

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

June 27, 1989

Research Triangle Park, North Carolina

The review meeting began at 8:30 a.m. on June 27 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) and Ellen Silbergeld. 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. Drs. Newberne and Silbergeld were unable to attend this meeting. These minutes have been reviewed and approved by all members of the Subcommittee and Panel present. They were written by Dr. Larry G. Hart, Executive Secretary.

When available, a limited number of final NTP Technical Reports for the studies may be obtained free of charge from the NTP Public Information Office, MD 82-04, P. O. Box 12233, Research Triangle Park, NC 27709. Telephone: (919) 541-3991; FTS: 629-3991. Subsequently, they may be purchased from the National Technical Information Service, U. S. Department of Commerce, 5285 Port Royal Road, Springfield, Virginia 22161, (703) 487-4650.

The next NTP technical reports peer review meeting will be held November 20-21, 1989, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, (919) 541-3971; FTS 629-3971.

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SUMMARY MINUTES-PEER REVIEW PANEL MEETING-6/27/89

Allyl Glycidyl Ether. Dr. G.A. Boorman, NIEHS, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of allyl glycidyl ether by reviewing the experimental design, results, and proposed conclusions:

Under the conditions of these 2-year inhalation studies, there was equivocal evidence of carcinogenic activity of allyl glycidyl ether for male Osborne-Mendel rats, based on the presence of one papillary adenoma of respiratory epithelial origin, one squamous cell carcinoma of respiratory epithelial origin, and one poorly differentiated adenocarcinoma of olfactory epithelial origin, all occurring in the nasal passage of males exposed at 10 ppm. There was no evidence of carcinogenic activity of allyl glycidyl ether for female rats. One papillary adenoma of the respiratory epithelium was present in a female rat exposed at 5 ppm. There was some evidence of carcinogenic activity of allyl glycidyl ether for male B6C3F1 mice, based on the presence of three adenomas of the respiratory epithelium, dysplasia in four males, and focal basal cell hyperplasia of the respiratory epithelium in seven males in the nasal passage of mice exposed at 10 ppm. There was equivocal evidence of carcinogenic activity of allyl glycidyl ether for female mice, based on the presence of one adenoma of the respiratory epithelium and focal basal cell hyperplasia of the respiratory epithelium in seven females exposed at 10 ppm.

Dr. Ashby, a principal reviewer, agreed with the conclusions. He expressed surprise that the chemical was not a more potent carcinogen, as suggested by its chemical structure, structural similarity to glycidol, and genotoxicity, and wondered if there might not have been a more marked expression of carcinogenic potential by a different route of exposure. He asked that there be cross reference to the potential carcinogenicity of glycidol and comparative discussion of the genetic toxicity in the Discussion. Dr. Boorman said this would be done.

Dr. Mirer, the second principal reviewer, agreed with the conclusions. He thought that the sensitivity of the study in male rats was reduced by excessive mortality which was unrelated to compound administration. Dr. Mirer commented on the reproductive studies, noting that exposure at 30 ppm appeared to have produced adverse effects in male rats. This was the lowest dose used in this assay; therefore, a no effect level was not established he noted. Dr. Boorman said reproductive studies would be given more emphasis; also, the reproductive toxicology group at NIEHS planned to study other chemicals in the glycidyl ether series including glycidol.

Dr. Lijinsky, the third principal reviewer, did not agree with the conclusions. He opined that these experiments were not designed to produce carcinogenic effects, mainly because of the limited dose of a high boiling point compound that can be administered by the inhalation route. Likewise, the reproductive studies suffered from the same limitation. Dr. Lijinsky considered the numbers of tumors observed to be too few to justify the levels of evidence chosen in male rats and mice. Dr. Boorman responded that the large numbers of pre-

neoplastic lesions, particularly in mice, made the difference. Dr. Popp and Dr. Garman supported the level of evidence in male mice; however, Dr. Popp was unsure as to whether he could support equivocal evidence in male rats and female mice. Dr. Lijinsky supported the suggestion made by Dr. Ashby for gavage studies of allyl glycidyl ether, especially since higher concentrations could be given and that would better allow comparison to the glycidol (NTP TR #374) experiments.

Dr. Gold said she considered the studies in male rats to be inadequate based on poor survival, particularly in the high dose group. Dr. Boorman noted that the low survival was due primarily to renal disease. Dr. J. Haartz, NIOSH, said the current NIOSH estimate of workers exposed is about 400.

Dr. Ashby moved: (a) that the Technical Report on allyl glycidyl ether be accepted with the conclusions as written for male rats and female mice, equivocal evidence of carcinogenic activity, for female rats, no evidence of carcinogenic activity, and for male mice, some evidence of carcinogenic activity, and (b) that a statement be added to the conclusions for male rats to indicate that the sensitivity for detecting a carcinogenic effect was reduced by excessive mortality. Dr. Garman seconded the motion, for which the vote resulted in a tie with four yes votes (Ashby, Garman, McKnight, Mirer) to four no votes (Gold, Klaassen, Lijinsky, Popp). Dr. Scala, the Chair, then cast the tie breaking vote in favor of acceptance.

Benzaldehyde. Dr. J.B. Bishop, NIEHS, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of benzaldehyde 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 benzaldehyde for male or female F344/N rats receiving 200 or 400 mg/kg per day. There was some evidence of carcinogenic activity of benzaldehyde for male and female B6C3F1 mice, as indicated by increased incidences of squamous cell papillomas and hyperplasia of the forestomach.

Dr. Garman, a principal reviewer, agreed with the conclusions. He appreciated the inclusion of tables providing comparison of incidence rates of forestomach tumors and genetic toxicity with other chemicals studied by the Program which induce these lesions. He asked for a more detailed description of the brain lesions seen in high dose rats in the 13-week studies. Dr. Bishop agreed.

