Board of Scientific Counselors
National Toxicology Program

Summary Minutes
from
Peer Reviews of Draft Technical Reports of Long-Term
Carcinogenesis Bioassays by the Technical Reports
Review Subcommittee and Panel of Experts

on

September 22, 1982
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: Dr. Norman Breslow, Robert Elashoff, Joseph Highland, Michael Holland, Frank Mirer, Robert Scala, Bernard Schwetz, Stan Vesselinovitch, and Mary Vore. Drs. Breslow and Highland were unable to attend the meeting.

Final NTP Technical Reports for these 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 February 28, 1983 in Research Triangle Park. For information, contact Dr. Larry G. Hart (919) 541-3971; FTS 629-3971.

Contents

Trichloroethylene (without Epichlorhydrin). Dr. Swenberg was a principal reviewer for the report on the bioassay of trichloroethylene (TCE). The report originally had been reviewed on June 16, 1982, but was deferred to allow for revision. He agreed with the modified conclusions as sent to the panel members on September 15 which stated that: "Under the conditions of this bioassay, epichlorohydrin-free trichloroethylene caused renal tubular-cell tumors in male F344/N rats, produced nephropathy in both sexes, shortened the survival time of males, and did not produce a carcinogenic response in females. This experiment was considered to be inadequate to evaluate the presence or absence of a carcinogenic response to trichloroethylene in male F344/N rats. Trichloroethylene induced a carcinogenic response among both sexes of $B6C3F_1$ mice, as evidenced by the appearance of hepatocellular carcinomas in males and females and hepatocellular adenomas in females." Dr. Swenberg questioned whether or not some further comment on the inadequacy of the rat study needed to be added to stress the exceeding of the MTD and the poor tissue accountability for pathology.

As a second principal reviewer, Dr. Elashoff agreed with the modified conclusions and raised several issues relating to statistical methodology. As a third principal reviewer, Dr. Harper agreed with the conclusions but wasn't sure the decreased survival of male rats could be attributed to an exceeding of the MTD. He was also concerned about the excessive number of apparent gavage errors.

In discussion from the floor, Dr. Z. Bell, PPG Industries, maintained that the current draft report was not adequate for assessing biological significance. He submitted copies of a detailed critique which he asked be made part of the meeting record, and requested that the draft report be returned for further revision and review at the next meeting. Mr. L. Schlossberg, Detrex Chemical Industries, read from reviews commissioned by him of the bioassay report which he interpreted as being inadequate in rats for establishing the carcinogenicity of TCE. Further, in his opinion the available epidemiologic and animal evidence indicate TCE is not a carcinogen. Mr. Schlossberg requested that the review of the bioassay be deferred until the data from other ongoing bioassays of TCE in different strains of rats are ready for review.

In discussion by other panel members, Dr. Mirer said the mouse findings should be placed first in the summary since the results are fairly clear and unequivocal. He opined that the large number of early deaths in the rat study actually increased the difficulty of detecting carcinogenic effects. He questioned how one interprets findings of an excess of tumors in the presence of frank organ damage, i.e., are the tumors secondary to the organ damage or do they result from a separate process? In support of the latter, he noted that although kidney damage was observed in female rats and mice of both sexes there was no concomitant increased incidence of renal tumors. Dr. Swenberg stated that the presentations made by the speakers from the floor related to the male rat findings and were in agreement with the NTP conclusions that the male rat study was inadequate. Both Drs. Swenberg and Holland emphasized that the purpose of the peer review was to evaluate the technical report, not the total evidence on the toxicology of the chemical, and to make recommendations as to the acceptability of the

report as a reflection of the experimental data.

Dr. Swenberg moved that the report on the bioassay of trichloroethylene be accepted with the minor revisions discussed. Dr. Harper seconded the motion and the report was approved by nine affirmative votes. There was one negative vote (Dr. Mirer).

