PEER REV

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

February 28, 1983
Research Triangle Park, North Carolina

The review meeting began at 9 a.m. in the Conference Center, Building 101, South Campus, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the Subcommittee are: Drs. Margaret Hitchcock (Chairperson), Curtis Harper, James Swenberg, and Alice Whittemore. Members of the Panel are: Drs. Louis Beliczky, Devra Davis, Robert Elashoff, Seymour Friess, Michael Holland, Robert Scala, Tom Slaga, John Van Ryzin, Stan Vesselinovitch, and Mary Vore. Drs. Slaga, Vesselinovitch, and Whittemore were unable to attend the meeting.

Final NTP Technical Reports for the approved bioassays will be available for sale in three to six months from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, (703) 487-4650.

The next NTP bioassay peer review meeting will be held June 29, 1983, in Research Triangle Park. For information contact Dr. Larry G. Hart (919) 541-3971; FTS 629-3971.

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Ethyl Acrylate. Dr. Swenberg, a principal reviewer for the report on the bioassay of ethyl acrylate, agreed with the stated conclusions that: "Under the conditions of these studies, ethyl acrylate was carcinogenic for the forestomach of F344/N rats and $B6C3F_1$ mice of either sex, causing squamous cell carcinomas in male rats and male mice, squamous cell papillomas in male and female rats and male mice, and squamous cell papillomas or carcinomas in male and female rats and male and female mice. Ethyl acrylate also caused irritation of the forestomach mucosa in male and female rats and mice." He stated that the report was well written, accurately reflecting the data of the study and interpreting the data openly and objectively. He opined that the section on human toxicity and exposure put the study in perspective for the reader. Dr. Swenberg said that while there was not a decrease in survival or body weight, he believed the maximum tolerated dose (MTD) was achieved because of inflammatory lesions in the forestomach.

As a second principal reviewer, Dr. Davis agreed with the conclusions and said the study design was sound. She said the gavage route was appropriate but bolus delivery may have provided a cofactor for carcinogenesis. She said the increased incidence of retinopathy and cataracts in high dose male and low dose female rats should be noted. Dr. Davis commented that the discussion was an excellent one that skillfully laid out the problems of conducting the study of lower molecular weight esters of acrylic acid.

As a third principal reviewer, Dr. Friess stated that while the evidence is clear for increased incidence of squamous cell carcinomas of the forestomach in male rats and mice, there is no statistical demonstration for these carcinomas in female rats and mice. He opined that the finding of increased incidence of squamous cell carcinomas with increased concentration of chemical in the bolus suggests a linkage of the effect with irritant/necrotic stresses rather than chemical initiation of carcinogenesis, and he said this view is further supported by lack of carcinogenic effect reported in a recently completed inhalation study. Dr. Friess commented on the negative trends in tumor incidence in both species and in seeking causation asked whether there were any dietary, stress or metabolic factors different in dosed than in control animals. He observed that the genetic variance or lack of genetic homogeneity of the $B6C3F_1$ stock in this study as well as in others did nothing to invalidate the study.

Dr. Beliczky questioned whether too much emphasis was given to the negative trends for tumor incidence while Dr. Davis noted the analogy with the divergent antitumor and carcinogenic effects of many cancer chemotherapeutic agents. Dr. Scala complimented NTP on inclusion of sentinel animal data in recent reports and requested some comment be made about the meaning or significance of the data.

There was considerable discussion among Panel members and staff about how the conclusion should be worded to reflect: (1) the differential degree of carcinogenic response between male and female animal groups, and (2) non-significant increases for squamous cell papillomas in female mice.
Dr. Swenberg stated that in view of forestomach tumors being a tissue and tumor type where there frequently is progression, the papillomas in female mice could not be ignored. The revised conclusion reads as follows: "Under the conditions of these studies, ethyl acrylate was carcinogenic for the

forestomach of F344/N rats and $B6C3F_1$ mice, causing squamous cell carcinomas in male rats and male mice, squamous cell papillomas in male and female rats and male mice, and squamous cell papillomas or carcinomas (combined) in male and female rats and mice. The evidence for carcinogenicity was greater in male than in female animals. Ethyl acrylate also caused irritation of the forestomach mucosa in male and female rats and mice."

