reviewer, stated that her main concern was the large number of purportedly accidental gavage-related deaths in rats. These apparent dose-related deaths raised important questions about how the animals in the different dose groups were treated, apart from the chemical effect. She said that if the "accidental" deaths were not a result of toxic effects of the chemical, more explanation needs to be given as to why the frequency of death increases so clearly with dose.

Dr. Gold, the second principal reviewer, agreed with the conclusion in male rats in principle but would like to see more discussion of the severity of nephropathy and incidence of hyperplasia in the animals with adenomas and adenocarcinomas-- and how these compared to animals without such tumors. Dr. Dieter reported that four male rats in the low and of these animals four in the high dose groups had tubular cell hyperplasia and none had tubular cell tumors. Among the seven animals with tumors, none had hyperplasia; while all had marked severity for nephropathy. Dr. Gold also thought the rat studies might be considered inadequate because of the large number of accidents and poor survival. She asked for a description of NTP policy on maintaining a dose level throughout a study rather than reducing the dose when there are survival problems. Dr. J. Huff, NIEHS, stated that there was no set policy on changing exposure concentrations during the experiments. Since the chemical is a food flavoring agent, Dr. Gold questioned why corn oil gavage was chosen as the route of administration rather than feed. Dr. Dieter said the chemical was not stable in food and a high enough concentration could not be obtained in water, and that microencapsulation would be the route of choice today.

Dr. Lijinsky, the third principal reviewer, said the study was conducted with less adequacy than typical and comments should be added about the nature of the gavage errors, and how many deaths in each group were affected. If gavage-related deaths were too numerous, then consideration should be given to repeating the study. He thought the confusion over the numbers of accidental deaths made it difficult to assess if the top doses in rats were optimal doses.

Dr. Dieter said apparent adverse effects of the gavage technique did not appear to be random, and a cluster of accidents occurred between weeks 48 and 53 of

the study. There was a similar pattern of early mortality in the studies on benzyl alcohol (NTP TR #343, In Press) which were conducted in the same laboratory. Dr. S. Eustis, NIEHS, added that the deaths were not due to simple mechanical trauma, i.e., there were no indications that gavage needles punctured esophaguses or tracheas, or material deposited directly in the lungs for all of these deaths. The material in the lungs resembled aspirated stomach contents. Dr. Huff commented that animals in gavage studies often quickly become aware and anxious about getting the chemical, whereas controls seem to be less so since only the vehicle is given. Dr. Ashby further speculated that the smell or irritant properties along with a depressant effect of the chemical could have contributed to animals being more difficult to handle, leading to more difficulty in administering the mixture, and, hence, a greater likelihood of gavage error. As to the decision to continue the studies in rats, Dr. Dieter noted that there was reasonable survival in all groups through week 80; a steady and increasing rate of mortality ensued after that time. The staff decided that the positive effects were of major importance despite markedly reduced survival, and whereas the study had these flaws, one could not discount the increases in chemically-induced neoplasms. Dr. Dieter opined that the later mortality was primarily due to a combination of nephropathy and chemical toxicity.

Dr. McKnight moved that the conclusion for male rats be changed to inadequate study of carcinogenic activity. Dr. Lijinsky seconded the motion, which was rejected by five no votes (Ashby, Garman, Klaassen, Perera, Popp) to four yes votes (Gold, Lijinsky, McKnight, Newberne) with one abstention (Gallo). Dr. Gallo's abstentions here and on subsequent votes were based on his having served as a consultant to the manufacturer of the chemical. Dr. Garman moved that the conclusion for male rats be accepted as written, some evidence of carcinogenic activity, with reservations concerning poor survival as written. Dr. Perera seconded the motion. Dr. Ashby moved to amend the motion to change the conclusion to equivocal evidence of carcinogenic activity; this was tabled for lack of a second. Dr. Garman's motion was rejected by five no votes (Ashby, Gold, Lijinsky, McKnight, Newberne) to three yes votes (Garman, Perera, Popp) with two abstentions (Gallo, Klaassen).