Dr. Ashby, the second principal reviewer, agreed with the conclusions. He noted that the conclusion of some evidence in male mice was based on the dose response trend and the dose-related increase in hyperplasias. Dr. Ashby spoke to the question of whether irritation leads to hyperplasia which in turn leads to tumors. Dr. J. Huff, NIEHS, indicated that this has been a long standing speculation, and the literature and NTP studies are replete with exceptions; for instance the benzaldehyde studies in mice showed little evidence of forestomach irritation.

Dr. Mirer, the third principal reviewer, agreed with the conclusions in female rats and male mice. He said the conclusion in male rats should be some evidence, or at a minimum, equivocal evidence of carcinogenic activity, based on increased incidences of pancreatic acinar cell adenomas with a significant trend and a significant pairwise comparison in the high dose group by the logistic regression test. Dr. Bishop noted that several control groups from this study laboratory have had some of the highest incidences of pancreatic adenomas observed in any NTP studies. This along with only a marginal increase at the high dose which was well within the historical control range supported a conclusion of no evidence. Dr. Mirer argued that the studies provide clear evidence in female mice if studies by the NTP or others can be shown that demonstrate progression of squamous papillomas of the forestomach to malignancy. Dr. S. Eustis, NIEHS, responded that there were no carcinomas to provide evidence of progression and there was only a marginal increase in papillomas. Finally, Dr. Mirer stated that the results indicate that female rats and mice of both sexes might have tolerated higher doses, and decreased survival in male rats might have compromised the sensitivity of the study for detecting neoplastic effects. Dr. Bishop replied that in high dose male rats, survival was greater than 70% at 18 months and greater than 50% up until the last two weeks of the study.

Dr. Garman moved that the Technical Report on benzaldehyde be accepted with addition in the conclusions of a statement that female rats and mice of both sexes might have tolerated higher doses and inclusion in Table 111-6 of statistical values for pancreatic hyperplasias in male rats, and with the conclusions as written for male and female rats, no evidence of carcinogenic activity, and for male and female mice, some evidence of carcinogenic activity.

Dr. Ashby seconded the motion. Dr. Mirer offered an amendment that the level of evidence in male rats be changed to equivocal evidence of carcinogenic activity based on increased incidences of adenomas and hyperplasias of the pancreas and of mononuclear cell leukemias. Dr. McKnight seconded the amendment which was defeated by six no votes to two yes votes (McKnight, Mirer). The original motion by Dr. Garman then was accepted by seven yes votes to one no vote (Klaassen).

d-Carvone. Dr. P.C. Chan, NIEHS, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of d-carvone 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 d-carvone for male or female B6C3F1 mice administered 375 or 750 mg/kg, 5 days per week.

Dr. Chan reported that a planned two-year study of d-carvone in F344 rats was terminated after 13 months and the tissues were not evaluated. The decision to terminate the rat study was made because of several performance problems in the laboratory that were unrelated to the mouse study.

Dr. Lijinsky, a principal reviewer, agreed with the conclusions. He found it worrisome that there was a higher mortality among control female mice than in the dosed groups which suggested that the animals were not randomized. Dr. Chan responded that the animals were properly randomized. Dr. S. Eustis, NIEHS, explained that the mortality was due to utero-ovarian infections and it appeared that the chemical may have prevented the infections. Dr. Lijinsky asked about the volumes of dose solution administered and whether they might not have been excessive in some cases. Dr. Chan pointed out that the volume of dose solution was constant among groups, 10 ml/kg. In view of the early termination of the rat study, Dr. Lijinsky thought speculation in the Discussion about possible renal effects of d-carvone in rats should be deleted.

Because Dr. Newberne, the second principal reviewer, was unable to attend the meeting, Dr. L. Hart, NIEHS, read his review into the record. Dr. Newberne agreed with the conclusions. He asked for more information to be added on reasons for the cancellation of the rat study. He said speculative discussion comparing renal effects of d-limonene in male rats with unknown renal effects of d-carvone should be deleted.

Dr. Klaassen, the third principal reviewer, agreed with the conclusions. He said reasons for cancelling and not reporting the rat study should be given near the front of the report. Dr. McKnight asked for clarification as to whether or not the problems with the rat study might have impinged on the mouse study. Dr. J. Huff, NIEHS, said the in-life portion of the mouse studies was completed and proceeding through the histopathology phase by the time the two-year rat studies were begun. About halfway through the studies, the NTP make the decision to terminate them based on the judgment that the collective performance of the contract laboratory was inadequate at that time. There was no evidence of fraud or deceit. Information on the prechronic studies in rats was included in the draft Report because they were done concomitant with the chronic studies in mice and are considered valid. Further, he said that based on the audit and knowledge of laboratory performance at the time the mouse studies were done, the Program believes the mouse studies are adequate for evaluation. Dr. Popp commented that the Audit Summary supported this belief. Dr. Ashby and Dr. Lijinsky proposed that all references to the rat studies be deleted from the report. Dr. Chan agreed. Dr. Scala said the discussion was helpful in providing assurances to the Panel concerning the reliability of the data from the mouse studies.

Dr. Lijinsky moved that the Technical Report on d-carvone be accepted with the revisions discussed, including deletion of all references to the rat studies as

well as the comparisons with d-limonene, and the conclusions as written for male and female mice, no evidence of carcinogenic activity. Dr. Klaassen seconded the motion. Dr. Gold offered an amendment to the motion to add a phrase to the conclusions stating that the mice may have been able to tolerate a higher dose. The vote on this amendment resulted in a tie with four no votes (Ashby, Klaassen, Lijinsky, Popp) and four yes votes (Garman, Gold, McKnight, Mirer). To resolve the tie, Dr. Scala, the Chair, voted no resulting in defeat of the amendment. The original motion by Dr. Lijinsky then was accepted unanimously with eight votes. It was suggested that the prechronic studies in rats could be published separately as a toxicity studies report.