Dr. Swenberg moved that the technical report on the bioassay of ethyl acrylate be accepted with the revisions discussed. Dr. Davis seconded the motion and the report was approved unanimously by the Peer Review Panel.

1,1,1-Trichloroethane. Dr. Vore was a principal reviewer for the report on the bioassay of 1,1,1-trichloroethane (TCE). A revised conclusion was presented to the review panel and read as follows: "Under the conditions of these studies, 1,1,1-trichloroethane was not carcinogenic for male F344/N rats. This study was inadequate for carcinogenesis evaluation in female F344/N rats because of the large number of accidental deaths and because the high dose was toxic. The association between the administration of 1,1,1-trichloroethane and the increased incidences of hepatocellular carcinomas in male $B6C3F_1$ mice was considered equivocal. 1,1,1-Trichloroethane was carcinogenic for female $B6C3F_1$ mice, causing an increased incidence of hepatocellular carcinomas." The only change from the conclusion printed in the draft report was to give more emphasis to the inadequacy of the study in female rats. Dr. Vore agreed with the revised conclusions. She opined that the high number of gavage errors in dosed groups vs. controls suggest, as noted previously for other halogenated hydrocarbon solvents, that the TCE contributed to the gavage error.

As a second principal reviewer, Dr. Harper agreed with the revised conclusion. He said that information on the 1977 NCI bioassay of TCE, which was judged inadequate due to low survival, should be given more prominence, and more detail should be included. He also commented on the high incidence of gavage errors, and said a protocol revision was needed for studies with chlorinated aliphatic compounds to minimize the occurrence of such errors. As a third principal reviewer, Dr. Beliczky also expressed concern with the large number of deaths due to gavage accidents. He inquired as to whether central nervous system effects had been studied. Dr. L. Birnbaum, NTP Chemical Manager, replied that nervous system effects were not assessed, other than daily clinical observations.

In discussion from the floor, Dr. T. Torkelson, Dow Chemical Company, said the section on mutagenesis needed to contain data already supplied to the EPA. He said Dow would submit the information to NTP. Dr. J. Moore, NTP, said the material would be considered for inclusion only if published, although in certain instances raw data might be acceptable.

Drs. Swenberg, Davis and Scala requested that more information be given in the report on latency (time-to-tumor) for different tumors in the historical control data base. Dr. J. Haseman, NTP, replied that sufficient information is in the newly defined NTP historical control data base to determine whether the preponderance of tumors of a particular type were late-appearing (i.e., seen at terminal kill) or were observed in animals dying prior to the end of the study. He opined that with the data currently available it would be difficult to provide a more comprehensive assessment of tumor latency. Dr. Scala questioned the validity of the study in male rats due to poor survival. Dr. Birnbaum said the study was adequate based on survival (greater that 50%). Several members commented on the low recovery of TCE from corn oil (35.9%). Dr. C. Jameson, NTP, responded that this low but reproducible recovery was due to a low extraction efficiency from corn oil and was taken into account in the calculations of actual dose concentrations. In response to concerns expressed by the Panel, NTP agreed to (1) include uncensored 78 week survival data, (2) provide a textual explanation of why decreased latency of interstitial cell tumors of the testes was not considered significant, and (3) mention in the abstract the increased incidence of malignant lymphomas and leukemias in male mice while noting that the increased incidence does not appear to be compound-related.

Dr. Vore moved that the technical report on the bioassay of 1,1,1-trichloroethane be accepted with the revisions discussed. Dr. Harper seconded the motion and the report was approved unanimously by the Peer Review Panel.

