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
December 9, 1985
Research Triangle Park, North Carolina

The review meeting began at 8:30 a.m. in the Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the Subcommittee were: Drs. Jerry Hook (Chairperson), Frederica Perera and James Swenberg. Members of the Panel of Experts were: Drs. John Crowley, Kim Hooper, Thomas Jones, Richard Kociba, David Kotelchuck, Franklin Mirer, Ian Purchase, Robert Scala, Steven Tannenbaum, and Bruce Turnbull. These minutes have been reviewed and approved by all members of the Subcommittee and Panel. 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 March 26, 1986, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, (919) 541-3971; FTS 629-3971.

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Ampicillin trihydrate. Dr. J. Dunnick, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of ampicillin trihydrate by reviewing the experimental designs, results, and proposed conclusions:

> Under the conditions of these 2-year gavage studies, there was equivocal evidence of carcinogenicity for ampicillin trihydrate in male F344/N rats as shown by an increased incidence of pheochromocytomas of the adrenal gland medulla and a marginally increased incidence of mononuclear cell leukemia. There was no evidence of carcinogenicity in female F344/N rats receiving 750 or 1,500 mg/kg ampicillin trihydrate or in male and female $B6C3F_1$ mice receiving 1,500 or 3,000 mg/kg per day. Nonneoplastic lesions of the forestomach were seen in male rats and in male and female mice.

Dr. Kociba, a principal reviewer for the draft technical report, agreed with the conclusions as written for female rats and male and female mice. However, he said the conclusion for male rats should be equivocal evidence of benign tumor induction based on the increased incidence of adrenal gland pheochromocytomas. He thought that, within the range of historical control incidence rates, the increased incidence of mononuclear cell leukemia was not compound related. Dr. Kociba said the design of both 13-week and 2-year studies would have been made more useful by inclusion of clinical pathology, more detailed clinical observations, and ampicillin blood levels, possibly being correlated with pharmacologic effects. He requested deletion of the last sentence in the conclusions, regarding nonneoplastic lesions, as being duplicative.

As second principal reviewer, Dr. Turnbull agreed with the conclusions for female rats and male and female mice. He said the evidence for any increase in mononuclear cell leukemia was weak and should not be part of the conclusion for male rats. He asked that the report indicate whether original and quality assurance (QA) pathology examinations were performed in a "blind" fashion with respect to dose group, and whether or not the QA examination was "blinded" to the original diagnoses. Dr. Eustis, NIEHS, indicated that the Program did not routinely endorse pathology diagnoses without awareness of all relevant information. During the PWG, however, there is "blind" pathology to some extent. This would be better explained in all future Technical Reports.

Most of the ensuing discussion dealt with the level of evidence of carcinogenicity in male rats and whether the increased incidences of adrenal gland medullary pheochromocytomas and mononuclear cell leukemia were related to administration of ampicillin trihydrate. Dr. Swenberg commented that the incidences of mononuclear cell leukemia in both low and high dose groups were almost double the historical control average (28% and 26%, respectively, versus 14%) and were at the top of the historical range. Thus, in his opinion, equivocal evidence of carcinogenicity was appropriate. Dr. Mirer argued that the positive trend test and statistically significant increases in mononuclear cell leukemia using the life table test supported a designation of some evidence of carcinogenicity.

Dr. Perera agreed. Dr. S. Eustis, NIEHS, said the highly variable incidence of mononuclear cell leukemia argued for the level chosen. In response to Dr. Perera, Dr. J. Huff, NIEHS, noted the decreased incidence of adrenal medullary hyperplasia, a precursor lesion to pheochromocytoma, in both dose groups which influenced choosing equivocal evidence of carcinogenicity. Dr. Turnbull questioned the appropriateness of the life table test for analysis in view of the numbers of rats with mononuclear cell leukemia surviving to the end of the study. Dr. J. Haseman, NIEHS, replied that mononuclear cell leukemia is generally considered by the NTP to be a fatal tumor, although he agreed that this determination is not clear-cut in this instance, since the leukemia incidences were similar in male rats dying prior to the end of the study and in the terminal kill animals.