In further discussion, most Panel members agreed that the tumor response in male rats was likely associated with chemical administration but considered the study in male rats to be confounded due to technical errors. Dr. Huff thought that the effects of gavage were receiving disproportionate attention, and urged that this issue not affect consideration of other aspects of the studies. He asked the Panel if indeed they believed the male rat study needed to be repeated to really answer public health concerns. Unconvinced, Dr. Klaassen made a motion to change the conclusion in male rats to inadequate study of carcinogenic activity. Dr. Ashby seconded the motion, which was accepted by six yes votes (Ashby, Gold, Klaassen, Lijinsky, McKnight, Newberne) to three no votes (Garman, Perera, Popp) with one abstention (Gallo). Dr. Klaassen moved that the conclusions be accepted as written for female rats and male and female mice, no evidence of carcinogenic activity. Dr. Popp seconded the motion, which was accepted by nine yes votes with one abstention (Gallo). In discussion following this vote, questions were raised about the inconsistency of judging the studies in female rats adequate when there was similarly poor survival, also likely due to gavage technique. Dr. Klaassen moved that the conclusion for female rats be changed to inadequate study of carcinogenic activity. Dr. Newberne seconded the motion, which was accepted by four yes votes (Ashby, Klaassen, Lijinsky,

Newberne) to two no votes (Garman, Perera) with four abstentions (Gallo, Gold, McKnight, Popp).

Dr. Scala asked Dr. Ashby to draft a statement which would convey to the NTP a sense of why a majority of the members of the Panel deemed the studies of alpha-methylbenzyl alcohol in rats to be inadequate. Such a statement was drafted, approved by the Panel members present on the day following the meeting, and presented to the NTP for action. The statement recommended that the NTP review the technical conduct of the studies in rats with two possible outcomes: (1) if the review confirms the technical adequacy of the overall study procedures, the levels of evidence as written in the report should be affirmed; or (2) if the NTP concludes the rat studies are flawed, then the studies should be reclassified as inadequate and future repeat studies should be considered.

Nalidixic Acid. Dr. R.E. Morrissey, NIEHS, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of nalidixic acid by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year feed studies, there was clear evidence of carcinogenic activity of nalidixic acid for F344/N rats, as indicated by increased incidences of preputial gland neoplasms in males and clitoral gland neoplasms in females. There was equivocal evidence of carcinogenic activity for male B6C3F1 mice fed diets containing nalidixic acid, as indicated by marginally increased incidences of subcutaneous tissue neoplasms. There was no evidence of carcinogenic activity for female B6C3F1 mice fed diets containing 2,000 or 4,000 ppm nalidixic acid for 2 years.

Dr. Klaassen, a principal reviewer, agreed with the conclusions. He pointed out that there was no dose reponse in tumor incidence in rats, indicated emphasis could be given to the "anti-carcinogenic" effects of nalidixic acid in female rats and, noted the greater survival of dosed groups of female rats. Dr. Morrissey said some of these negative effects may have been related to the decreased body weight gain observed in dosed female rats. Dr. Klaassen asked whether there are human equivalents of preputial and clitoral glands. Dr. Morrissey replied that as specific organs, they are not found as such in humans, but prepuce and clitoris are.

Dr. Ashby, the second principal reviewer, agreed with the conclusions. He commented on observations from the NTP database that of all the chemicals that induce preputial or clitoral neoplasms, nalidixic acid is the only one that is neither genotoxic nor a multi-organ carcinogen. He speculated that this was a classic case to pursue to examine secondary mechanisms of the chemical induction of cancer, in that the chemical might be inducing changes in homeostatic mechanisms resulting in either increases or decreases in tumor incidences. Dr. Gallo commented that the specific tumors increased or decreased in rats had estrogen receptors and wondered if nalidixic acid might be modifying estrogen metabolism in some manner.

Dr. Newberne, the third principal reviewer, agreed with the conclusions. He noted the lack of a dose-reponse in male and female rats. He thought the discussion concerning possible mechanisms for severe degeneration of germinal epithelium of seminiferous tubules of the testis in high dose male rats from the 13-week studies to be speculative. Likewise, he had similar comments concerning discussion of retinal degeneration and cataracts in dosed groups of both male and female rats from the two-year studies. There ensued a discussion as to whether the eye lesions were associated with chemical administration or with light. Dr. J. Huff, NIEHS, pointed out that the low dose animals were housed on the racks in the animal rooms above the high dose animals and the incidences of eye lesions did not appear to be associated with cage location.