1,2-Dichloropropane. Dr. Friess was a principal reviewer for the report on the bioassay of 1,2-dichloropropane. A revised conclusion was presented to the review panel and read as follows: "Under the conditions of these studies, 1,2-dichloropropane was carcinogenic for female F344/N rats, causing an increased incidence of adenocarcinoma of the mammary gland concurrent with decreased survival and body weight gain. There was no evidence of carcinogenicity for male F344/N rats. 1,2-Dichloropropane should be considered carcinogenic for male and female B6C3F$_1$ mice, causing an increased incidence of hepatocellular adenomas." He agreed with the conclusions in rats but had reservations about the conclusions in mice since there were significant increases in hepatocellular adenomas but not in carcinomas. Dr. Friess said it was unfortunate that the genetic homogeneity of the parent C3H mice was less than optimum, and that the maximum tolerated dose (MTD) was exceeded for both female mice and rats, but neither finding would invalidate the bioassay.

As a second principal reviewer, Dr. Swenberg said that the conclusions are more equivocal than presented in the abstract since the increased weight loss and stress may be responsible for the increase in adenocarcinomas of the mammary gland. He stated that no mention is made in the discussion as to the possible consequences of exceeding the MTD and how this may have affected the results. With regard to the mouse liver tumors, he said there was little question that there was an increase in adenomas; he asked that some discussion center on possible mechanism. Dr. Swenberg opined that the report overinterpreted the data.

As a third principal reviewer, Dr. Beliczky said the mammary adenocarcinomas in female rats appear to be the major significant finding. Although increases in liver adenomas in mice were significant, he stated that combining liver adenomas and carcinomas was not realistic. [As stated by the NTP, combining liver tumors was considered appropriate since benign and malignant neoplasms may represent stages of a progression.] He said that assumptions and speculative judgement were introduced into the discussion to apparently make more of a case for carcinogenicity than just the mammary adenocarcinomas in female rats. More discussion was needed regarding mutagenic testing and screening for chromosome aberrations and sister chromatid exchanges. Dr. Beliczky said that doses selected were too high, survivorship was decreased, potential effects on genetic integrity were introduced into the study and comparison with more potent chemical carcinogens was inappropriate.

Dr. J. Lamb, NTP Chemical Manager, said changes would be made in the abstract with regard to combining liver adenomas and carcinomas, including more discussion of the necrogenic activity of 1,2-dichloropropane; there would be a fuller discussion using literature references on how toxicity and possibly an altered nutritional state may relate to development of mammary tumors and for this experiment in female rats.

Dr. Swenberg reiterated that the evidence was equivocal for carcinogenicity, and at best, there was probable or possible evidence for carcinogenicity. He requested a pathology reexamination of the mammary tumors. Dr. G. Boorman, NTP, said a review and grading would be done and included in the revised report. Dr. Holland cautioned against belaboring continually the issue of MTD but rather it should be considered in relation to the quality of the science

and the quality of the data. Dr. Moore said it would be useful to learn whether the study was appropriate for a carcinogenic interpretation based on the mammary tumors. Dr. Haseman pointed out that NTP historical control data on mammary adenocarcinomas indicate that these are rare and late-appearing tumors. Dr. Swenberg commented that tumors induced by known mammary carcinogens are early appearing (strain and species not specified). Dr. Scala pointed out two other possible factors which could confound interpretation of the data, one being an unacceptable degree of temperature excursion and the other being a mixing of species in the same animal room. Dr. Davis said sentinel animal data should be included.

Dr. Swenberg moved that the technical report on the bioassay of 1,2-dichloropropane be deferred for revision. Dr. Friess seconded the motion and the report was deferred until the next meeting of the Peer Review Panel.

Chlorobenzene. Dr. Scala, a principal reviewer for the report on the bioassay of chlorobenzene, agreed with the conclusion that: "Under the conditions of these studies, chlorobenzene administration increased the occurrence of neoplastic nodules of the liver in male F344/N rats, providing some but not clear evidence of carcinogenic activity. Carcinogenic effects of chlorobenzene were not observed in female F344/N rats or in male or female B6C3F$_1$ mice." However, his preference was that the last sentence read: "No carcinogenic effects were observed in female F344/N rats or in male or female B6C3F$_1$ mice administered chlorobenzene." He stated that the extrapolation of the effects of chlorobenzene to humans based on structure and rodent toxicity as comparable to benzene should be labelled as speculation.