3,3'-Dimethoxybenzidine Dihydrochloride. Dr. D.L. Morgan, NIEHS, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of 3,3'-dimethoxybenzidine dihydrochloride by reviewing the experimental design, results, and proposed conclusions:

Under the conditions of these 21-month drinking water studies, there was clear evidence of carcinogenic activity of 3,3'-dimethoxybenzidine dihydrochloride for male F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal gland, preputial gland, oral cavity, intestine, liver, and mesothelium. Increased incidences of astrocytomas of the brain may have been related to chemical administration. There was clear evidence of carcinogenic activity of 3,3'-dimethoxybenzidine dihydrochloride for female F344/N rats, as indicated by benign and malignant neoplasms of the Zymbal gland, clitoral gland, and mammary gland. Increases in neoplasms of the skin, oral cavity, large intestine, liver, and uterus/cervix were also considered to be related to chemical administration of 3,3'-dimethoxybenzidine dihydrochloride.

Dr. Morgan explained that the studies were intended to last 24 months but were terminated after 21 months because of rapidly declining survival of exposed animals due to neoplasia.

Dr. McKnight, a principal reviewer, agreed with the conclusions. She commented that for skin tumors, the statistical analysis would be more accurate if based on the time at which a tumor first appeared in each animal, rather than the time at which each animal died with a tumor, but noted that in this study that change of analysis would not affect the conclusions.

Dr. Popp, the second principal reviewer, agreed with the conclusions. He pointed out that the Introduction provides information that indicates the chemical had been shown previously to be carcinogenic in experimental animals, and thought that the rationale for doing the current study could be added here as well to avoid potential confusion for the reader. Dr. Popp noted the observation of foci in the livers of rats after nine months treatment which suggested the chemical might be a hepatocarcinogen, yet there was a relatively weak liver tumor response at 21 months. Dr. Morgan speculated that the early animal deaths may have sufficiently shortened the time available for progression of foci to detectable tumors.

Dr. Gold, the third principal reviewer, agreed with the conclusions. She also requested mentioning the rationale for performing the current study in light of the findings from the earlier studies. She opined that some of the earlier studies were not adequate by current standards. Dr. Morgan said the rationale for the study would be stated earlier in the Introduction and the inadequacies of the earlier studies would be noted. Dr. Gold asked that NIOSH data from the current National Occupational Exposure Survey (NOES) be appended indicating estimated numbers of U.S. workers exposed to the chemical. Dr. Scala questioned the accuracy of the exposure estimates. Dr. H. Matthews, NIEHS, proposed that the numbers of workers exposed to 3,3'-dimethoxybenzidine was likely to be greater than the survey estimates since NTP studies have shown, at least in animals, that dyes derived from benzidine or its congeners were metabolically reduced in the body almost completely to the parent compound. Dr. Gold also suggested that the results from the study in mice conducted at NCTR be included in the report.

Dr. Mirer said another rationale for the NTP study could be that there is no tumor site concordance between humans and animals. Dr. J.Huff, NIEHS, responded that there were no epidemiology studies on this congener to enable determination of concordance. He added that there is a comparable neoplastic site (urinary bladder) in humans and dogs exposed to the parent chemical, benzidine.

Dr. McKnight moved that the Technical Report on 3,3'-dimethoxybenzidine be accepted with the revisions discussed and the conclusions as written for male and female rats, clear evidence of carcinogenic activity. Dr. Popp seconded the motion, which was accepted unanimously with eight votes.

Glycidol. Dr. R.D. Irwin, NIEHS, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of glycidol by reviewing the experimental design, results, and proposed conclusions:

Under the conditions of these 2-year gavage studies, there was clear evidence of carcinogenic activity of glycidol for male and female F344/N rats and male and female B6C3F1 mice, based on increased incidence of a variety of neoplasms at numerous tissue sites.

Dr. Irwin noted that because survival of chemically exposed rats was reduced by the early lethality caused by neoplasia, the usual convention of expressing tumor incidence might underestimate the true incidence that would have occurred in the absence of such reduced survival. Therefore, tumor analyses for rats were based on the effective number of rats in each group, i.e., the number of rats alive at the time that the first rat died or was killed in a moribund condition with the tumor at a particular site.

Dr. Popp, a principal reviewer, agreed with the conclusions. He also agreed with the approach described by Dr. Irwin based on the effective number of animals although he thought the rationale could be highlighted better in the Materials and Methods section. He asked for an explanation of the poor survival in control rats. Dr. Irwin indicated there was no ready explanation, and this would be noted in the report.

Dr. McKnight, the second principal reviewer, agreed with the conclusions. She thought the tumor sites supporting the level of evidence should be listed in the conclusions. She said the statement that exposure to chemical accelerated the development of advanced stages of mononuclear cell leukemia should either be given more support or omitted. Dr. Irwin agreed, and said the effect on leukemias would be given less emphasis.

Dr. Garman, the third principal reviewer, agreed with the conclusions. He expressed concern about the presentation of the histopathology portion of the report in that the sections dealing with microscopic lesions were rather sketchy, being restricted primarily to statements of lesion frequencies. Dr. S. Eustis, NIEHS, responded that extensive histologic descriptions had not been prepared because the studies were overwhelmingly positive and a large percentage of the neoplasms were malignant. He said brief histologic descriptions would be added.

Dr. Gold suggested adding more details as to which target sites support the evaluation of clear evidence of carcinogenic activity and which do not. Dr. J. Huff, NIEHS, explained that the NTP philosophy was to arrive at an overall conclusion for each experiment. With so many tumor sites contributing to the level of evidence and because of competing risks, summary tables A and B were put in the Abstract to indicate the tumors that collectively contributed to clear evidence in rats and mice. Dr. Huff indicated that the concluding statement would be divided into a sentence for rats and one for mice, and referrals would be made to the lists of neoplasms given in the Abstracts. Dr. Ashby noted that assignment of a level of evidence to each tumor site would be helpful in examining carcinogenic mechanisms. Dr. Gold asked that estimates of worker exposure from the National Occupational Exposure Survey (NOES) be added to the report.