Dr. Hooper moved that the conclusions in the Technical Report on ampicillin trihydrate be accepted as written for female rats and male and female mice, no evidence of carcinogenicity. Dr. Mirer seconded the motion and it was approved unanimously with eleven affirmative votes. Dr. Kociba moved that the phrase "and marginally increased incidence of mononuclear cell leukemia" be deleted from the first sentence of the conclusion as supporting equivocal evidence of carcinogenicity in male rats. Dr. Swenberg seconded the motion and it was defeated by six negative votes (Drs. Hooper, Mirer, Perera, Scala, Swenberg, and Tannenbaum) to five affirmative votes (Drs. Crowley, Jones, Kociba, Purchase, and Turnbull). Dr. Swenberg then moved that the conclusions as written for male rats, equivocal evidence of carcinogenicity, be accepted. Dr. Tannenbaum seconded the motion, and it was approved by six affirmative votes to one negative vote (Dr. Kociba) with four abstentions (Drs. Crowley, Jones, Purchase, and Turnbull).

Chlorpheniramine Maleate. Dr. R. Melnick, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of chlorpheniramine maleate by reviewing the experimental designs, results, and proposed conclusions:

> Under the conditions of these studies, there was no evidence of carcinogenicity for F344/N rats or for B6C3F$_1$ mice of either sex administered chlorpheniramine maleate in distilled water by gavage, 5 days per week for 2 years. Due to high mortality in high dose female rats and high dose male mice, the sensitivity of these groups to detect a carcinogenic response was reduced. Chlorpheniramine maleate had a proliferative effect on the thyroid gland of female mice, as shown by the increased incidences of follicular cell cysts and hyperplasia in both low dose and high dose groups.

Dr. Turnbull, a principal reviewer for the draft Technical Report, in general agreed with the conclusions as stated, although he expressed concern about whether the study had enough power to detect an effect, primarily because of the decreased survival in high dose female rats and male mice. He said the estimated maximum tolerated dose (EMTD) was exceeded in both groups and possibly at both dose levels in female mice. Dr. Turnbull noted that it is much more difficult to substantiate a negative result than a positive result when there is reduced statistical power. Some consideration should be given to classifying the conclusions in female rats and male and female mice as inadequate study of carcinogenicity.

As a second principal reviewer, Dr. Purchase agreed with the conclusions but also agreed with Dr. Turnbull that there might be some debate and consideration given to categorizing the studies in mice as inadequate. He said the importance of high mortality and reduced body weight in some of the groups on interpretation of the study should be given more emphasis in the report. Dr. Purchase noted that there is considerable information on the metabolism and pharmacokinetics of this chemical in humans and animals which should have been used in choosing the dose levels and which could aid in placing the results of the animal studies in the context of human hazard. Dr. Melnick said there would be additional discussion on the possibility of metabolism being altered in mice as a function of treatment, as well as comparing what is known about chlorpheniramine pharmacokinetics in rodents versus humans.

As a third principal reviewer, Dr. Tannanbaum agreed with the conclusions. He commented that there was no attempt to analyze for nitrosamine contamination, and had this been a positive study, this omission could have compromised interpretation. Further, unlike humans, the rat lacks the capacity for endogenous nitrosation. Dr. Melnick said nitrosation studies had been considered but were not performed.

Discussion was focused on the issue of adequacy of the high dose studies in female rats and male mice. Excessive mortality in the original male mouse groups had required starting another study at lower dose levels. Dr. Melnick stated that survival in low dose groups of male mice and female rats was considered to be adequate to detect carcinogenic response. Dr. Perera and Dr.

Hooper supported a designation of inadequate study for both groups. In particular, Dr. Hooper commented on the marginally significant increases in adrenal capsular adenomas in male mice, lesions he opined might have been more pronounced had there been a dose group intermediate between the low and high doses. Dr. Swenberg reminded the Panel that as a guideline if there was 50% survival after 78 weeks, the study was adequate. Dr. Huff indicated that most organizations suggest 50% survival in all groups at the end of the studies to designate adequacy.

Dr. Turnbull moved that the findings in male mice be considered an inadequate study of carcinogenicity based on the conduct of the study, e.g., the two starts, and, secondarily, the exceeding of the estimated maximum tolerated dose (EMTD). Dr. Hooper seconded the motion. In discussion after the motion, Dr. Purchase stated that he now felt the available information supported the study being adequate to assess a carcinogenic response. The motion was defeated by eight negative votes to three affirmative votes (Dr. Crowley, Dr. Perera, and Dr. Turnbull). Dr. Hooper then moved that the study in male mice be considered equivocal evidence of carcinogenicity based on the increases in adrenal capsular adenomas. Dr. Swenberg seconded the motion, and it was defeated by 10 negative votes to 1 affirmative vote (Dr. Hooper). Dr. Purchase moved that the conclusions as written for male mice, no evidence of carcinogenicity, be accepted. Dr. Jones seconded the motion, and it was approved by eight affirmative votes to three negative votes (Dr. Crowley, Dr. Perera, and Dr. Turnbull). Dr. Purchase moved that the conclusions as written for male and female rats and female mice, no evidence of carcinogenicity, be accepted. Dr. Kociba seconded the motion, and it was approved unanimously with 11 affirmative votes.