As a second principal reviewer, Dr. Vore agreed with the conclusions. She praised the discussion of the rationale for dose selection for the chronic studies, and thought the discussion on the metabolism of chlorobenzene was nicely done. As a third principal reviewer, Dr. Van Ryzin agreed in general with the conclusions. He said the evidence for carcinogenic activity was not strong, being based on significant increases in neoplastic nodules in male rats at the high dose only. He stressed the decrease observed in carcinomas in male rats as well as the equivocally significant results when neoplastic nodules and carcinomas are combined. Dr. Van Ryzin questioned whether the maximum tolerated doses were achieved. He suggested that the finding of a renal tubular cell adenocarcinoma in a high-dose female rat and transitional cell papillomas of the urinary bladder in a low and high dose male rat might be emphasized because of their rarity and low historical control incidence.

In discussion from the floor, Dr. C. R. Stack, Chlorobenzenes Program Panel of the Chemical Manufacturers Association, said her group questioned the analogy drawn between chlorobenzene toxicity and benzene toxicity. She asked that the Chlorobenzenes Program Panel have the opportunity to provide written comments on the report subsequent to the meeting. Dr. Moore said that comments received within 30 days would be accepted on any of the reports reviewed at this meeting.

Dr. W. Kluwe, NTP Chemical Manager, said that, in view of NTP findings and other reports indicating that some of the prechronic toxicology of chlorobenzene is similar to that of benzene, statements should be in the report. Dr. Scala agreed as long as the discussion is labelled as speculation. In response to concerns expressed by Dr. Van Ryzin, Dr. Kluwe said that the first sentence of the conclusion as printed on the review form was in error while the conclusion printed in the draft report was correct and indicated that neoplastic nodules in the liver were significantly increased in high-dose male rats only. The first sentence of the conclusion read thusly: "Under the conditions of this bioassay, chlorobenzene administration increased the occurrence of neoplastic nodules of the liver in high dose (120 mg/kg/day) male F344/N rats, providing some but not clear evidence of carcinogenic activity of chlorobenzene in male rats."

Dr. Scala moved that the technical report on the bioassay of chlorobenzene be accepted with revisions discussed. Dr. Elashoff seconded the motion and the report was approved unanimously by the Peer Review Panel.

Diglycidyl Resorcinol Ether. Dr. Holland was a principal reviewer for the report on the bioassay of diglycidyl resorcinol ether (DGRE). The conclusion stated that: "Under the conditions of these studies, diglycidyl resorcinol ether caused hyperkeratosis and hyperplasia of the forestomach in rats and mice. DGRE was carcinogenic for male and female F344/N rats and male and female B6C3F$_1$ mice, causing both benign and malignant neoplasms of the forestomach." Dr. Holland wondered what the significance of positive PVM titers in rats might be with regard to the treatment-related bronchopneumonia, and to the quality of the animals used in the bioassay. He observed that there were two reported negative skin painting studies with DGRE, including one by himself and coworkers, and in view of the irritant properties of DGRE, he suggested that the forestomach tumors likely resulted from an indirect or local toxic effect of DGRE.

As a second principal review, Dr. Scala agreed with the conclusions. He criticized the poor quality of animal husbandry and environmental controls at the laboratory performing the bioassay, and cited the high virus titers in the animals used and the excessive temperature and humidity fluctuations. Further, the failure to kill some concurrent control animals at the time of large numbers of deaths in the test groups reflected poorly on laboratory management. As a third principal reviewer, Dr. Elashoff agreed with the conclusions. He asked whether the presence of 19% impurities in the test compound would restrict interpretability of the study. He agreed with the report that the high and early mortality in high-dose rat groups in the primary study led to divergent or contradictory findings among statistical tests yielding confusing information.