In comments from the audience, Dr. Donald McFee, representing Occusafe, a private consulting firm, suggested that there be some discussion on the possible effects of the six percent impurities, especially alpha-chlorohydrin and diglycidyl ether. Mr. Ralph Johnson, Vice President of Environmental Affairs, Dixie Chemical Company, the sole domestic manufacturer of glycidol, commented on human exposure studies they had done at all customer sites. He said the studies indicated about 70 persons were exposed annually at levels not exceeding two parts per million. A copy of their report was given to Dr. Irwin after the meeting.

Dr. Popp moved that the Technical Report on glycidol be accepted with the conclusions as written for male and female rats and mice, clear evidence of carcinogenic activity. Dr. Garman seconded the motion, which was accepted unanimously with eight votes.

Succinic Anhydride. Dr. R.L. Melnick, NIEHS, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of succinic anhydride 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 succinic anhydride for male or female F344/N rats given 50 or 100 mg/kg succinic anhydride. There was no evidence of carcinogenic activity for male B6C3F1 mice given 38 or 75 mg/kg succinic anhydride or for female B6C3F1 mice given 75 or 150 mg/kg.

Because Dr. Newberne, a principal reviewer, was unable to attend the meeting, Dr. L. Hart, NIEHS, read his review into the record. Dr. Newberne agreed with the conclusions, but questioned the gavage route of exposure. Dr. Melnick said the route used was selected because that is the human route of exposure. Dr. Newberne stated that the usage data were available and including the weight or percentage used in foods would be helpful.

Dr. Gold, the second principal reviewer, agreed with the conclusions. She commented on the finding of oil droplets in the lungs of some rats and mice on retrospective examination. She suggested that the possible role of gavage accidents in early deaths or moribund states needed further discussion and that it should be clearly stated that survival was considered adequate to detect a carcinogenic effect. Dr. Melnick pointed out that there were sufficient numbers of animals to evaluate carcinogenicity, as stated in the discussion, results and abstract. Dr. Gold also thought that more information on human exposure should be added including more recent estimates on worker exposure from the NIOSH National Occupational Exposure Survey (NOES). Dr. Melnick said the NOES data would be included in the report.

Dr. McKnight, the third principal reviewer, agreed with the conclusions. She said she could support a conclusion of equivocal evidence of carcinogenic activity in male rats based on a statistically significant positive dose-related trend in the incidence of keratoacanthomas of the skin. Dr. Melnick explained that the incidence in high dose animals was not significantly different from control values and the incidence was slightly lower than the highest historical incidence observed in male corn oil gavage control F344 rats. With regard to early deaths due to gavage accidents, Dr. McKnight noted there was a clear dose-related trend, presumably because dosed animals become harder to handle and are thus more prone to accident. Thus, she proposed that these "accidental" deaths could be classified as deaths due to toxic effects of the chemical as it is administered under study conditions. Dr. Melnick said an additional survival curve of total survival without censoring for gavage accidents would be included.

Dr. Gold moved that the Technical Report on succinic anhydride be accepted with the conclusions as written for male and female rats and mice, no evidence of carcinogenic activity. Dr. Popp seconded the motion which was accepted by seven yes votes with one abstention (McKnight).

TOXICITY STUDIES

Cobalt Sulfate Heptahydrate. Dr. J.R. Bucher, NIEHS, NTP Study Scientist, introduced the short-term toxicity studies of cobalt sulfate heptahydrate by reviewing the rationale, experimental design, and results. Cobalt sulfate aerosols were administered by whole body inhalation exposure to groups of F344/N rats and B6C3F1 mice of both sexes for 16-days or 13-weeks. In 16-day studies, all rats and mice exposed to the top concentration of 200 mg/cubic meter died, while in 13-week studies, all rats and all but 2/10 male mice exposed to the top concentration of 30 mg/cubic meter survived to the end of the studies. A no-observed-adverse-effect level was not reached with either species. In the 16-day studies, lesions were observed in the respiratory tracts of rats and mice including degeneration of the olfactory epithelium and necrotizing inflammation in the nose, larynx, trachea, and bronchi. Regenerative lesions, including peribronchial and septal fibrosis in the lung, were also observed in both species. In the 13-week studies, lesions in the respiratory tract included olfactory epithelium degeneration, squamous metaplasia of the respiratory epithelium, and inflammation in the nose of rats and mice. In the larynx, inflammation, necrosis, and squamous metaplasia occurred in both species, and ulcers and inflammatory polyps occurred in rats. In the lung, histiocytic infiltrates, bronchiolar regeneration, peribronchiolar and septal fibrosis, and epithelial hyperplasia in the alveoli were observed in both rats and mice. The tissue affected at the lowest concentration was the larynx.

Dr. Garman, a principal reviewer, said the draft report was well prepared. In particular, he thought the histopathology portion of the Results section to be commendable for the detailed description of the localization of the lesions at the suborgan level and for the high quality photomicrographs of the lesions. He suggested changes to the discussion that emphasized the unique susceptibility to chemical injury of the area of the larynx affected by cobalt. He said the aerosol used needed to be better characterized in the report, especially the pH in view of the irritation observed. Dr. Bucher noted that the aerosol was a dry aerosol and the pH was concentration dependent and not as acidic as predicted so there was less concern that effects seen were due to the acidity.

Dr. Klaassen, a second principal reviewer, said the report that cobalt activates the enzyme, heme oxygenase, should be corrected to indicate that cobalt induces the enzyme.

Dr. Mirer stated that cobalt is an extremely important compound from an industrial health point of view, and the lowest level tested in this study is only slightly above the allowed occupational health limit. He said there was some indication that respiratory effects of cobalt in humans may have allergic aspects. Dr. Mirer asked whether the NTP planned to do chronic studies. Dr. Bucher said no decision had been made on further studies. Dr. R. Griesemer, NIEHS, said the Panel's advice would be helpful in setting priorities. Dr. Scala said that seeing no objections, the Panel would pass along the report with its comments.

