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

November 6, 1987
Research Triangle Park, North Carolina

The review meeting began at 9:00 a.m. in the Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the Subcommittee are: Drs. Robert Scala (Chairperson), Michael Gallo and Frederica Perera. Members of the Panel of Experts are: Drs. John Ashby, Charles Capen, Vernon Chinchilli, Kim Hooper, Donald Hughes, William Lijinsky, Franklin Mirer, James Popp, and Andrew Sivak. Drs. Gallo and Chinchilli were unable to attend this meeting although written reviews by Dr. Chinchilli were read and entered into the record. 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 April 18 and 19, 1988, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, (919) 541-3971; FTS: 629-3971.

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Benzyl Alcohol. Dr. M. P. Dieter, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of benzyl alcohol 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 benzyl alcohol for male or female F344/N rats dosed with 200 or 400 mg/kg. Survival in both dose groups of female rats was 50% that of vehicle controls, primarily due to an increased number of gavage-related deaths. There was no evidence of carcinogenic activity of benzyl alcohol for male or female B6C3F$_1$ mice dosed with 100 or 200 mg/kg for 2 years.

Dr. Ashby, a principal reviewer, agreed with the conclusions. He noted the positive genetic toxicity findings reported for gene mutations in mouse lymphoma cells and for chromosome aberrations in Chinese hamster ovary cells. However, Dr. Ashby stated that benzyl alcohol was a classic example of a non-genotoxic non-carcinogen which appears to be genotoxic in vitro because of inadequate criteria for the conduct of in vitro genotoxicity assays. He said the discussion should be revised to reflect this.

Dr. Hughes, the second principal reviewer, agreed with the conclusions. He commented on aspects of the conduct of the study, specifically the higher incidence of accidental gavage deaths in dosed rats compared to controls, and the dosing error which occurred with male and female mice during week 80. He wondered whether either might have compromised the integrity of the study. Dr. Dieter replied that the gavage accidents appeared to be due to a combination of faulty gavage procedure and the anesthetic properties of benzyl alcohol. He felt that although the increased mortality somewhat reduced the sensitivity, there was no question that the results were negative for rats, and that the misdosing with alpha methyl benzyl alcohol did not affect the outcome in mice. Dr. Haseman added that there was no hint of increased tumor incidences in dosed rats.

Dr. Chinchilli, the third principal reviewer, was unable to attend the meeting. Dr. L. Hart, NIEHS, read his review. Dr. Chinchilli agreed with the proposed conclusions. In other discussions, Dr. Mirer and Dr. Ashby noted the similarities in chemical structure and metabolic pathways between benzyl alcohol and benzyl acetate and asked for enhanced discussion of this comparison. Dr. Perera asked for clarification of a change in interpretation of NTP genetic toxicity results originally reported in the conclusions as "weakly positive" for sister chromatid exchanges (SCEs) in Chinese hamster ovary cells. A revision of this section that was distributed to the Panel termed the findings for SCEs as "equivocal." She also asked why a paragraph discussing the genotoxicity of metabolites of benzyl alcohol had been deleted in the revised section. Dr. J. Bishop, NIEHS, responded that the overall conclusion for SCE has always been equivocal. Both with and without metabolic activation, one of the two trials in each of these situations was weakly positive because a significant increase was observed only at the highest dose. The original Discussion paragraph on the genotoxicity of benzyl alcohol described the results of cytogenetic tests as demonstrating induction of both SCEs and chromosome

aberrations. He said it should have noted that the SCE response was judged equivocal as correctly stated in the Abstract and Introduction sections. Dr. Bishop said information on the genotoxicity of the metabolites of benzyl alcohol was already found in the Introduction. However, paragraphs would be added to the Discussion describing the genotoxicity of alpha methyl benzyl alcohol and benzyl acetate. Dr. Scala suggested that Panel members, particularly Dr. Ashby and Dr. Perera, convey their comments on the revised text to Dr. Bishop or Dr. Dieter.

Dr. Ashby moved that the Technical Report on benzyl alcohol be accepted with revisions as discussed and with the conclusions as written for male and female rats and mice, no evidence of carcinogenic activity. Dr. Hughes seconded the motion, which was approved unanimously with nine votes.