Dr. E. McConnell, NTP Chemical Manager, speculated that the bronchopneumonia was probably due to aspiration of food resulting from gastric dysfunction caused by the tumors. Dr. Holland said the technical grade nature of the compound should be clearly noted in the summary. NTP indicated this would be given in the title. There was further discussion by Drs. Friess and Holland as to whether the forestomach tumors might have been due to secondary or irritant effects of DGRE as opposed to a specific chemical/tissue interaction. Dr. McConnell said he would give more emphasis to the irritant properties of DGRE.

Dr. Holland moved that the technical report on the bioassay of diglycidyl resorcinol ether be accepted with revisions discussed. Dr. Elashoff seconded the motion and the report was approved unanimously by the Peer Review Panel.

Ethoxylated Dodecyl Alcohol. Dr. Hitchcock, a principal reviewer for the report on the bioassay of ethoxylated dodecyl alcohol, agreed with the stated conclusions that: "Under the conditions of this bioassay, ethoxylated dodecyl alcohol was not carcinogenic for F344/N rats or B6C3F$_1$ mice of either sex." She thought less significance should be given to the reduction in food intake in rats with respect to both weight loss and incidence of nephropathy.

As a second principal reviewer, Dr. Holland wondered why special stains were not done on representative pathology slides so that the composition of the pigment in the kidneys could be determined. He also felt the food intake discussion should be shortened. As a third principal reviewer, Dr. Van Ryzin agreed with the conclusions, and all three reviewers remarked that the report was well written.

Dr. Swenberg objected to the inclusion of a few uncommon tumors in the abstract, meaning squamous cell papillomas or carcinomas of the stomach and adrenal cortical adenomas in mice. He also stated these tumors may or may not be related to compound administration. Dr. Davis said that because this chemical is used in spermicidal preparations these uncommon tumors should be mentioned in the abstract. Dr. Swenberg maintained there was no dose response and the tumors were chance findings. Dr. Holland asked NTP for a policy on inclusion of information in the abstract on rare tumors where statistical significance was lacking. Dr. Moore replied there could be no rigid rule but rather the decision should be left to the discretion of the chemical manager to include if he thought there might be a relationship with chemical administration. Dr. Haseman said control data on both adrenal and stomach tumors from five recent studies at the testing laboratory for ethoxylated dodecyl alcohol could be added to the report to help the chemical manager in making this decision. Dr. Moore said revisions would include a statement on the identity of the pigment deposited in the kidney based on special stains; mutagenicity data with appropriate appendices; and discussion of possible associations of renal effects with the chemical would be labelled clearly as being speculative.

Dr. Holland moved that the technical report on the bioassay of ethoxylated dodecyl alcohol be accepted with the revisions discussed. Dr. Elashoff seconded the motion and the report was approved by eight affirmative votes. There were two negative votes (Drs. Friess and Swenberg), and one abstention (Dr. Scala).

Asbestos, crocidolite. Dr. Harper, a principal reviewer for the report on the bioassay of crocidolite asbestos, agreed with the stated conclusions that: "Under the conditions of this study, crocidolite asbestos was not overtly toxic and did not cause a carcinogenic response when ingested at a level of 1% in the diet by male and female Fischer 344/N rats for their lifetime." He said that offspring of asbestos-fed mothers are slightly smaller at weaning than offspring of control mothers, and this fact should be emphasized.

As a second principal reviewer, Dr. Elashoff agreed with the conclusions. He noted that the justifiable use of historical control data on the incidences of thyroid C-cell adenomas and carcinomas in male rats likely averted a probable false positive finding in this study. He expressed interest and concern as to the design considerations and tradeoffs that led to use of a single dose group, the stated dose level, and the stated sample size. Dr. McConnell described the rationale for the design of this and the other asbestos studies in the series. Due to the high level of oral exposure (1% in diet) and the life-time duration of the study, the design committee decided to recommend larger but fewer dose groups.