Dichloromethane (Methylene Chloride). Dr. Holland, a principal reviewer for the report on the bioassay of dichloromethane (DCM) agreed with the stated conclusions that: "Under the conditions of the bioassay, DCM was carcinogenic for both male and female F344/N rats, inducing hepatic neoplastic nodules and adrenal cortical adenomas in both sexes and pancreatic acinar-cell adenomas in males. The corn oil vehicle may have contributed to the incidences of pancreatic tumors. DCM was carcinogenic for both male and female B6C3F$_1$ mice, inducing hepatocellular carcinomas in both sexes. Thyroid C-cell carcinomas in male F344/N rats and leukemia and alveolar/bronchiolar adenomas in female B6C3F$_1$ mice may have been associated with the administration of DCM". He said the summary or conclusions should be more explicit about the potential role of corn oil, particularly in the case of pancreatic lesions. Dr. Holland stated that what he considered to be a deficiency for this and other bioassays is that historical control data are not treated statistically the same as the contemporary data, e.g., adjusting for intercurrent mortality. He asked that clinical, gross, and microscopic criteria be established to allow for more consistant diagnoses of death due to gavage errors.

As a second principal reviewer, Dr. Scala agreed with the conclusions, although he commented that significantly reduced survival of high-dose rats of both sexes, and female mice should be noted. He said this bioassay is an example of an apparent inadequate dose selection using the prechronic studies; in view of the impact of dose selection on the adequacy of a bioassay, he suggested subjecting dose selection to a peer review process. He said the question of whether or not the maximum tolerated dose (MTD) was exceeded should be discussed more fully, and may be an important issue with any bioassay where there is significantly decreased survival in any treatment group. Dr. Scala also commented on the excessive numbers of gavage-associated deaths and their effect on diminishing sensitivity of the bioassay.

As a third principal reviewer, Dr. Mirer stated that the use of age adjusted statistical tests should be addressed in the discussion in consistent fashion, as should the combining of tumors. As regards the latter, he was referring to the incidence of hepatocellular adenomas and carcinomas in treated mice when combining these organ site tumors decreases or eliminates statistical significance seen with carcinomas alone in some statistical tests. Dr. Elashoff stated that combining tumors in the same organ, e.g., adenomas and carcinomas of the liver, was appropriate if the incidence trends were in the same direction for each tumor, i.e., both increasing or both decreasing in relation to control rates. Dr. Mirer was also concerned by the apparently high incidence of gavage-associated deaths, and the unresolved issue of whether chemical toxicity was involved in view of dose related trends in gavage death.

From the floor, Dr. J. Kirschman, General Foods and chairman of the National Coffee Association (NCA) Special Task Force on Methylene Chloride, reported that a NCA sponsored two-year bioassay of DCM given in drinking water to F344 rats showed no compound related increases in tumors. He voiced the opinion that the NTP study was unacceptable for use in assessing potential risk to humans due to excessive mortality in the animal groups. Dr. R. Squire, a private consultant representing the NCA, discussed his analysis of the pathology slides for male rat pancreata and female rat livers from the NTP study. He believed there were proliferative effects associated with

the corn oil vehicle on the pancreas and potential co-carcinogenic effects of corn oil and DCM. He suggested that oil gavage be re-examined as a general procedure for toxicity testing. Dr. L. Golberg, also a private consultant representing the NCA, spoke to the differences in the pharmacokinetics of DCM between corn oil gavage and administration in drinking water, and suggested that organ and tissue levels of DCM would be higher and the biologic half-life longer after gavage. These potential differences should be considered when assessing exposure to DCM under "real life" conditions. Mr. W. Goodrich, Institute of Shortening and Edible Oils, cautioned against condemning corn oil or dietary fat as cancer causing when based on inadequate scientific data. [The NTP has been investigating the effect of vehicle in all testing laboratories. The data are being evaluated and will be presented to the Board of Scientific Counselors in the future.]

Dr. J. Haseman, NTP, responded to comments by Drs. Holland and Mirer. He stated that NTP does not give survival-adjusted rates for historical control data because (1) historical control data are included primarily for descriptive purposes and are generally not used in a formal testing framework, (2) the survival-adjusted rates currently reported by the NTP assume the tumors in question were the cause of death, and (3) survival differences among historical control groups generally were not large. He further stated that NTP is studying the problem of how to use historical control data in a formal statistical analysis, and when this problem is resolved, it is likely that NTP technical reports will contain more detailed information on historical controls. Dr. Holland said it would still be useful to have adjusted rates.

Dr. Schwetz agreed with Dr. Scala that the issue of the MTD should be discussed in the abstract of every bioassay report. He called for more consistant use of untreated controls in comparisons with vehicle controls and dose groups. He had questions about the criteria for diagnosis of adrenal cortical adenomas vs. hyperplasia. Dr. Swenberg stated that in view of the differences between tumor rates in vehicle vs. untreated controls, untreated control data should be included in the tumor incidence tables. Drs. J. Moore, NTP, and Haseman agreed.