Dimethylvinyl Chloride. Dr. B. Schwetz, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of dimethylvinyl chloride (DMVC) by reviewing the experimantal designs, results, and proposed conclusions:

> Under the conditions of these 2-year gavage studies, there was clear evidence of carcinogenicity of dimethylvinyl chloride for both sexes of F344/N rats and $B6C3F_1$ mice. This was based on increased incidences of neoplasms of the nasal cavity, oral cavity, esophagus, and forestomach of male and female F344/N rats. $B6C3F_1$ mice showed increased incidences of squamous cell neoplasms of the forestomach in males and females and squamous cell carcinomas of the preputial gland in males.

Dr. Crowley, a principal reviewer for the draft technical report agreed with the conclusions as written. He asked for clarification or more definition of the relationship between tumors and early mortality in the dose groups vs. a relationship with compound-induced toxicity.

As a second principal reviewer, Dr. Perera agreed with the conclusions. Her principal criticisms had to do with there being insufficient and incomplete presentation and discussion in the text of tumor incidence data displayed in the tables. She cited a number of examples and noted especially insufficient comment about increased tumor incidences in low dose groups. Dr. Schwetz responded that for some tumors, especially those with increases in the low dose groups, there was little or no mention in the text because of only marginally increased incidence, lack of a high dose effect, or primarily a benign nature of the tumors. Dr. Perera noted that although Harderian gland tumors were significantly increased in incidence and with a positive trend in female mice, there was considered to be an "uncertain relationship" to chemical administration. She questioned this interpretation.

As a third principal reviewer, Dr. Purchase also agreed with the conclusions. He thought there was very good evidence that early mortality was due to malignant neoplasms, noting that virtually all animals dying after about 60-70 weeks had malignant neoplasms. Dr. Schwetz replied that most of the dosed rats dying without nasal tumors died earlier than those with such tumors, citing this as an example of why the causes of death were probably a combination of cancer and DMVC toxicity. Dr. Purchase said the argument that dimethylvinyl chloride acts directly on the nasal mucosa was weaker than one suggesting that the nasal lesions were a result of systemic absorption and gave reasons in support of an indirect effect. Dr. Scala said the lack of kidney or bladder tumors in mice also spoke to the tumorigenicity occurring through systemic rather than local effects.

In other discussion, Dr. Scala commented on the introduction of information on chemical metabolism and immunotoxicology studies for the first time in the discussion without prior presentation of data in the results section.

Dr. Crowley moved that the Technical Report on dimethylvinyl chloride be accepted with the conclusions as written for rats and mice of both sexes, clear evidence of carcinogenicity. Dr. Hooper seconded the motion and it was approved unanimously with eleven affirmative votes.

Methyl Methacrylate. Dr. P. Chan, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of methyl methacrylate by reviewing the experimental designs, results, and proposed conclusions:

> Under the conditions of these 2-year inhalation studies, there was no evidence of carcinogenicity for male F344/N rats exposed at 500 or 1,000 ppm, for female F344/N rats exposed at 250 or 500 ppm, or for male and female B6C3F$_1$ mice exposed at 500 or 1,000 ppm. Inhalation of methyl methacrylate was associated with inflammation of the nasal cavity and degeneration of the olfactory sensory epithelium in male and female rats and mice; epithelial hyperplasia of the nasal cavity was also observed in exposed mice.

Dr. Scala, a principal reviewer for the draft technical report, agreed with the conclusions as written. He said discussion of the results of the mutagenicity tests needed a better focus. The reader was left uncertain as to what the investigators concluded about the results.