Drs. Ashby and McKnight wondered why there was not some evidence of carcinogenic activity in male mice based on the increases in subcutaneous tissue tumors. Dr. S. Eustis, NIEHS, explained that pairwise comparisons (for high dose vs. control) and the trend test showed only marginal statistical significance, and, further, the incidence of tumors was within the historical control range for the laboratory.

Dr. Klaassen moved that the Technical Report on nalidixic acid be accepted with the conclusions as written for male and female rats, clear evidence of carcinogenic activity, for male mice, equivocal evidence of carcinogenic activity, and for female mice, no evidence of carcinogenic activity. Dr. Gold seconded the motion, which was accepted by nine yes votes to one no vote (Klaassen).

Phenylbutazone. Dr. F.W. Kari, NIEHS, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of phenylbutazone by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year gavage studies, there was equivocal evidence of carcinogenic activity of phenylbutazone for male F344/N rats, as shown by the occurrence of small numbers of renal tubular cell adenomas and carcinomas. There was some evidence of carcinogenic activity for female F344/N rats, as shown by the occurrence of two transitional cell carcinomas in the top dose group. Tubular cell adenomas and carcinomas may have been associated with the administration of phenylbutazone to female rats. There was some evidence of carcinogenic activity for male B6C3F1 mice, as shown by the increased incidence of hepatocellular adenomas or carcinomas (combined). There was no evidence of carcinogenic activity for female B6C3F1 mice administered phenylbutazone in corn oil by gavage at doses of 150 or 300 mg/kg.
>
> Phenylbutazone was also nephrotoxic to rats, as shown by the dose-related increase in the severity of age-related nephropathy, necrosis of the renal papilla, and mineralization of the collecting ducts in the papilla.

Dr. Kari reported that kidneys from this and several other studies were step-sectioned to evaluate potential renal lesions more rigorously. In this special examination, additional tubular cell adenomas were diagnosed in four low dose and one high dose male rats and in three low dose and one high dose female rats. Kidney tumors were not observed in any of the control rats in either the single or multiple sections.

Dr. Ashby, a principal reviewer, agreed in principle with the conclusions. His major concern was that the small and variable numbers of different types of renal tumors in rats along with the additional tumors observed after step-sectioning made interpretation somewhat more complicated. Dr. J. Huff, NIEHS, emphasized that the kidney is clearly a target organ for toxicity in both laboratory animals and humans. In particular, Dr. Ashby found difficult distinguishing equivocal evidence in male rats from some evidence in female rats. Regarding liver tumors, having in male mice both high concurrent and historical control incidences, Dr. Ashby was unconvinced that there was chemical induction of neoplasia. Dr. Kari responded that the liver was clearly a target organ and the incidence observed in the high dose group was nearly double the concurrent control incidence and was outside the historical control range. Dr. Ashby stated that from the available genetic toxicity data, phenylbutazone appeared to be a specific clastogen.

Dr. Lijinsky, the second principal reviewer, agreed with Dr. Ashby that the levels of evidence for male and female rats could have been the same or even reversed. He praised the decision to do additional step-sections of the kidneys in rats. Dr. Kari commented that for renal tubular cell tumors in male rats, the statistical evaluation indicated marginal significance for both dose response trend and pairwise comparisons. Although the additional sections uncovered tumors in dose groups, the limited database for step sections did not enable drawing a clear relationship between the tumor incidence and chemical exposure. In female rats, the level of some evidence was based on the occurrence of the two transitional cell carcinomas, tumors that have never been

observed in nearly 2100 vehicle controls nor in about 1600 untreated control female rats. Dr. Huff mentioned that kidney tumors of any type in Fischer rats are particularly uncommon. The incidence of liver neoplasms in high dose male mice compared with controls indicated only equivocal evidence in Dr. Lijinsky's view. He wondered whether the poor survival in high dose female rats might not have been due to gavage errors, and questioned use of the gavage route including the choice of oil rather than water as the vehicle.

Dr. Garman, the third principal reviewer, agreed with the conclusions. He said the photomicrographs of the renal neoplasms included in the Technical Report were quite helpful, especially in supporting the conclusion in female rats. He asked for more documentation in the report as to specifics of the pathology working group (PWG) process, in particular organs examined and numbers within an organ group.