Dr. Holland moved to accept the report with the proviso that the conclusion should more clearly state that corn oil alone is capable of producing proliferative changes in the pancreas. Dr. Moore replied that the apparent corn oil effect was seen in some bioassays but not in others so one must be careful about stating that there was a cause/effect relationship. Dr. Swenberg opined there might be a contaminant in the oil which could be responsible for the lesions. Dr. Scala said that for him to accept the report there had to be clear statements about the MTD question and the gavage error issue.

Dr. Scala moved that the report on the bioassay of dichloromethane be accepted with the revisions discussed. Dr. Elashoff seconded the motion. Dr. Moore said that the revised technical report would be sent to the Peer Review Panel members for their examination; if any panel member has remaining concerns the report will be brought back to the next review meeting. The report was approved by nine affirmative votes with one abstention (Dr. Schwetz).

Asbestos, amosite. Dr. Vesselinovitch was a principal reviewer for the report on the bioassay of asbestos, amosite. The conclusion stated that: "Under the conditions of this bioassay, amosite asbestos was not carcinogenic or toxic when ingested at a 1% level in the diet by male and female Fischer 344/N rats. The cocarcinogenic studies using 1,2-dimethylhydrazine dihydrochloride (DMH) were considered inadequate because of the high rate of DMH induced intestinal neoplasia in both the amosite asbestos and non-amosite exposed groups." His only question about the conclusion was that there was a statistically significant increase in C-cell carcinomas of the thyroid in amosite treated males; even though combined carcinomas and adenomas were not significantly increased compared to controls, he wondered if some mention should be made in the conclusion. He asked why there was only one dose level and how the level was chosen.

As a second principal reviewer, Dr. Harper said he agreed with the conclusions as stated in the report. As a third principal reviewer, Dr. Whittemore agreed with the conclusions but expressed concern as to whether the MTD was achieved. She said the lack of pretests to determine the MTD and choice of dose level needed further explanation. She commented that none of the tables gave exact p-values nor was it always clear as to what type of test the p-value was for. Finally, she noted there was large numbers of typographical errors, omissions and ambiguous statements. Dr. Schwetz also questioned the experimental design, including use of only one dose and the preweaning gavage regimen.

In response to the questions about the experimental design, Dr. Moore explained that this study, as well as other oral asbestos ingestion studies in rats, represented a consensus design put together by a group of scientists within the Department of Health, Education and Welfare. One dose was used so as to expose a larger than usual number of animals, and the one percent dose level in the diet represented the highest dose thought reasonable to administer from a biological standpoint, and one considered to represent a tolerated dose. The preweaning gavage was added to provide exposure of animals at a time when there might be increased permeability of the gut which could allow enhanced penetration of fibers.

In other discussion, Dr. Scala objected to ascribing effects of asbestos on neonatal animals via lactational exposure when fibers were not measured in the milk. Dr. Whittemore asked to see the report again after revisions had been made and Dr. Moore agreed.

Dr. Whittemore moved that the report on the bioassay of amosite asbestos be accepted with agreed on revisions. Dr. Harper seconded the motion and the technical report was approved with nine affirmative votes with one abstention (Dr. Swenberg).

Tremolite. Dr. Vesselinovitch, as a principal reviewer for the report on the bioassay of tremolite, agreed with the conclusion that: "Under the conditions of this bioassay, nonfibrous tremolite was not toxic and did not cause a carcinogenic response when ingested at a level of 1% in the diet by male and female Fischer 344 rats for their lifetime." He said his questions regarding the experimental design had been answered during the review of the amosite report. As a second principal reviewer, Dr. Harper said the conclusion statement should include the observation that the offspring of mothers exposed to tremolite were of normal size at birth but were smaller than control animals at weaning and remained smaller throughout their life.

As a third principal reviewer, Dr. Scala had a number of comments on various aspects of the experimental design, on sample purity, and on analytical methodology. He stressed the need to discuss whether or not an MTD had been achieved, and noted that possible toxic effects may be seen in early weight gain differences between control and exposed neonatal animals which were never made up. Dr. Scala asked why the study was done in the first place, especially, if the major carcinogenic potential of this class of minerals has to do with their fibrous nature, then what can be gained from testing a nonfibrous material?

