

Board of Scientific Counselors  
National Toxicology Program

Summary Minutes  
from

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

March 26, 1986  
Research Triangle Park, North Carolina

The review meeting began at 8:30 a.m. in the Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the Subcommittee were: Drs. Jerry Hook (Chairperson), Frederica Perera and James Swenberg. Members of the Panel of Experts were: Drs. Charles Capen, Vernon Chinchilli, John Crowley, Kim Hooper, Donald Hughes, Franklin Mirer, James Popp, Ian Purchase, Robert Scala, and Andrew Sivak. Dr. Purchase was unable to attend this meeting. These minutes have been reviewed and approved by all members of the Subcommittee and Panel present at the meeting. They were written by Dr. Larry G. Hart, Executive Secretary.

When available, final NTP Technical Reports for the studies may be purchased from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, Virginia 22161, (703) 487-4650.

The next NTP technical reports peer review meeting will be held August 19, 1986, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, (919) 541-3971; FTS 629-3971.

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Boric Acid. Dr. M. P. Dieter, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of boric acid in mice by reviewing the experimental design, results, and proposed conclusions:

Under the conditions of these 2-year feed studies, there was no evidence of carcinogenicity of boric acid at doses of 2,500 and 5,000 ppm for male or female B6C<sub>3</sub>F<sub>1</sub> mice.

Dr. Perera, a principal reviewer, agreed with the conclusions as written. She said the conclusion should note that survival was significantly decreased in high and low dose males limiting the sensitivity of the assay. Dr. Dieter agreed that this would be done.

As a second principal reviewer, Dr. Capen agreed with the conclusions.

As a third principal reviewer, Dr. Scala also agreed with the conclusions. He noted that mean feed consumption measurements for group housed animals may have little value.

In other discussions, Dr. Hooper and Dr. Mirer asked for a fuller discussion of the nonneoplastic toxicity, in particular, the reproductive toxicity, and said inclusion of occupational exposure levels would be useful if they can be obtained. Dr. Dieter said the discussion would be expanded and workplace exposure levels would be sought.

Dr. Perera moved that the Technical Report on boric acid be accepted with the conclusions as written for male and female mice, no evidence of carcinogenicity. Dr. Capen seconded the motion and it was approved by ten affirmative votes with one abstention (Dr. Hughes).

1,4-Dichlorobenzene. Dr. J. A. Goldstein, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of 1,4-dichlorobenzene by reviewing the experimental design, results, and proposed conclusions:

Under the conditions of these 2-year gavage studies, 1,4-dichlorobenzene produced clear evidence of carcinogenicity for male F344/N rats, as shown by an increased incidence of renal tubular cell adenocarcinomas. There was no evidence of carcinogenicity for female F344/N rats receiving doses of 300 or 600 mg/kg. There was clear evidence of carcinogenicity for both male and female B6C3F<sub>1</sub> mice, as shown by increased incidences of hepatocellular carcinomas and hepatocellular adenomas. Marginal increases were observed in the incidences of pheochromocytomas of the adrenal gland in male mice. Nonneoplastic effects in the kidney of male and female rats, in the liver of male and female mice, and in the thyroid gland and adrenal gland of male mice were also associated with the administration of 1,4-dichlorobenzene.

Dr. Swenberg, a principal reviewer, agreed with the conclusion in female rats, no evidence of carcinogenicity. He proposed that the conclusions in male rats and male and female mice be changed to some evidence of carcinogenicity based on what he viewed as: (1) occurrence of tumors only in tissues where there was considerable toxicity; (2) lack of genotoxicity in a battery of short term tests; and (3) a negative finding for carcinogenicity in a previous inhalation study. Dr. S. Eustis, NIEHS, commented that the incidence of nonneoplastic lesions in mice was high but the toxicity (single cell necrosis) was not severe but generally minimal to mild. Dr. Swenberg thought that the inhalation route would have been more appropriate than the gavage route since it is the predominant route of human exposure. Dr. Goldstein responded that the oral route was a valid route since the chemical is found in drinking water and also in many waste dumps. Regarding the negative findings in the previous inhalation study, she reported that the exposure period in rats was only 76 weeks vs. 102 in the present studies while the earlier inhalation study in mice suffered from high early mortality due to fighting and a respiratory infection. Therefore, exposures were terminated at 61 weeks and surviving mice were sacrificed at 51 to 61 weeks (males) or 75 to 76 weeks (females). Dr. Goldstein concluded that it would be difficult to compare the two studies since there were so many differences between them including length of exposure as well as route of exposure and possibly the doses received.