α-Methyldopa Sesquihydrate. Dr. J. K. Dunnick, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of α-methyldopa sesquihydrate by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year feed studies, there was no evidence of carcinogenic activity of α-methyldopa sesquihydrate for male or female F344/N rats fed diets containing 3,100 or 6,300 ppm. There was equivocal evidence of carcinogenic activity of α-methyldopa sesquihydrate for male B6C3F$_1$ mice as shown by three dosed mice having uncommon tubular cell tumors of the kidney. There was no evidence of carcinogenic activity of α-methyldopa sesquihydrate for female B6C3F$_1$ mice fed diets containing 6,300 or 12,500 ppm. Nonneoplastic lesions of the kidney including karyomegaly were observed in dosed female mice. Decreased incidences of several tumor types (in the adrenal gland in male rats, uterus in female rats, liver in male and female mice, and anterior pituitary gland in female mice) were considered related to α-methyldopa sesquihydrate exposure.

Dr. Hughes, a principal reviewer, agreed with the conclusions. He thought the dose levels used in prechronic studies of up to 100,000 ppm or 10 percent of the diet to be excessive and levels that high should be discouraged. Dr. Dunnick noted that NTP guidelines allowed a maximum level of five percent for two-year studies. The higher level was used in the fourteen-day studies to help identify potential target organ toxicity.

Dr. Chinchilli, the second principal reviewer, was unable to attend. Dr. L. Hart, NIEHS, read his review. Dr. Chinchilli agreed in principle with the conclusions. However, he felt a statistical analysis incorporating historical control data should be conducted for the tubular cell tumors of the kidney in male mice. He said it was difficult to put in perspective the relevance of responses in the current experiment (control, 0/50, low dose, 2/50, and high dose, 1/50) when compared to the historical control rate of 0.3% (6/2029). He didn't believe results of such an analysis would drastically change the stated conclusions.

Dr. J. Haseman, NIEHS, stated that the NTP does not generally use historical control data in a formal testing framework, due in part to changing rates over time, particularly for the more common tumors. If the historical control rate were used as the basis for comparison, then there would be a significant ($P<0.05$) increase in tumor incidence in the low dose group, but not in the high dose group.

Dr. Perera, the third principal reviewer, agreed with the conclusions. She asked for discussion of the relationship between tubular cell hyperplasia and tubular cell adenomas/adenocarcinomas in the kidney of male mice. She also asked if there was a rationale for combining these endpoints as has been done for hyperplasia and tumors at other sites. Dr. Dunnick said the Discussion would be expanded and references cited along with NTP results that support the progression of hyperplasia to neoplasia in renal tubular cells.

Dr. Sivak commented that the inclusion of a comparison of dose rates given to animals with usual dose rates used therapeutically in humans was useful and important information. Dr. Popp requested that the nephropathy and cysts observed in dosed rats be included under non-neoplastic effects in the Summary Table. Dr. Dunnick agreed that these effects were both biologically and statistically significant and would be added to the Table.

Dr. Hughes moved that the Technical Report on α-methyldopa sesquihydrate be accepted with revisions as discussed and with the conclusions as written for male and female rats and female mice, no evidence of carcinogenic activity, and for male mice, equivocal evidence of carcinogenic activity. Dr. Capen seconded the motion. Dr. Perera proposed an amendment that a statement be included after the conclusion for male mice that there was an increase in hyperplasia in the dosed groups. The amended motion failed for lack of a second. The original motion then was approved unanimously with nine votes.

Roxarsone. Dr. K. M. Abdo, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of roxarsone by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year feed studies, there was equivocal evidence of carcinogenic activity of roxarsone for male F344/N rats as indicated by a marginally increased incidence of adenomas of the exocrine pancreas. There was no evidence of carcinogenic activity for female F344/N rats fed diets containing 50 or 100 ppm roxarsone for 2 years. There was no evidence of carcinogenic activity for male or female B6C3F$_1$ mice fed diets containing 100 or 200 ppm roxarsone for 2 years.