As a second principal reviewer, Dr. Mirer agreed with the conclusions as written. He asked for comment on the significance of the dose-related increases in cytoplasmic vacuolization of the adrenal cortex and focal hyperplasia of the adrenal gland medulla in rats, with the dose-related trend most apparent in female rats. Dr. S. Eustis, NIEHS, stated that the vacuolization probably represented lipid accumulation, and the adrenal gland lesions were not considered biologically important. Dr. Mirer questioned the statement that methyl methacrylate was a weak mutagen. Dr. Chan agreed to delete the adjective.

As a third principal reviewer, Dr. Swenberg also agreed with the conclusions as written. He asked that the results and discussion be expanded in the description of the olfactory epithelial degeneration, particularly with regard to the 13-week studies and any sensory nerve damage. Dr. Chan responded that the degeneration of the olfactory epithelium was observed during the 13-week studies, but nerve damage was not seen. Dr. Eustis added that the olfactory lesions, although present at high incidence in the 2-year studies, were not severe and were generally focal or multifocal, not diffuse.

Dr. Scala moved that the Technical Report on methyl methacrylate 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 by 10 affirmative votes with one abstention (Dr. Purchase).

Oxytetracyline Hydrochloride. Dr. K. Abdo, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of oxytetracycline hydrochloride by reviewing the experimental designs, results, and proposed conclusions:

> Under the conditions of these 2-year feed studies of oxytetracycline hydrochloride, there was equivocal evidence of carcinogenicity in male F344/N rats as indicated by increased incidences of pheochromocytomas of the adrenal gland. There was equivocal evidence of carcinogenicity in female F344/N rats fed diets containing oxytetracycline hydrochloride, as indicated by increased incidences of neoplasms of the pituitary gland. There was no evidence of carcinogenicity in male or female B6C3F$_1$ mice fed diets containing 6,300 or 12,500 ppm oxytetracycline hydrochloride for 2 years.

Dr. Jones, a principal reviewer for the draft technical report, agreed with the conclusions as written.

As a second principal reviewer, Dr. Perera did not agree with the conclusions in rats. She stated that in males both a positive trend for pheochromocytomas and, using the incidental tumor test, significant increases in pheochromocytomas in the high dose group compared with controls provided adequate support for raising the conclusion to some evidence of carcinogenicity. Likewise in females, a positive trend for pituitary neoplasms and a significantly increased incidence of neoplasms in the high dose group compared with controls by the incidental tumor test supported raising the conclusion to some evidence of carcinogenicity. Dr. Abdo explained the rationale for the levels of evidence used. He said both the adrenal and pituitary tumors have high and variable spontaneous rates in untreated rats, and, secondly, the increases were not considered to be overwhelming. Also, no increases were observed in the low dose groups. Dr. Turnbull questioned calling the increase in pheochromocytomas in male rats statistically significant as they are common tumors, and the P value was greater than 0.01 (0.026). Dr. Huff, NIEHS, indicated this was a marginal increase and one that staff believed did not fit the category of no evidence of carcinogenicity.

As a third principal reviewer, Dr. Kociba agreed with the conclusions in mice and with the level of evidence in rats. However, because the conclusions in rats were based on increases in benign tumors, he stated the conclusions for both sexes should be equivocal evidence of benign tumor induction. Dr. McConnell mentioned that pheochromocytomas are benign neoplasms; for the pituitary neoplasms there were two adenocarcinomas in the control versus 10 in the exposed groups. Dr. Huff reminded the Panel that the morphologic type of neoplasms were always given in the conclusion.

In related discussion, Dr. Perera questioned the discounting of statistically significant results (adrenal pheochromocytomas in rats) because neither the trend nor the high dose incidence was significant by a newer statistical test, logistic regression analysis. She asked that this decision be better justified here and whenever statistically significant results are downgraded to equivocal evidence of carcinogenicity. Dr. Haseman explained that logistic

regression was employed because it does not require the utilization of time intervals and that there was some indication that, for this particular tumor, the survival patterns observed and the specific time intervals used by the incidental tumor test may have overstated the statistical significance. He opined that the increased tumor incidence may have been related to the greater survival in the high dose group (38/50) relative to controls (22/50).

Dr. Jones moved that the Technical Report on oxytetracycline hydrochloride be accepted with the conclusions as written for male and female rats, equivocal evidence of carcinogenicity, and for male and female mice, no evidence of carcinogenicity. Dr. Swenberg seconded the motion and it was approved by nine affirmative votes to one negative vote (Dr. Turnbull) with one abstention (Dr. Purchase). (Note: Dr. Kociba asked that the record show his basis for voting affirmative for equivocal evidence of carcinogenicity in male rats was based on the written comments in his review.)