As a second principal reviewer, Dr. Crowley agreed with the conclusions as written. He thought that based on the prechronic studies, the top dose in male rats in the chronic study was probably too high.

As a third principal reviewer, Dr. Hooper agreed with the conclusions as written. He said the conclusions in mice were supported by the finding of rare hepatoblastomas in high dose males. In view of the similarity of renal toxicity in male rats to that observed with other hydrocarbons, Dr. Hooper asked whether there was evidence on the role of the alpha-2-microglobulin in these effects. Dr. Eustis commented that although the

accumulation of hyaline droplets in renal tubules (presumably alpha-2-microglobulin) is seen earlier than most of the renal lesions there is no direct evidence for a cause and effect relationship between the protein and the later appearing lesions. Dr. Hooper suggested that inclusion of concentration levels of 1,4-dichlorobenzene in home use as mothballs and commercial levels as a toilet disinfectant would be useful. Dr. Goldstein said this information would be included if available.

In further discussion, Dr. Mirer cautioned against giving too much weight to comparing results with those of the previous inhalation study since the protocols and other aspects of the study were different from the NTP study. With regard to concomitant toxicity and tumors in the same organ, he stated that it would be departure from past practice for the Panel to lower the level of evidence because of concurrent toxicity. Dr. Swenberg said that after hearing clarifying remarks on the mouse liver tumors and the rareness of the hepatoblastomas found in high dose male mice, he would agree that the level of evidence in male and female mice should remain clear evidence of carcinogenicity.

Dr. J. Barter, representing the Chlorobenzene Producers Association, made several comments on the Technical Report. He opined that the differences reported between the inhalation study and the NTP oral study were important and should not be overlooked. He noted that there was considerable scientific debate about the relevance of mouse liver tumors as predictive for human cancer in view of the high and variable spontaneous incidences of such tumors. Finally, he said the association believed human occupational exposure numbers cited were serious overestimates. Dr. J. Huff, NIEHS, mentioned that liver neoplasia was not uniformly high in mice; the background rates for male B6C3F<sub>1</sub> mice are high but the female mouse has a relatively low rate in comparison. He asked for more accurate information on occupational exposures if available.

Dr. Perera felt too much attention was being given to other studies while emphasis should be placed on the NTP studies being reviewed. Dr. Swenberg argued that the previous inhalation studies as well as information on apparent lack of genotoxicity of the chemical were relevant to determining the strength of evidence in the present studies.

Dr. Hooper moved that the Technical Report on 1,4-dichlorobenzene be accepted with the conclusions as written, clear evidence of carcinogenicity for male rats and male and female mice, and no evidence of carcinogenicity for female rats. Dr. Perera seconded the motion and it was approved by ten affirmative votes to one negative vote (Dr. Swenberg).

Pentachloronitrobenzene. Dr. J. K. Dunnick, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of pentachloronitrobenzene in mice by reviewing the experimental design, results, and proposed conclusions:

Under the conditions of these 2-year feed studies, there was no evidence of carcinogenicity in either male or female B67C3F<sub>1</sub> mice receiving 2,500 ppm or 5,000 ppm of pentachloronitrobenzene. Infection is considered to have decreased survival of the female mice and thus reduced the sensitivity for determining the presence or absence of a carcinogenic response.

Dr. Hooper, a principal reviewer, agreed with the conclusions in male mice, and based on adequate survival in the low dose groups of female mice, he could agree with the conclusions in female mice. Noting that the widespread ovarian infection with Klebsiella limited survival of high dose female mice compromising the power of the study to detect the presence or absence of a carcinogenic effect, Dr. Hooper suggested that the second sentence of the conclusions be modified to read: "Poor survival among high dose females limited the sensitivity of this bioassay to detect a carcinogenic effect." Dr. Perera said the conclusion in females should be changed to inadequate study of carcinogenicity. Dr. Swenberg disagreed noting that under the guidelines used by the Panel for some time a study was considered adequate if survival was greater than 50% after 78 weeks; in this case, survival in high dose females was 60% at 78 weeks. Dr. Huff indicated that most organizations that utilize carcinogenicity findings from laboratory experiments require 50% survival at the end of a negative study. The 18 month (78 weeks) - 50% survival guideline was valid when studies were limited to this time interval in certain strains of mice that did not ordinarily survive to two years.