1,2-Dichloroethane. Dr. D.L. Morgan, NIEHS, NTP Study Scientist, introduced the short-term toxicity studies of 1,2-dichloroethane by reviewing the rationale, experimental design, and results. The chemical was administered to B6C3F1 mice, F344/N rats, Osborne-Mendel rats, and Sprague-Dawley rats in drinking water for 13 weeks to investigate potential differences in species and strain susceptibility. In addition, F344 rats were given 1,2-dichloroethane by gavage in corn oil to compare toxicity resulting from bolus administration with that of continuous exposure. Few toxic effects were observed in rats administered the chemical in drinking water, most likely because a high enough dose could not be achieved due to limitations in solubility and palatability. Based on organ weight increases, the kidney and liver appeared to be target organs, although histologic evidence of toxicity was found only in kidneys of female F344 rats and male mice. Similar doses (in mg/kg) by gavage resulted in greater toxicity to F344 rats than after administration in drinking water. Necrosis of the cerebellum and thymus, as well as hyperplasia, inflammation and mineralization of the forestomach were observed in rats that died or were killed in moribund condition.

Dr. Klaassen, a principal reviewer, commented that stating the rationale for the study earlier in the report, especially in the Abstract, would be helpful. Dr. Morgan agreed and said it would be put in the Abstract.

Dr. Popp, a second principal reviewer, said the report was clearly written and adequately presented the background and current experiments. He inquired as to the rationale for a separate group of animals for evaluation of clinical pathology parameters. Dr. Morgan replied that this was done because of uncertainty about the effects of bleeding on animal response to the chemical.

Dr. Mirer observed that if the comparative route studies were aimed at determining if there was saturation of metabolic mechanisms, the question was not answered. He said this could be more directly addressed by an absorption and distribution study. Dr. Gold said there should be newer human exposure data available. Dr. J. Haartz, NIOSH, said newer exposure data was available and she would send it to Dr. Morgan. Dr. L. Zeise, Calif. Dept. of Health Services, suggested that it would be helpful to include discussion of how the delivered dose was calculated in the drinking water studies. Dr. Bucher said this information would be included in this report and in reports of future drinking water studies.

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

alpha-Methylbenzyl Alcohol - Reevaluation. The NTP toxicology and carcinogenesis studies of alpha-methylbenzyl alcohol in F344/N rats and B6C3F1 mice (draft Technical Report No. 369) were peer reviewed by the Panel on March 13, 1989. Because of poor survival among rats of both sexes due mainly to a cluster of possibly accidental deaths around the midpoint of the studies and excessive dose-related mortality during the last quarter, the Panel was unable to ascertain to their satisfaction whether these occurrences were isolated events, or whether they represented a more generic technical inadequacy of the studies in rats. Therefore, the Panel asked the NTP to review the technical conduct of the two-year studies of alpha-methylbenzyl alcohol 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 (some evidence of carcinogenic activity in males and no evidence of carcinogenic activity in females) should be affirmed; or (2) if the NTP concludes that the rat studies are flawed, then they should be reclassified as inadequate studies of carcinogenic activity. The survival of mice was not an issue and the conclusions for mice were accepted by the Panel at the previous meeting.

Dr. S. Eustis, NIEHS, began with a general discussion of the problem of deaths resulting from poor gavage technique and how determination is made as to whether or not gavage was a factor in an animal's death. Speaking to the alpha-methylbenzyl alcohol studies, Dr. Eustis reported that dosing records, temperature and humidity data, clinical observations, and individual animal data records were reviewed by Dr. Dieter, the Study Scientist, and pathology staff. In addition, pathology diagnoses and slides from all early death animals were re-examined to determine as accurately as possible the causes of death. This review revealed no evidence that environmental conditions, infectious disease or other previously unreported factors contributed to the lowered survival in low and high dose male rats and high dose female rats. The majority of the early deaths recorded as accidental were associated with evidence that gavage was a factor. Review of the pathology data and slides confirmed that the preponderance of early deaths in dosed male rats could be attributed to exacerbation of chronic nephropathy by the chemical. In female rats, reduced survival in the high dose group seemed to be due primarily to the gavage-related deaths. In summary, Dr. Eustis stated that after reviewing all pertinent clinical records and audit reports on these studies, the staff believed that the conduct of the studies was technically adequate.

He then described further investigations on the kidneys of male rats. Three additional sections from each animal were taken to aid in the interpretation of the pathology data. From the extra sections, additional tubular cell neoplasms were observed in low and high dose male rats. The incidences of tubular cell neoplasms found during the original evaluation of one section per kidney were: controls, 0/50; low dose, 2/50; and high dose, 5/50. The overall incidence of tubular cell neoplasms observed in the original evaluation and the additional sections combined was: controls, 1/50; low dose, 13/50; and high dose, 14/50. The increased tumor incidence was significant ($p < 0.001$) for both low and high dose groups compared with controls.

Dr. Scala commented that unless the Panel or the NTP wanted to recommend changing the level of evidence in male rats, no further action was required. However, he thought a sense of the Panel motion would be appropriate for the record. Dr. Popp moved that based upon the additional studies and evaluation

that the NTP has done and the report presented by Dr. Eustis, the previously raised questions concerning the conduct of the study were now answered to the satisfaction of the Panel. Further, the original levels of evidence, some evidence of carcinogenic activity in male rats, and no evidence of carcinogenic activity in female rats, were still appropriate. Dr. Lijinsky seconded the motion. After further discussion, Dr. Mirer offered an amendment intended to confine the sense of the Panel action to the conduct of the study by deleting the sentence concerning the levels of evidence. Dr. Gold seconded the amendment, which was accepted unanimously with eight votes. Dr. Popp's motion minus the deleted sentence was then accepted unanimously with eight votes.

Dr. Scala concluded the review of alpha-methylbenzyl alcohol by asking that if the Program intends to change the levels of evidence in male rats, then that recommendation should be brought back to the Panel for evaluation.