Dr. E. McConnell, NTP chemical manager, responded that a bioassay with a non-fibrous material was needed for comparison of toxicity with those using fibrous (asbestos) forms. Further, there has been a large human exposure to tremolite. Dr. Moore noted that this form of crystalline tremolite was used widely and was found in food and pharmaceutical products. Further, he added the tremolite used has a fibrous contaminant which could be produced from the crushing process or just be there naturally. He said the true fiber count for this contaminant should be indicated in the report. Dr. R. Shapiro, NIEHS, said the tremolite used was chosen to duplicate the commercial material to which humans had been exposed in the past.

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

Allyl Isovalerate. Dr. Schwetz was a principal reviewer for the report on the bioassay of allyl isovalerate. The conclusions stated that: "Under the conditions of this bioassay, allyl isovalerate was carcinogenic for F344/N rats and B6C3F$_1$ mice, causing increased incidences of hematopoietic system lesions (mononuclear cell leukemia in male rats and lymphoma in female mice). Further, the increased incidence of two relatively rare tumors in these species may have been related to the administration of allyl isovalerate -- acinar-cell adenomas of the pancreas in male F344/N rats and squamous-cell papillomas in the gastric mucosa in male B6C3F$_1$ mice. Allyl isovalerate was not mutagenic for Salmonella typhimurium tester strains TA 98, 100, 1535, and 1537 (with or without metabolic activation using preincubation suspension)." Dr. Schwetz said the abstract should mention the significant decreases observed in male mice of hepatocellular carcinomas, alveolar/bronchiolar adenomas or carcinomas, and follicular-cell adenomas of the thyroid. Dr. Schwetz observed that the historical control data supplied supported the positive findings for lymphomas in female mice but indicated he thought there was an equivocal result for leukemias in male rats when the control range across laboratories was considered. He also stated that the genital tract infection which caused a significant number of deaths in female mice should be better defined and characterized in the results section and the possible impact on the outcome of the study in females discussed.

As a second primary reviewer, Dr. Vore agreed with the conclusion which separated incidences of hematopoietic lesions in rats and mice. She mentioned the number of rats killed accidentally, the number of female mice likely dying of infections, and the fact that the maximum tolerated dose appears not to have been attained.

As a third primary reviewer, Dr. Swenberg commented that for leukemias in male rats the incidence in high dose animals was within the historical control range, and therefore likely not of biological significance. With regard to lymphomas in female mice, the findings were at best equivocal. He doubted the biological significance for squamous cell papillomas of the mouse stomach, preputial gland tumors in rats, and that the increased incidence of pancreatic adenomas in male rats were accounted for by chemical induction. In sum, he said, there is little, if any, evidence of carcinogenicity with allyl isovalerate.

Dr. Huff, NTP, stated that the NTP policy was to compare treated groups with (in order of preference) i) concurrent controls, ii) laboratory specific historical controls, and iii) historical controls across laboratories. Due to a considerable laboratory to laboratory variation the NTP generally uses across laboratory historic rates only for rare tumors. The interlaboratory composite historic control tumor data were, in Dr. Huff's opinion, inappropriate for routine comparisons with individual carcinogenesis bioassays, and thus, inappropriate for making interpretive evaluations. Comparing the incidences of hematopoietic lesions in dosed rats and mice with both the concurrent controls and with the laboratory specific historic controls, Dr. Huff emphasized a clear dose response and a high dose effect in two (male rats and female mice) of the four experiments and some evidence of a similar trend in the other two studies (female rats and male mice).

In further discussion, Dr. Elashoff said comparison of historical control data with concurrent controls was difficult because adjusted incidences i.e., correction for survivorship, were used for concurrent control comparisons but not for the historical controls. Dr. J. Haseman, NTP, said for the data on allyl isovalerate there was little variability with regard to leukemias in rats in other control groups from the same testing laboratory. Dr. Holland said the criteria for diagnosing leukemia may vary tremendously from laboratory to laboratory. Thus, he would ignore the historical control data in arriving at any decision about the merits, or lack thereof, of the findings on hematopoietic lesions. There was discussion by Dr. E. McConnell, NTP, about appropriateness of combining leukemias and lymphomas in rats for statistical purposes.