As a second principal reviewer, Dr. Mirer agreed with the conclusions. In view of the previous compromised NCI study having been negative, he wondered whether this study should have been done. Dr. Mirer commented on the reduced and variable number of tissues from low dose male mice that were examined histopathologically. If this represented a change in protocol it should be emphasized in the methods. Dr. E. McConnell, NIEHS, replied that a reduced histopathology protocol was adopted and presented to the Board of Scientific Counselors in 1982. Under this protocol, tissues from low dose animals were examined microscopically only when there was reason to suspect there might be lesions, that is, if lesions were observed at gross necropsy or microscopic lesions were seen in a particular tissue in top dose animals. Dr. Eustis indicated this would be more clearly stated in the pathology methods section. Dr. Mirer asked that more discussion be included on the nonneoplastic lesions in female mice which might be the only compound related effects in the study. Dr. Dunnick replied that this would be expanded in the discussion and where appropriate, in the abstract.

As a third principal reviewer, Dr. Sivak agreed with the conclusions stating that he believed the survival in the high dose group to be adequate for interpretation of the study.

Dr. Hooper moved that the Technical Report on pentachloronitrobenzene be accepted with the modification of the last sentence of the conclusions as proposed by him. Dr. Swenberg seconded the motion and after discussion the motion was defeated by ten negative votes to one affirmative vote (Dr. Hooper). Dr. Hooper then moved to accept the report with the conclusions including the last sentence as written, no evidence of carcinogenicity, for mice of both sexes. Dr. Mirer seconded the motion and it was approved by ten affirmative votes to one negative vote (Dr. Swenberg).

Phenylephrine Hydrochloride. Dr. J. R. Bucher, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of phenylephrine hydrochloride by reviewing the experimental design, results, and proposed conclusions:

Under the conditions of these 2-year studies, there was no evidence of carcinogenicity of phenylephrine hydrochloride in male or female F344/N rats given 620 or 1,250 ppm in feed or in male or female mice given 1,250 or 2,500 ppm in feed. Survival of high dose male rats was greater than that of controls, and the incidences of mononuclear cell leukemia and pheochromocytomas in male rats were lower in exposed groups than in control male rats.

Dr. Popp, a principal reviewer, agreed with the conclusions as written. He noted that the rationale for studying the chemical was as a response to a recommendation it be included as part of a class study of benzyl alcohols with sympathomimetic activity. Certain members of this class have been associated with increased incidence of mesovarial leiomyomas. Thus, he asked why the ovary was not handled as a target organ for pathology. Dr. Bucher said the mesovarial tumors were observed on gross examination in the other studies. A statement would be added indicating such tumors were not seen grossly or microscopically in the current studies.

As a second principal reviewer, Dr. Sivak agreed with the conclusions and thought that modest reductions in weight at the high doses in both mice and rats indicated probable attainment of appropriate maximum tolerated doses. He stated that the reduction in adrenal lipoid degeneration and focal hyperplasia in treated male and female rats compared with untreated controls should be reported since the adrenal glands are likely target organs for the chemical.

As a third principal reviewer, Dr. Perera also agreed with the conclusions and the conduct of the study. She suggested that some mention might be made in the abstract of the nonneoplastic effects seen in the liver and prostate in rats, and in the liver in mice.

In other discussion, Dr. Scala expressed concern about the randomization of the animals noting the variation in initial body weights among animals from various groups. Dr. J. Haseman, NIEHS, said proper randomization was apparently used, but since the variability also has occurred in other studies an explanation will be sought. Dr. Mirer said it would be useful to have survival adjusted tumor rates also cited in the abstract and discussion since the life table analysis is based on the adjusted rate. Dr. Haseman agreed that this would be helpful when there were survival differences, and the tumors in question were lethal. Dr. Huff stated that this would be done for all studies where appropriate and informative.

Dr. Popp moved that the Technical Report on phenylephrine hydrochloride be accepted with the conclusions as written for rats and mice of both sexes, no evidence of carcinogenicity. Dr. Swenberg seconded the motion and it was approved unanimously with eleven affirmative votes.