Genetic Control of Inbred Rodents Produced for the NTP Studies - Dr. G. N. Rao NIEHS, began by stating that assuming chemical treatment is constant, much of the variability between animals of a group could be a combination of genetic factors, experimental factors, and environmental factors (Attachment 1).

The sources of genetic variability in inbred strains could be at least three: (1) residual heterozygosity; (2) genetic contamination; and (3) genetic mutations (Attachment 1, p. 1).

Dr. Rao discussed steps taken to prevent these sources of variation including (1) preventing formation of sublines (Attachment 1, p. 2), and (2) monitoring for genetic contamination by using two classes of tests, one being biochemical markers, markers selected to detect contamination of related strains, and the second is the skin grafting procedure (Attachment 1, p. 3).

Biochemical markers for mice are shown in Attachment 1, p. 4. Looked at are 13 different biochemical markers, plus two visible markers related to coat color, a total of 15 markers located on 11 chromosomes. These were selected because these are variable in different strains. With regard to the rat, nine different markers are used to differentiate different strains and detect any contamination, plus the coat color, a total of ten markers (Attachment 1, p. 5). The six-year experience based on these tests is that there is no genetic contamination of the NTP rat and mouse colonies.

With regard to genetic mutation, the same classes of tests are used. The findings for skin grafting are summarized in Attachment 1, pp. 8-9. Based on the skin graft procedure in effect now for three years, we can conclude that there were no major mutations during the last three years in the NTP rat and mouse colonies.

Results of a six year experience with biochemical markers are summarized in Attachment 1, pp. 10-11.

Dr. Rao concluded that there has been no genetic contamination and no major mutations in the NTP rat and mouse colonies during the past six years.

**GENETIC CONTROL OF INBRED RODENTS
PRODUCED FOR THE NTP STUDIES**

SOURCES OF GENETIC VARIATION IN INBRED STRAINS

- **RESIDUAL HETEROZYGOSITY WITH DIFFERENT AND DIFFERING INCREASE OF HOMOZYGOSITY IN INDIVIDUAL SUBLINES**
- **GENETIC CONTAMINATION: the unintentional or unobserved crossing of an alien genome into an established inbred strain**
- **GENETIC MUTATION**

PREVENT FORMATION OF SUBLINES

- **PERIODIC REDERIVATION WITH PEDIGREED STOCK FROM NIH GENETIC COLONIES (1981, 1984, 1987, . . .)**
- **LIMIT THE NUMBER OF GENERATIONS**

GENETIC CONTAMINATION

- **BIOCHEMICAL MARKERS**
- **SKIN GRAFTING**

BIOCHEMICAL MARKERS

SPECIFIC LOCI FOR GENETIC MONITORING OF MICE

LOCUS	CHROMOSOME	DESCRIPTION	TISSUE ANALYZED
IDH-1	1	Isocitric dehydrogenase	Kidney
PEP-3	1	Peptidase	Kidney
CAR-2	3	RBC carbonic anhydrase	RBC
GPD-1	4	Glucose-6-phosphate dehydrogenase	Kidney
PGM-1	5	Phosphoglucomutase	Kidney
LDR-1	6	Lactate dehydrogenase regulator	RBC
GPI-1	7	Glucose phophate isomerase	Kidney
HBB	7	Hemoglobin-beta chain	RBC
ES-1	8	Esterase	Kidney
MOD-1	9	Malic enzyme	Kidney
TRF	9	Transferrin	Serum
ES-3	11	Esterase	Kidney
ES-10	14	Esterase	RBC
A	2	Agouti-nonagouti	Coat color
B	4	Black-brown	Coat color

BIOCHEMICAL MARKERS

SPECIFIC LOCI FOR GENETIC MONITORING OF RATS

LOCUS	DESCRIPTION	TISSUE	LINKAGE GROUP
ES-1	Esterase-1	Plasma	V
ES-2	Esterase-2	Plasma	V
ES-3	Esterase-3	Liver	V
ES-4	Esterase-4	Kidney	V
ES-6	Esterase-6	Kidney	?
HBB	Hemoglobin	RBC	I
LAP-1	Leucine aminopeptidase-1	Kidney	?
PEP-3	Dipeptidase	Kidney Liver	X
FH-1	Fumerate hydratase	Kidney	X
C	Colored albino	Coat color	I

SIX
~~FIVE~~-YEAR EXPERIENCE

**No Genetic Contamination of
NTP Rat and Mouse Colonies**

GENETIC MUTATION

● SKIN GRAFTING

● BIOCHEMICAL MARKERS

SKIN GRAFTING

No skin graft rejections

- **Within the same colony**
- **Between the colonies rederived from NIH genetic colonies three years apart**

No major mutations during the last three years in the NTP rat and mouse colonies.

**C57BL/6N
C3H/HeN MTV-
F344/N**

**C57BL/6J
C3H/HeJ MTV-
F344/Ncr**

**Detected one to two loss mutations in 4 of 30
C57BL/6J mice indicating some degree of
genetic inhomogeneity within the C57BL/6
colony at Jackson Laboratory**

BIOCHEMICAL MARKERS

Six-Year Experience--Mice

- C57BL/6 x B6C3F1 back cross in one of 125 breeding cages.
- Variant pattern of glucose phosphate isomerase in one C57BL/6 indicating possible spontaneous mutation.
- Malic enzyme protein variant similar to the protein in C3H in one B6C3F1 mouse. This could be due to the expression of residual heterozygosity or a spontaneous mutation.
- Variants of esterase activity in B6C3F1 possibly due to a mutation at an unidentified esterase locus.

BIOCHEMICAL MARKERS

Six-Year Experience--F344 Rats

- **One esterase variant consistent with a mutation at an unidentified esterase locus**

CONCLUSION

No genetic contamination and no major mutations in the NTP rat and mouse colonies during the past six years.