Dr. K. Hooper, a principal reviewer, agreed with the conclusions. He asked for discussion as to why a dose-related increase of clitoral gland adenomas was considered unrelated to chemical administration.

Dr. Capen, the second principal reviewer, agreed with the conclusions. He suggested that comment be added as to whether neoplastic or non-neoplastic lesions were observed in the pancreata of rodents in a previous study with roxarsone. Dr. Abdo said there was no mention in the earlier study that the pancreas was one of the organs examined.

As a third principal reviewer, Dr. Sivak agreed in principle with the conclusions although he considered the occurrence of clitoral adenomas in female rats supportive of equivocal evidence of carcinogenic activity especially in view of increased hyperplasias in treated groups. In response to Dr. Hooper and Dr. Sivak, Dr. Abdo said the incidences of clitoral gland lesions were not significantly different from controls even when hyperplasias were included. Dr. S. Eustis, NIEHS, reported that greater emphasis was placed on the pancreatic lesions than on the clitoral gland lesions because of comparisons with their respective historical control rates. Further, the historical control data for clitoral gland tumors given in the Report is based on microscopic examination of tumors only observed grossly. In this study, all clitoral glands were evaluated histopathologically; therefore, he said direct comparisons with historical controls are misleading. The discussion would be expanded to include this added information.

Dr. Hooper moved that the Technical Report on roxarsone be accepted with the revisions discussed and with the conclusions as written for male rats, equivocal evidence of carcinogenic activity, and for female rats and male and female mice, no evidence of carcinogenic activity. Dr. Sivak seconded the motion, which was approved unanimously with nine votes.

Tetracycline Hydrochloride. Dr. D. D. Dietz, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of tetracycline hydrochloride by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year feed studies, there was no evidence of carcinogenic activity of tetracycline hydrochloride for male or female F344/N rats and B6C3F$_1$ mice fed diets containing 12,500 or 25,000 ppm. Tetracycline hydrochloride-dosed female rats and male mice showed greater survival rates than the respective controls during these studies. Dosed female mice showed a concomitant decrease in body weight and in the incidence of hepatocellular adenomas and carcinomas.

Dr. Sivak, a principal reviewer, agreed with the conclusions. He asked for clarification of why reduced incidence of hepatocellular tumors in mice was cited in the conclusions while significantly reduced incidences of lymphomas (male mice), Harderian gland tumors (male mice) and pituitary adenomas (male rats) were not. Dr. Dietz explained that the apparent reduced incidences in these other tumors were not cited either because control incidences were high or because decreases in tumor incidence were offset by increases in hyperplasias (pituitary). Dr. J. Haseman, NIEHS, added that the negative trend for liver neoplasms was easily the most striking decrease in tumor incidence. Dr. Sivak inquired about the stability of tetracycline on storage in feed in view of the chemical's extreme light sensitivity. Dr. Dietz said a one-week study of tetracycline stability in feed was done under ambient conditions and no breakdown was found. Dr. Sivak thought it would be useful to show the relationship between the doses used in these studies and the usual human exposures. Dr. Dietz said the comparisons would be included.

Dr. Lijinsky, the second principal reviewer, agreed with the conclusions. Although he considered this to be a good study, he noted that among female mice, the deficit in weight gain was greater in the first three months of the chronic study than in the 13-week subchronic study. He felt this might suggest a difference in animal treatment or, perhaps, a toxic effect not uncovered in the prechronic study.

As a third principal reviewer, Dr. Popp agreed with the conclusions. He questioned the choice of 25,000 ppm as the high dose for rats when this dietary concentration resulted in liver and bone marrow lesions in the 13-week studies. Dr. Dietz said the bone marrow atrophy did not seem to be dose related and the liver lesions were not considered to be life threatening and did not serve as a factor in dose setting.

Dr. Sivak moved that the Technical Report on tetracycline hydrochloride be accepted with revisions as discussed and with the conclusions as written for male and female rats and mice, no evidence of carcinogenic activity. Dr. Lijinsky seconded the motion, which was approved unanimously with nine votes.