Trichloroethylene. Dr. J. Mennear, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of trichloroethylene (TCE) by reviewing the experimental designs, results, and proposed conclusions:

> These two-year gavage studies of trichloroethylene in male and female ACI, August, Marshall, and Osborne-Mendel rats are considered to be inadequate studies of carcinogenicity because of insufficient survival in dosed animals and incomplete documentation of the conduct of the studies. However, under the conditions of these studies trichloroethylene administration was strongly associated with renal tubular cell cytomegaly and karyomegaly, and toxic nephropathy in both sexes of the four strains. In addition, an increased incidence of renal tubular cell neoplasms in male Osborne-Mendel rats, and possibly in female ACI and female August rats, and an increased incidence of testicular interstitial cell tumors in male Marshall rats may have been associated with the administration of trichloroethylene.

Dr. Hooper, a principal reviewer for the draft technical report, agreed with the conclusions for the four rat strains that these were inadequate studies of carcinogenicity. He discussed deficiencies in study design, conduct, and record keeping. With regard to the large numbers of "accidental" or "gavage-related" deaths, he argued that gavage trauma is lethal when accompanied by the toxicity of TCE. Dr. Hooper proposed that inhalation studies be designed in both rats and mice to confirm or deny the assertion that TCE is a tissue- and species-specific carcinogen (mouse liver). He asked that reference be included to a 1983 Japanese inhalation study in which TCE was reported to produce pulmonary adenocarcinomas in female ICR mice.

As a second principal reviewer, Dr. Swenberg agreed with the conclusions but stated that these studies were inadequately designed, failing to utilize a previous study with Osborne-Mendel rats in dose selection. Beginning with the abstract, it should be stated that the doses used exceeded the maximum tolerated dose in all four strains of rats. He detailed a number of suggested revisions. However, he said the last sentence regarding kidney tumors should be deleted as the findings described have not been reproduced in several negative studies and are difficult to assess due to the severe renal toxicity.

As a third principal reviewer, Dr. Crowley also suggested that the last sentence should be deleted from the conclusions. He also asked for clarification concerning the relationship of gavage trauma with chemical toxicity in causation of "accidental" deaths. Dr. Mennear responded that the mortality originally termed "gavage-related deaths" were likely to be associated with toxicity of TCE.

Dr. E. McConnell, NIEHS, said that despite the deficiencies in these studies, the NTP considered the toxic renal lesions to be real and consistent with effects seen with other halogenated solvents.

In other discussion, Dr. Tannenbaum pointed out that the major metabolite of TCE in humans and rats is trichloroacetic acid which could be the cause of the nephrotoxicity, and, thus, raising the question of whether there might be a threshold effect. Dr. Mirer cautioned against minimizing the importance of the renal toxicity in view of the fact that doses used were in the same range as the occupationally permitted exposure levels in air. Dr. Perera spoke against deleting the last sentence of the conclusions, arguing that if the nontumor toxic effects should not be dismissed despite the deficiencies of the study, then neither should the neoplastic effects. Dr. McConnell said the toxic renal lesions were observed at very high incidence while the neoplastic changes were found at very low incidence. Dr. Swenberg stated that conclusions about carcinogenicity cannot be drawn from an inadequate study of carcinogenicity.

Dr. Scala said the audit report findings suggest these studies are flawed and should not be published as an NTP Technical Report. The data on the kidney lesions could be reported out separately. Dr. Jones said the information should be made readily available. Dr. Purchase thought it difficult to recommend publication or not based on the present report. He suggested that the report be redrafted and brought back to the Panel.

Dr. Hooper moved that the report be referred for extensive revision and then brought back to the Panel. Dr. Swenberg seconded the motion. Dr. Kociba requested that more information from the Audit Report be included in the redraft. Dr. Swenberg asked that information also be provided on the findings from an independent audit by the Halogenated Solvent Industry Alliance (HSIA). Dr. J. Huff, NIEHS, said a revised technical report could be ready for consideration at the summer 1986 meeting. Dr. Hook summarized the motion to say that the technical report would be rewritten, adding a more extensive summary of the findings from audits conducted by both the NTP and HSIA and returned to the Panel for review. The Panel approved the motion by nine affirmative votes with two abstentions (Dr. Kociba and Dr. Purchase).