Light Intensity Associated Ophthalmitis of Fischer 344 Rats in Long-Term Studies - Dr. G. N. Rao, NIEHS, noted that if the genetic variability can be controlled, then experimental and environmental factors will be the major causes for variability between animals of a group. Light is one of the major environmental factors for animals. Often albino rats and mice are known to be more sensitive to light than pigmented animals. Dr. Rao then shared NTP experience on light intensity associated ophthalmitis or ocular lesions other than retinal degeneration of Fisher 344 rats in long-term studies (Attachment 2).

The NIH guide for the care and use of animals, 1978 edition, recommended 75 to 125 foot candles light intensity in the animal room. Laboratories conducting studies for the NTP have light intensities as high as 150 foot candles in the animal rooms. Rats in the NTP studies are group housed at five per cage in hanging dryer type poly carbonate cages (Attachment 2, p. 2). These are very clear plastic cages, and they are as transparent as clear glass. And when the animal room is illuminated from the ceiling lights, there will be higher lighting to the animals in the cages in the top row to some extent, while the side columns will be exposed to higher light intensities than the animals in the inside cages and the bottom rows. And indeed, in the NTP studies quite a few animals in the top row have developed opaque or cloudy eyes.

Attachment 2, pp. 3-4 shows a six-row cage rack with five cages per row, used for the dose arrangement in one of the testing facilities. The high-dose male animals were in the top two rows, high dose females in the middle rows, low dose males in the bottom rows, and in the second rack the rest of the animals. The information presented here is a summary of three different studies combined, those on benzyl acetate, geranyl acetate and zearalenone. Shown is the number of animals with lesions over the number of animals started in the study combined for the three studies in each of these cages. Lesions were diagnosed as cataracts by histologic examination. In the top row some 65 percent of all the animals had the lesions, while less than ten percent in the bottom rows and inside column cages had lesions. In the second rack of the same set of studies, some 70 percent of in the top row alone had opaque eyes or cataracts. Very few in the other rows had lesions.

Attachment 2, pp. 5-6 shows the prevalence of lesions in a second facility. This is combined information from two different studies, those on erythromycin stearate and 2-amino-5-nitrophenol. In this facility they housed animals by dose in two rows together, but with all the males in one rack and all the females in a different rack. When combined from two different studies, some 80 percent or more of the animals in the top row had cloudy or opaque eyes, a few were found on the side column, while very few in the bottom rows and the interior cages had eye lesions.

Dr. Rao noted that some of the contract facilities had light intensities as high as 150 foot candles. Attachment 2, p. 7 shows very high light intensities in the top row and in the side cages with light intensity substantially lower at the interior, inside cages, and the bottom rows. This is at 120 foot candles five feet from the floor.

If the light intensity is changed to 80 foot candles at five feet from the floor, there is a substantial decrease, but still a higher light intensity in the top row and side cages, but not in inside cages or the bottom (Attachment 2, p. 8).

Attachment 2, p. 9 shows a combination of the light intensity versus eye lesions. This is the light intensity inside the cages, assuming a hundred and twenty foot candles at five feet from the floor. And these are the eye lesions observed in 2-amino-5-nitrophenol study listed as opaque or cloudy eyes at the 22nd month of the study. These were the number of animals with the lesions, number that are surviving at the time of observation. Almost all on the top row had eye lesions, a good number in the side column had eye lesions, whereas in the interior cages and the bottom rows, very few had eye lesions.

Dr. Rao said these studies established that indeed the eye lesions were associated with the light intensity. So, the laboratory animal program implemented several procedures to decrease the light intensity associated with eye lesions (Attachment 2, p. 10). One was to decrease the room light intensity to less than 50 foot candles at five feet from the floor. Attachment 2, p. 11 shows the type of light intensity seen. In all cases there are less than 15 foot candles inside the cage. Based on our experience lesions have not been seen in the cages where the light intensities are around this or less than this. So, this light intensity is expected not to cause any substantial increase in the eye lesions.

In addition, instead of housing the animals in rows combined in two horizontal rows together, the animals are now housed in columns, from top to bottom, so that -- environmental factors could be equalized between study groups.

Further, instead of placing all the animals of a treatment group in two columns together, each column should be randomized between two or three rats used for a given study so that there would be a reasonable distribution of the environmental effects.

Also required are rotation of cages from top to bottom every two weeks when the rats are changed. And by this process the animals in any given cage will not be exposed to the higher light intensity of the top cage, the top row, for more than one-sixth of the total time of the study.

Using these procedures the light intensity of ocular lesions has been reduced in NTP studies to a very low level, to less than ten percent. Occasionally, randomly distributed eye lesions are seen, and these are possibly inherent to the albino animals, and these are mostly aging associated cataracts.

**LIGHT INTENSITY ASSOCIATED OPHTHALMITIS
OF FISCHER 344 RATS IN LONG-TERM STUDIES**

**ILLUMINATION OF 75-125 FT. CANDLES
(807-1,345 LUMENS/M²) IS RECOMMENDED**

(NIH GUIDE, 1978)

Cage or Column

Row

1

2

3

4

5

1

2

3

4

5

6

PREVALENCE OF EYE LESIONS BY CAGE POSITION AND TREATMENT
GROUP FOR THREE STUDIES AT LABORATORY 1^a

RACK 1							
ROW	COLUMN					TREATMENT	TOTAL BY ROW (Σ)
	1	2	3	4	5		
1	8 ^b /15 ^c	10/15	13/15	8/15	10/15	High dose males	49/75 (65)
2	0/15	2/15	2/15	0/15	1/15		
3	1/15	0/15	0/15	2/15	1/15	High dose females	4/75 (5)
4	1/15	1/15	1/15	0/15	0/15		
5	0/15	0/15	1/15	2/15	2/15	Low dose males	5/75 (7)
6	3/15	2/15	0/15	1/15	0/15		

^a Lesions diagnosed as cataracts by histologic evaluation at the end of the three studies with chemicals Benzyl acetate, Geranyl acetate, Zearalenone.