Dr. Schwetz moved that the report on the bioassay of allyl isovalerate be accepted subject to the written and verbal revisions discussed. Dr. Vore seconded the motion. Dr. Swenberg requested that he have the opportunity to see the revised draft. The technical report was approved by nine affirmative votes with one abstention (Dr. Holland).

1,2-Dichlorobenzene. Dr. Scala, a principal reviewer for the report on the bioassay of 1,2-dichlorobenzene, agreed with the conclusion that: "Under the conditions of this bioassay, 1,2-dichlorobenzene was not carcinogenic for male or female F344/N rats or B6C3F$_1$ mice; however, the maximum tolerated dose was probably not achieved in the present study." He said the MTD must have been nearly achieved, and there was a risk of significant liver and/or kidney damage at higher doses. He called attention to the highly variable survival rates between the two thirteen week studies in mice, and asked for further discussion of these results.

As a second principal reviewer, Dr. Whittemore agreed with the conclusions as stated. She said the large survival differences in mice between the two 14-day studies deserved more comment. She noted a dose-related trend in kidney tubular regeneration in male mice and asked for discussion of the biological significance. A discussion followed on whether or not a MTD had been achieved. Dr. Holland said the statement in the conclusion should be stricken, while Dr. Swenberg said the survival curve, particularly for male rats, indicated a MTD had been reached. NTP agreed to remove the comment on MTD from the conclusion. There was discussion, primarily between Dr. Schwetz and Dr. J. Goldstein, NTP, about the degree and significance of porphyria or porphyrin excretion observed in the 13 week studies. Dr. Vore asked that a statement be included in the report about the increase in porphyrin excretion.

Dr. Scala moved that the report on the bioassay of 1,2-dichlorobenzene be accepted with the revisions discussed. Dr. Vore seconded the motion and the technical report was approved unanimously by the Peer Review Panel.

2,6-Xylidine. Dr. Swenberg, a principal reviewer for the report on the bioassay of 2,6-xylidine, agreed with the conclusion that: "Under the conditions of this bioassay, 2,6-xylidine was carcinogenic for Charles River CD rats of each sex, causing increases in the incidences of both adenomas and carcinomas in the nasal cavity. A rhabdomyosarcoma, a rare tumor of the nasal cavity, was observed in dosed rats of both sexes. In addition the increased incidences of subcutaneous fibromas and fibrosarcomas in male and female rats and an increased incidence of neoplastic nodules in the livers of female rats may have been related to the administration of 2,6-xylidine." Since the two-year bioassay was in rats only, he questioned whether subchronic data in $B6C3F_1$ mice should be included. The NTP indicated that this was the most appropriate place to publish these data. Dr. Swenberg said some of the statistics used were confusing and wondered about the significance of using a p-value of 0.06; asked for more details on the various studies done on stability and volatility of the chemical in feed; and provided a list of references to other systemically administered agents that have induced nasal cavity tumors. In response to Dr. Swenberg's criticism, Dr. Haseman said NTP would revise the format for presenting the statistical analyses of tumor incidence data and make it consistent with the format used in most other NTP bioassay reports.

Dr. M. Kornreich, NTP, said NTP conducted three prechronic studies by gavage in rats and mice, while the original studies done by EPA included two prechronic studies and a two-year bioassay using different protocols, a different route of administration, and different strain of rat. The information from all of these studies were included since the NTP thought it would be useful to understanding the toxicology of 2,6-xylidine. She acknowledged there was considerable loss of chemical due to evaporation from the dosed feed, and to a lesser extent through chemical reaction with food components, resulting in an uncertain dose per rat. Mr. R. Smith, Ethyl Corporation, and Dr. C. Jameson, NTP, described studies done by Ethyl and NTP to measure loss of compound from dosed feed.

As a second principal reviewer, Dr. Schwetz said that although he agreed with the conclusions regarding the nasal tumors, it needed to be pointed out that the actual dose level was not determined in the chronic study and the route of exposure was mixed (oral and vapor inhalation). Dr. Schwetz commented that there was too much information included that should be reported elsewhere, especially the data from studies in mice. Further, he said there were many areas where scientific terminology used was imprecise or incorrect.