Tetrakis (Hydroxymethyl) Phosphonium Sulfate (THPS) and Tetrakis (Hydroxymethyl) Phosphonium Chloride (THPC). Dr. C. W. Jameson, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of THPS and THPC by reviewing the experimental design, results, and proposed conclusions:

Under the conditions of these 2-year gavage studies, there was no evidence of carcinogenicity of THPS in both sexes of F344/N rats or B6C3F<sub>1</sub> mice given 5 or 10 mg/kg. There was no evidence of carcinogenicity of THPC in both sexes of F344/N rats given 3.75 or 7.5 mg/kg, in male B6C3F<sub>1</sub> mice given 7.5 or 15 mg/kg, or in female B6C3F<sub>1</sub> mice given 15 or 30 mg/kg.

Dr. Scala, a principal reviewer, agreed with the conclusions as written. He was pleased that the Chemical Manager emphasized the dosing errors but expressed concern about the effect that these dosing mixups in some animal groups may have had on the validity of the study, and asked for more explanation. Dr. Scala had a number of questions about dose selection in the prechronic phase which he discussed with Dr. Jameson after the review. He noted that since THPS and THPC differ in their chemical structure only by an anion yet show differences in toxic effects some speculative discussion would be helpful.

As a second principal reviewer, Dr. Crowley agreed with the conclusions but also expressed concern about the possible effects of the dosing mixups. In view of the elevated rates of mononuclear cell leukemia in male rats at the end of the study, he wondered if the life table test was the appropriate statistical test for interpreting the data. Dr. Eustis emphasized that mononuclear cell leukemia takes several months to develop and is considered a fatal disease. Dr. Haseman said this was a good illustration of the difficulty of choosing the most appropriate statistical test, and this uncertainty was considered in the overall evaluation of the studies. He also noted that in many earlier studies where leukemia was an endpoint there was strong evidence that it was a fatal tumor. Dr. Huff added that leukemia was often a late developing neoplasm.

As a third principal reviewer, Dr. Hughes also agreed with the conclusions. He concurred with the other reviewers in calling for more explanation on the dosing mixups. He thought the rationale for choosing the gavage route of exposure not to be particularly convincing, especially since information on absorption, distribution, metabolism, and excretion was not available. Dr. Hughes commented that the section in the introduction on the reported initiation/promotion studies with THPC was potentially misleading because he viewed THPC as a "suspected" promoter rather than a promoter per se.

In response to the reviewer's concerns about the dosing mixups, Dr. Jameson said that the laboratory technicians inadvertently switched vials of THPS and THPC for dosing mice on only three days at about the midpoint of the study. This represents less than 0.6% (3/520) of the gavage days. There were no observed adverse effects, and the NTP considered the incident to have no

Xylenes (Mixed). Dr. W. C. Eastin, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of xylenes (mixed) by reviewing the experimental design, results, and proposed conclusions:

Under the conditions of these 2-year gavage studies, there was no evidence of carcinogenicity of xylenes (mixed) in male or female F344/N rats given 250 or 500 mg/kg or in male or female mice given 500 or 1,000 mg/kg.

Dr. Popp, a principal reviewer, agreed with the conclusions as written. He asked that a rationale be given for using the gavage route of exposure, and in parallel, the common or most important route of human exposure.

As a second principal reviewer, Dr. Mirer agreed with the conclusions. He expressed concern that perhaps higher doses could have been given and thus a maximum tolerated dose was not achieved for female rats and male and female mice, even though the choice of dose was well justified. Dr. Eastin indicated the doses were appropriate based on the 90 day studies results, and that the marginally lower body weights in male rats gave some indication that higher doses were likely not possible.

As a third principal reviewer, Dr. Chinchilli also agreed with the conclusions. He asked that the randomization scheme and the process for animal cage rotation be described in the methods section. Dr. Eastin said that a statement would be added about how randomization is done. Cages were not being rotated at the time of these studies although cage rotation is practiced with more recent studies. Dr. Huff stated that this information would be added to the Methods section in all Technical Reports.

Dr. Mirer moved that the Technical Report on xylenes (mixed) be accepted with the conclusions as written for rats and mice of both sexes, no evidence of carcinogenicity. Dr. Popp seconded the motion and it was approved by ten affirmative votes with one abstention (Dr. Scala).

impact on the outcome of the studies. Documentation from the laboratory indicated this was an isolated incident. More information would be given in the text of the report.

Dr. Scala moved that the Technical Report on THPS and THPC with the conclusions as written for rats and mice of both sexes, no evidence of carcinogenicity, be accepted subject to inclusion of the more detailed explanation of the dosing mixups as presented by Dr. Jameson. Dr. Popp seconded the motion and it was approved unanimously with eleven affirmative votes.