As a third principal reviewer, Dr. Davis said the thyroid tumors were a carcinogenic response, but because of the control rates in the other life-time asbestos studies the results may not be important. She commented that additional data on tumor incidences in recent control groups, data on food contaminants for both experimental and control groups, as well as rat-month studies would provide valuable information for further interpretation of the results from the current study. Dr. Davis noted that by incorporating bulk rather than fractionated asbestos into the feed, a majority of the fibers were much longer than fibers to which humans are usually exposed in drinking water, and there is an inverse correlation between fiber length and toxicity and biological translocation. She expressed concern that there were no specifications in the report of engineering and safety practices regarding production of the asbestos and preparation of the pellets used in the diet. She recommended that environmental and occupational monitoring be done of firms preparing test substances. She said the increased longevity and decreased body weights in crocidolite exposed animals merited more emphasis, and speculated the decreased weight might have been due in part to more rapid gastrointestinal transit time produced by the high fiber diet. Dr. Davis urged that due consideration be given to new studies using smaller fibers which are size fractionated, and further, exposure of animals should be through drinking water.

There was considerable discussion among panel members and NTP staff concerning the aspect ratio for the fibers used in the study which was greater than the optimal aspect ratios for biological translocation and carcinogenicity. With regard to dose preparation and route of exposure, Dr. McConnell said that had ground or fractionated fibers been used there could have been a potential safety hazard as well as some undesired inhalation exposure. Administration in water would have been more hazardous to labroatory personnel and because of settling of fibers the dosage would have been uncertain. In terms of fiber size, he stated that fibers of the size range used were potent carcinogens for the pleural cavity. He said that NTP could do an ashing study on tissues such as liver, kidney and lymph nodes to see if fibers were present. Dr. Scala

opined that useful information could be gained by grinding up or dissolving some of the asbestos pellets and examining the fiber composition. Dr. Moore said data was available which characterizes the proportion of fibers by length and width using electron microscopic examination. Dr. Swenberg asked for the rates for thyroid C-cell tumors in recent lifetime studies. Dr. Haseman replied that in four recent lifetime studies carried out at this laboratory these rates were 20, 21, 21 and 25 percent which taken together were similar to the 27 percent rate in the crocidolite asbestos exposed animals. Dr. Davis requested that the wording of the last sentence of the second paragraph of the abstract be reworded to say that "this slight increase [in tumor incidence] was not regarded as being biologically important" with "important" replacing "significant".

Dr. Davis then moved that the technical report on the bioassay of crocidolite asbestos be accepted with the additions and revisions discussed. Dr. Harper seconded the motion and the report was approved unanimously by the Peer Review Panel.

Benzyl Acetate. The NTP draft technical report on the bioassay of benzyl acetate was reviewed and approved by the Peer Review Panel on June 16, 1982. The draft report concluded that benzyl acetate was associated with an increased incidence of pancreatic adenomas in male F344/N rats. Subsequently the NTP discovered that in some instances an increase in proliferative lesions of the exocrine pancreas (hyperplasias and adenomas) may occur more frequently in vehicle controls compared with untreated controls. The possibility exists that the increased incidence in pancreatic adenomas may have been influenced by the administration of benzyl acetate and the oil vehicle. An announcement was made at the September 22, 1982, meeting of the Peer Review Panel that NTP would make appropriate revisions in the report and circulate them for comments to members of the Panel.

On January 12, 1983, a letter was sent to all Peer Review Panel members who were present at the review on June 16, 1982. The letter and attachments detailed the proposed changes made in the benzyl acetate technical report, primarily in the abstract, discussion, and conclusion sections. An explanatory note about the reexamination of pancreas slides from nearly 2000 animals was included. By February 21, 1983, all Panel members had responded by letter or by telephone that they approved of the proposed changes and agreed with the conclusions.

At the request of the Fragrance Manufacturers Association (FMA) and the Flavor and Extract Manufacturers Association (FEMA), NTP decided to delay printing and distribution of a final report to allow these organizations a further opportunity to furnish written submissions as to the reasons they think the conclusions in the report should be revised. Copies of this submission along with the revised report will be sent to the Peer Review Panel for their review. A FMA/FEMA representative will be given up to 15 minutes for an oral presentation to the Panel at their June meeting.