^b Number with lesions, combined from the three studies.

^c Number started, combined from the three studies.

PREVALENCE OF EYE LESIONS BY CAGE POSITION AND TREATMENT
 GROUP FOR THREE STUDIES AT LABORATORY 1 (cont'd)

RACK 2

ROW	COLUMN					TREATMENT	TOTAL BY ROW (Σ)
	1	2	3	4	5		
1	8/15	11/15	12/15	11/15	9/15	Low dose females	51/75 (68)
2	2/15	1/15	1/15	0/15	1/15		5/75 (7)
3	0/15	0/15	0/15	0/15	0/15	Control males	0/75 (0)
4	0/15	1/15	0/15	0/15	0/15		1/75 (1)
5	0/15	1/15	0/15	1/15	0/15	Control females	2/75 (3)
6	0/15	0/15	2/15	0/15	1/15		3/75 (4)

PREVALENCE OF EYE LESIONS IN MALE RATS BY CAGE
POSITION FOR TWO STUDIES AT LABORATORY 2^a

ROW	COLUMN					TOTAL BY TREATMENT ROW (%)
	1	2	3	4	5	
1	5 ^b /5 ^c	6/9	6/7	9/9	8/8	Low dose 34/38 (89)
2	4/6	2/9	1/7	1/5	5/8	
3	2/7	1/6	0/7	1/5	1/8	High dose 5/33 (15)
4	2/8	1/9	0/6	1/8	3/10	
5	2/7	0/9	1/9	0/8	3/9	Control 6/42 (14)
6	0/9	0/9	0/7	0/8	1/8	
Total by column (%)	15/42 (36)	10/51 (20)	8/43 (19)	12/43 (26)	21/51 (42)	

^a Observations listed as opaque or cloudy eyes at 22-23 month of two studies.

^b Number with lesions, combined from the two studies.

^c Number surviving at the time of observation, combined from the two studies.

PREVALENCE OF EYE LESIONS IN FEMALE RATS BY CAGE POSITION FOR TWO STUDIES AT LABORATORY 2

ROW	COLUMN					TREATMENT	TOTAL BY ROW (Σ)
	1	2	3	4	5		
1	7/9	5/7	7/7	7/10	7/7	Low dose	33/40 (80)
2	5/9	2/9	3/8	2/7	4/8		16/41 (39)
3	0/9	1/10	2/8	0/7	1/7	High dose	4/41 (10)
4	1/6	1/10	1/10	0/9	1/7		4/42 (10)
5	0/8	0/8	0/9	0/9	1/8	Control	1/42 (2)
6	1/6	0/7	0/8	0/9	0/8		1/38 (3)

LIGHT INTENSITY IN THE CAGES AT A LIGHT INTENSITY OF 120 FT-CANDLES IN THE ROOM¹

LIGHT INTENSITY IN THE CAGES^{2,3}

	COLUMN				
ROW	1	2	3	4	5
1	50	37	30	37	50
2	40	28	20	28	40
3	30	21	15	21	30
4	20	15	10	15	20
5	16	11	6	11	16
6	8	5	3	5	8

¹ 5 feet from the floor.

² Inside front of the cages under ideal conditions where the cage rack is parallel to the ceiling light fixtures.

³ Foot-candles (1 ft-candle = 10.8 lumens/m²)

LIGHT INTENSITY IN THE CAGES AT A LIGHT
INTENSITY OF 80 FT-CANDLES IN THE ROOM¹

LIGHT INTENSITY IN THE CAGES^{2,3}

	COLUMN				
ROW	1	2	3	4	5
1	30	23	18	23	30
2	20	15	11	15	20
3	15	11	8	11	15
4	12	9	6	9	12
5	10	7	4	7	10
6	5	3.5	2	3.5	5

¹ 5 feet from the floor.

² Inside front of the cages under ideal conditions where the cage rack is parallel to the ceiling light fixtures.

³ Foot-candles (1 ft-candle = 10.8 lumens/m²)

PREVALENCE OF EYE LESIONS BY CAGE POSITION
AND LIGHT INTENSITY IN MALE F344 RATS

		COLUMN				
		1	2	3	4	5
ROW	1	50 ^a 4 ^b /4 ^c	37 5/5	30 4/4	37 5/5	50 5/5
	2	40 3/4	28 1/5	20 0/3	28 0/3	40 4/5
	3	30 1/3	21 1/4	15 0/4	21 1/4	30 1/4
	4	20 1/4	15 0/4	10 0/4	15 1/4	20 1/5
	5	16 0/4	11 0/5	6 0/5	11 0/5	16 0/5
	6	8 0/5	5 0/5	3 0/5	5 0/5	8 1/5

^a Ft-candles inside front of the cages under ideal conditions where the cage rack is parallel to the ceiling light fixtures and the room light intensity is 120 ft-candles at 5 feet from the floor.

^b Number with lesions.

^c Number surviving at the time of observation at 22nd month of the study with 2-Amino-5-nitrophenol in laboratory 2.

**PROCEDURES TO DECREASE THE PREVALENCE OF EYE
LESIONS IN F344 RATS**

- **Decrease the room light intensity to 40-50 ft-candles at 5' from the floor.**
- **Rotate the cages within the same column at two-week intervals.**
- **Do not use top row, if possible.**

LIGHT INTENSITY IN THE CAGES AT A LIGHT INTENSITY OF 40 FT-CANDLES IN THE ROOM¹

LIGHT INTENSITY IN THE CAGES^{2,3}

	COLUMN				
ROW	1	2	3	4	5
1	12	10	7	10	12
2	10	7	5	7	10
3	8	6	4	6	8
4	6	4.5	3	4.5	6
5	4	3	2	3	4
6	2	1.5	1.0	1.5	2

^a 5 feet from the floor.

^b Inside front of the cages under ideal conditions where the cage rack is parallel to the ceiling light fixtures.

^c Foot-candles (1 ft-candle = 10.8 lumens/m²)