Further discussion centered primarily on the rat kidney tumors and the levels of evidence. Dr. Lijinsky noted that in his laboratory a transitional cell papilloma of the renal pelvis had been seen in a vehicle (corn oil) control female F344 rat. Dr. J. Haseman, NIEHS, said the historical control database was used informally to support the level of evidence in female rats. Dr. S. Eustis, NIEHS, commented that the anaplastic carcinoma of apparent uncertain origin seen in the kidney of a low dose female rat was more likely of transitional cell than tubular cell origin. Dr. McKnight asked that a table be included in the text to report statistical significances for trend and pairwise comparisons of the composite incidences for renal tubular cell adenomas and carcinomas (combined) that were identified by both single- and step-section evaluations.

Dr. Ashby moved that the conclusion for male rats be accepted as written, equivocal evidence of carcinogenic activity. Dr. Garman seconded the motion, which was accepted unanimously with 10 votes. Dr. Ashby moved that the conclusion for female rats be accepted as written, some evidence of carcinogenic activity. Dr. Garman seconded the motion, which was accepted by seven yes votes (Ashby, Gallo, Garman, Klaassen, Lijinsky, Perera, Popp) to three no votes (Gold, McKnight, Newberne). Dr. Ashby moved that the conclusion for male mice be changed to equivocal evidence of carcinogenic activity. Dr. Lijinsky seconded the motion, which was rejected by six no votes (Garman, Gold, Klaassen, McKnight, Perera, Popp) to four yes votes (Ashby, Gallo, Lijinsky, Newberne). Dr. Ashby then moved to accept the motion as written, some evidence of carcinogenic activity. Dr. Perera seconded the motion, which was accepted by eight yes votes with two abstentions (Lijinsky, Newberne). Dr. Ashby moved that the conclusion for female mice be accepted as written, no evidence of carcinogenic activity. Dr. Gallo seconded the motion, which was accepted unanimously with 10 votes.

Toluene. Dr. J.E. Huff, NIEHS, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of toluene by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year inhalation studies, there was no evidence of carcinogenic activity for male or female F344/N rats exposed to toluene at concentrations of 600 or 1,200 ppm. There was no evidence of carcinogenic activity for male or female B6C3F1 mice exposed by inhalation to toluene at concentrations of 120, 600, or 1,200 ppm for 2 years.
>
> Male and female mice might have been able to endure somewhat higher exposure concentrations without compromising health or longevity.

Dr. Gallo, a principal reviewer, agreed with the conclusions. He thought the dose selection for both rats and mice was correct based on organ weight changes and biologic activity after 14 to 15 weeks exposure. He disagreed that mice might have been able to tolerate a higher dose. Dr. Gallo stated that dicussion on the comparative metabolism of benzene and the alkylbenzenes was excellent although some discussion on the area of toluene (and xylene) modifying the metabolism of benzene would enhance this section.

Dr. Popp, the second principal reviewer, agreed with the conclusions, and considered the dose selection quite appropriate based on the available information. He said the only question of carcinogenicity based on the original pathology concerned the incidence of kidney tumors; thus, the approach of making additional step-sections of male rat kidneys was appropriate and should be commended. Decreased survival in male mice groups might best be put in perspective in relation to survival in other inhalation studies including any information on intra- or interlaboratory variation.

Dr. Lijinsky, the third principal reviewer, opined that the use of the inhalation route of exposure was inappropriate because it prevented a maximum dose being given to the animals. He noted that prechronic studies had been done by the gavage route and asked why this route was not used for the two-year studies. As a corollary, he commented that a carcinogenic effect was demonstrated by the gavage route for benzene but not by inhalation exposure. Dr. Huff responded that the 14-15 week studies were done by two routes for comparative purposes, and the inhalation route was chosen largely because it was particularly relevant to human exposure and because metabolism patterns in rodents were similar by either route. Regarding toluene, Dr. Huff stated that he would communicate with Dr. C. Maltoni in Italy and attempt to obtain more details about his gavage experiments and expecially if any toxic and neoplastic lesions were considered to be related to toluene exposure. Regarding benzene, he further noted that Dr. Maltoni has shown benzene to be a multi-organ carcinogen after inhalation exposure.