As a third principal reviewer, Dr. Vore agreed with the conclusions as stated. She requested that a statement be made concerning the high mortality in high-dose male rats. Death did not appear to be due to nasal tumors since the incidence of tumors in female rats was very similar to that in males, yet there was no increased mortality in female rats. She opined that there was a low probability that inhalation was a realistic route of exposure, and did not need to be invoked as a mechanism for the nasal tumors.

In response to a question by Dr. Mirer about data on fertility and birth defects, Dr. Kornreich said the chronic bioassay was done in conjunction with a multigeneration reproduction study. Dr. Scala emphasized his concern about the lack of quantitation of the doses, and commented on the distinct possibility

of topical and inhalation exposure being involved in effects seen. Dr. Holland said that if we applied the same degree of concern about the actual dose to the target tissue in all bioassays, we would have a considerable dilemma. Further, he said delaying release of the data pending the acquisition of more detailed information about the proportion of exposure of the nasal route versus the oral route would be unwarranted. Dr. Hitchcock said data developed by NTP and others on rate of loss of chemical through volatility from diet should be highlighted in the report and some reasonable estimate of exposure derived. Dr. Vore requested the mouse data not be deleted but placed in the appendix.

Dr. Swenberg moved that the report on the bioassay of 2,6-xylidine be accepted with the revisions indicated. Dr. Vore seconded the motion. Dr. Scala requested an opportunity for the Panel to read a revised statement dealing with the question of dose and route before the report is published. The technical report was approved unanimously by the Peer Review Panel.

Toluene Diisocyanate. Dr. Elashoff, a principal reviewer for the report on the bioassay of toluene diisocyanate (TDI), agreed with the conclusions that: "Under the conditions of this bioassay, toluene diisocyanate was carcinogenic for F344/N rats, causing subcutaneous fibromas and fibrosarcomas (combined) in males and females, pancreatic acinar-cell adenomas in males, and pancreatic islet-cell adenomas, neoplastic nodules of the liver, and mammary gland fibroadenomas in females. Toluene diisocyanate was carcinogenic for female $B6C3F_1$ mice, causing hemangiomas or hemangiosarcomas (combined), as well as hepatocellular adenomas, but TDI was not carcinogenic for the male mice." He noted that due to the common lethal nontumorigenic toxicity, the Life Table and Incidental Tumor Tests are the appropriate statistical analyses. As a second principal reviewer, Dr. Mirer said the conclusions were appropriate. He criticized the storage of gavage solutions at room temperature in view of the reactivity of TDI with corn oil. He said the compound related, dose related toxicity indicated the doses may have been too high in rats yet this did not compromise the conclusions.

As a third principal reviewer, Dr. Holland accepted the abstract as written. He commented that the interpretation of the bioassay was complicated by the instability of the material in contact with water as in the gastrointestinal tract. However, since it is probable that similar degradation would occur if exposure were by the respiratory route, the data were, in his opinion, a valid representation of TDI potential tumorigenicity. Dr. M. Dieter, NTP, said the corn oil was not anhydrous or kept in a dessicator, and rates of degradation of TDI would be given in the text.

In discussion from the floor, Dr. L. Rampy, Dow Chemical Company and representing the International Isocyanate Institute, reiterated and added to comments sent to the review panel and NTP staff prior to the meeting. First, he said the occupational route of exposure to TDI is inhalation; thus, the gavage route is improper, with toxicity, spectrum of metabolites and other factors likely to differ between the routes. He said that TDI would be converted after oral dosing to toluene diamine, a known animal carcinogen. He voiced other interpretive deficiencies in this study, and requested that the report be rejected until the validity of the study could be established with a more complete understanding of the study's strengths and weaknesses.

Dr. Swenberg stated the study should have been stopped at one year due to excessive mortality and exceeding the MTD. He said that bronchial pneumonia may have been related to early deaths. Dr. Holland said the question of toxicity and exceeding the MTD along with gavage errors seems to recur in the bioassays. The point, he said, is that the effect of reducing a sample size in a positive study is of less overall concern than the effect of reducing sample size in the case of a negative study. Dr. Elashoff noted that, in the case of TDI, despite reduction of power and sensitivity due to early mortality, the tumorigenic effects were such that there was significance both in trend tests and pairwise comparisons at a number of target sites.

Dr. Scala stated that there needed to be a statement(s) in the conclusion about exceeding the MTD in rats, and possibly in male mice. Dr. Mirer requested that there be in the abstract a discussion about the reactivity of TDI with corn oil.

Dr. Elashoff moved that the report on the bioassay of toluene diisocyanate be accepted conditional on the revisions, written and discussed. Dr. Holland seconded the motion and the technical report was approved by nine affirmative votes with one abstention (Dr. Schwetz).

Geranyl Acetate. Dr. Hitchcock, a principal reviewer for the report on the bioassay of geranyl acetate, agreed with the conclusions that: "Under the conditions of this bioassay, geranyl acetate was not carcinogenic for F344/N rats or B6C3F$_1$ mice of either sex, although in male rats increased incidences of squamous cell papillomas of the skin and tubular-cell adenomas of the kidney may have been related to administration of the test chemical. The reduced survival observed in high-dose male rats, high-dose male mice, and high- and low-dose female mice lowered the sensitivity of this bioassay for detecting increased tumor incidences in these groups." She noted that the chronic study was not completed for high-dose mice of both sexes because of gavage errors in the ninety-first week. She said the thirteen-week study clearly resulted in an overestimate of the MTD for male rats in the chronic study. As a second principal reviewer, Dr. Elashoff agreed with the conclusions, and with Dr. Hitchcock that the doses were too high in the chronic rat study.

In discussion, Dr. Scala said there needed to be more discussion in the report about exceeding the MTD, and its impact on the usefulness of the bioassay results. Dr. Mirer said kidney and liver toxicity may well relate to reduced survival and, therefore, mention should be made in the conclusions. Dr. Moore said since renal tubular-cell tumors ordinarily appear late in the rodent's life, we would not have seen them in many high-dose rats due to the early mortality. Dr. Swenberg suggested the low dose in this instance becomes an MTD.

Dr. Elashoff moved that the report on the bioassay of geranyl acetate be accepted. Dr. Mirer seconded the motion and the technical report was approved by nine affirmative votes with one negative vote (Dr. Scala).

Sodium 2-Ethylhexyl Alcohol Sulfate. Dr. Vore, a principal reviewer for the report on the bioassay of sodium 2-ethylhexyl alcohol sulfate, agreed with the conclusions that: "Under the conditions of this bioassay, sodium 2-ethylhexyl sulfate was not carcinogenic for F344/N rats or for male B6C3F$_1$ mice. The increased incidence of hepatocellular carcinoma in female B6C3F$_1$ mice may have been associated with administration of sodium 2-ethylhexyl sulfate. Renal transitional-cell papillomas in one high-dose male and one high-dose female F344/N rat, as well as a renal tubular-cell adenoma in a second high-dose female F344/N rat, may have been related to administration of sodium 2-ethylhexyl sulfate." She commented that although a MTD may not have been attained in male mice, this did not appear to have a significant effect on the conclusions. She said discussion of the structural similarity of the 2-ethylhexyl side chain in di (2-ethylhexyl) adipate, di (2-ethylhexyl) phthalate, and sodium 2-ethylhexyl sulfate must be balanced by a discussion of the differing physical-chemical properties between the present compound and the other two.

As a second principal reviewer, Dr. Schwetz agreed with the conclusions of the report. Regarding mixing of dose solutions with the feed, he questioned whether the test agent was uniformly mixed in the diet. He noted noted differences between the two-year and 13-week studies including source of animals, types of feed and mode of housing. These were all variables which should have been controlled. He said the abstract should indicate that dose levels for female mice were double those for male mice.

As a third principal reviewer, Dr. Whittemore said the conclusion should state that a high incidence of chronic focal nephritis was observed in high-dose male rats, but not in control or low dose males. She questioned the negative conclusion concerning kidney tumors in the rat. She said the lack of statistical significance for these tumors in dosed rats when compared with historical controls needs comment in view of their rarity. Dr. Swenberg responded by saying the singular tubular adenoma and transitional cell carcinoma could not be combined as they are of different cell types, and, there was kidney toxicity so the neoplasms were probably secondary to increased cell turnover because of toxicity. Dr. Whittemore replied she was not suggesting combining tumors but rather highlighting rare tumors even though statistical significance was lacking.

Dr. Vore moved that the report on the bioassay of sodium 2-ethylhexyl alcohol sulfate be accepted with corrections and suggestions. Dr. Schwetz seconded the motion and the technical report was approved by nine affirmative votes with one abstention (Dr. Whittemore).