There was some discussion about the usefulness of adding 10 animals to some groups to be sacrificed at 15 months. Dr. Scala noted that this had been a recommendation by the NTP Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation in their 1984 report with the rationale being to obtain a broader look at chronic toxicity unobscured by geriatic changes. Dr. Huff said this earlier evaluation was also helpful in preparing for evaluations after two years by identifying putative target organs early. Dr. Perera suggested that the NTP

evaluate the usefulness of the interim evaluation and Dr. Huff agreed. Dr. Ashby commented on the finding of five tubular cell adenomas in male rat control animals after step sectioning and urged caution in use of data from step sectioning until there is a fairly large database.

In response to Dr. Lijinsky's contention that the study was inadequate because a high enough dose was not given, Dr. Scala asked if there was more discussion on this point. Dr. S. Eustis, NIEHS, said the evidence of renal toxicity in both male and female rats spoke to there being sufficient exposure. Dr. Ashby thought the inhalation route to be the most appropriate. Dr. R. Griesemer, NIEHS, agreed and said that under the conditions used, the studies were adequately done and reported.

Dr. Gallo moved that the Technical Report on toluene be accepted with the conclusions as written for rats and mice of both sexes, no evidence of carcinogenic activity, but with deletion of the statement that, 'Male and female mice might have been able to endure somewhat higher exposure concentrations without compromising health or longevity'. Dr. Popp seconded the motion, which was accepted with nine votes and one abstention (Lijinsky).

4-Vinyl-1-Cyclohexene Diepoxide. Dr. R.S. Chhabra, NIEHS, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of 4-vinyl-1-cyclohexene diepoxide by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year dermal studies, there was clear evidence of carcinogenic activity of 4-vinyl-1-cyclohexene diepoxide for male and female F344/N rats, as shown by squamous cell and basal cell neoplasms of the skin. There was clear evidence of carcinogenic activity of 4-vinyl-1-cyclohexene diepoxide for B6C3F1 mice, as shown by squamous cell carcinomas of the skin in males and squamous cell carcinomas of the skin and ovarian neoplasms in females; increased incidences of lung neoplasms in females may also have been related to chemical application.

Dr. Gold, a principal reviewer, agreed with the conclusions. She suggested that if the few skin tumors reported as not being found directly at the site of application were near to the site and believed related to chemical administration, then they should be included with those at the site for purposes of evaluation. Dr. Chhabra said the tumors away from but adjacent to the site of application were attributed to inadvertant spread of the chemical from the site, and that the tumors would be combined for analysis. Dr. Gold said that for reasons of clarity findings for lung neoplasms in female mice should be stated as equivocal evidence of carcinogenic activity. Dr. Chhabra commented that only the highest level of evidence per experiment is stated. Dr. J. Huff, NIEHS, added that the lung tumors in female mice were duely labelled as "may have been related", and thus, were not an integral part of the selected level of evidence.

Dr. Ashby, the second principal reviewer, agreed with the conclusions. He thought the discussion unnecessarily complicated in trying to come to terms with the ovarian tumors. Dr. Chhabra responded that there did seem to be a differential retention of the chemical in the ovaries and a relationship between chemical metabolism and activity in the tissue. Dr. R. Griesemer, NIEHS, observed that the finding of ovarian atrophy and neoplasia was an important toxic event and deserved appropriate discussion.

In response to a request by Dr. Perera, Dr. Chhabra said he would try to obtain more recent information from the producer on potential human exposure to the chemical. There was some discussion as to whether the irritant properties of the chemical contrasted with the alkylating activity might have played a role in skin tumor initiation.

Dr. Gold moved that the Technical Report on 4-vinyl-1-cyclohexene diepoxide be accepted with the conclusions as written for male and female rats and mice, clear evidence of carcinogenic activity. Dr. Gallo seconded the motion, which was accepted by nine yes votes with one abstention (Garman). Dr. Garman's abstention was for reason of company affiliation.

TOXICITY STUDIES

Acetone. Dr. D.D. Dietz, NIEHS, NTP Study Scientist, introduced the short-term toxicity studies of acetone by reviewing the rationale, experimental design, and results. Acetone was administered in drinking water to groups of F344/N rats and B6C3F1 mice of both sexes for 14-days or 13-weeks. Results show that minimally toxic drinking water concentrations are estimated to be 20,000 ppm acetone for male rats and mice and 50,000 ppm for female mice. No toxic effects were identified for female rats. While the testis, kidney, and hematopoietic system were identified as target organs in male rats, and the liver was the target organ for male and female mice based on the histopathology findings, the results confirm that acetone is only mildly toxic at doses up to 100,000 ppm in drinking water.

Dr. Gallo, a principal reviewer, said clinical pathology should have been done and should be the norm for these 13-week studies. He opined that lack of palatability at the higher concentrations clearly caused a depression in water consumption resulting in the dose received being less than the intended dose. He commented on the wide ranges in temperature and relative humidity in the animal facilities. Dr. Dietz said the temperature range was misleading as there was just one day in the 13-week studies where the temperature was out of specifications.

Dr. Klaassen, a second principal reviewer, also reiterated the need for clinical chemistry measurements. He commented that results and discussion should be separate sections.

Dr. Scala said that seeing no objections, the Panel would accept the Technical Report with the modifications as discussed.

Hexachloro-1,3-Butadiene. Dr. S.H. Yang, NIEHS, NTP Study Scientist, introduced the short-term toxicity studies of hexachloro-1,3-butadiene (HCBD) by reviewing the rationale, experimental design, and results. Two-week and 13-week toxicity studies of HCBD incorporated in the diet were conducted in male and female B6C3F1 mice. The most important toxic responses observed were renal tubular necrosis and/or regeneration. Female mice were more susceptible to the toxicity of HCBD than male mice. Based on histopathologic evaluations, a no-observed-adverse-effect level was not established in female mice in the 13-week studies, even at the lowest dose of 1 ppm. In contrast, a no-observed-effect level of approximately 10 ppm was estimated for male mice.

Dr. Klaassen, a principal reviewer, had several editorial suggestions and commented that nearly all the conclusions were drawn from the histopathological data, and it would be useful also to use other measures, e.g., clinical chemistry. Dr. Yang said no clinical pathology was done.

Dr. Scala, a second principal reviewer, stated that a clearer exposition of the kidney pathology with more extensive tabulation would have been desirable. Dr. Yang agreed to add a table. Dr. Scala asked whether the unexplained losses of HCBD from the feed in the animal room might simply have resulted from volatilization. Dr. Yang replied that volatilization did appear to be responsible for the losses.

Dr. Griesemer stated that the goal of this and the other short-term toxicity studies to be reviewed was to design and conduct stand-alone toxicologic characterization experiments. Dr. Scala concluded by saying that barring any objections, the Panel would pass the Technical Report along with comments to the Program.

n-Hexane. Dr. J.K. Dunnick, NIEHS, NTP Study Scientist, introduced the short-term toxicity studies of n-hexane by reviewing the rationale, experimental design, and results. Thirteen-week studies were conducted by the inhalation route in B6C3F1 mice. Rat studies were not performed since the Chemical Industry Institute of Toxicology (CIIT) had done 13-week studies in F344/N rats. Two basic protocols were used: (1) exposure for 6 hours/day, 5 days/week in doses ranging up to 10,000 ppm; and (2) exposure for 22 hours/day, 5 days/week at a dose of 1000 ppm. All mice lived to the end of the studies, and only minimal toxicity was observed. Paranodal swellings seen in nerves at 1,000 ppm (22 hours/day studies) and at 10,000 ppm were considered to be minimal nerve damage that would not result in paralysis. Exposure-related lesions of the nasal cavity occurred after n-hexane exposure, but minimal or no effects were seen at 1,000 ppm or below.

Dr. Popp, a principal reviewer, said the report was generally well written. No significant changes in format were needed. He said the designation of animals for neuropathology examination and rationale for groups selected for this examination required clarification, and that the discussion should conclude by stressing the similarity between the findings in the mice studies and those from the CIIT studies in rats. Dr. Dunnick said these changes would be made.

Dr. Garman, a second principal reviewer, said there needed to be clarification of the numbers of animals from which nerve fibers were examined and the numbers of teased nerve fibers examined per animal. He asked that statements be made as to which of the nasal turbinate lesions were biologically significant. Dr. Dunnick said a table would be added with a grading of the lesions in the nasal cavity that would show the lesions in the high dose group (10,000 ppm) to be most prominent.

Dr. Scala commented that it was important to distinguish between n-hexane used in the studies and n-hexane as a component of commercial hexane. Most hexane sold and used is the latter, which contains about 40% n-hexane. Dr. Scala concluded by saying that, barring no objections, the Panel would pass the Technical Report along with comments to the Program.