Board of Scientific Counselors
National Toxicology Program

Summary Minutes
from

Peer Review of Draft Technical Reports of Long-Term
Toxicology and Carcinogenesis Studies and Short-Term Toxicity Studies
by the Technical Reports Review Subcommittee

on

November 21, 1991

Research Triangle Park, North Carolina

The review meeting began at 8:30 a.m. on November 21 in the Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the Subcommittee are: Drs. Curtis Klaassen (Chairperson), Paul Bailey, Gary Carlson, Kowetha Davidson, Harold Davis, Robert Garman, Jay Goodman, David Hayden, Daniel Longnecker, Barbara McKnight, Ellen Silbergeld, Matthew van Zwieten, Lauren Zeise, and Mr. Louis Beliczky. Drs. Davis, Longnecker, and Silbergeld were unable to attend the meeting. These minutes have been reviewed and approved by all members of the Subcommittee present. They were written by Dr. Larry G. Hart, Executive Secretary.

When available, a limited number of final NTP Technical Reports for the studies may be obtained free of charge from the NTP Central Data Management, MD A0-01, P. O. Box 12233, Research Triangle Park, NC 27709. Telephone: 919/541-3419; FTS: 629-3419. Subsequently, they may be purchased from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, Virginia 22161, 703/487-4650.

The next NTP technical reports peer review meeting will be held June 23-24, 1992, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, 919/541-3971; FTS 629-3971.

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SUMMARY MINUTES
TECHNICAL REPORTS REVIEW SUBCOMMITTEE MEETING

November 21, 1991

1,3-Butadiene. Dr. R.L. Melnick, NIEHS, introduced the toxicology and carcinogenesis studies of 1,3-butadiene in B6C3F1 mice by discussing the uses and rationale for study including the results of a previous NTP study in mice, describing the experimental design for both standard and stop studies which were intended to assess the relationship of exposure duration vs. concentration on carcinogenicity, reporting on survival and toxicity especially to the hematopoietic system and gonads in both sexes, and presenting data on nonneoplastic and neoplastic lesions caused by 1,3-butadiene at multiple sites in both sexes. Dr. Melnick reported on the expression of K-ras oncogenes in liver tumors and said that the K-ras is the most commonly detected oncogene in human cancers. Dr. C. Shackelford, NIEHS, provided a morphological description of hemangiosarcomas of the heart induced by 1,3-butadiene. The conclusions were that:

> The previous inhalation studies of 1,3-butadiene in male and female B6C3F1 mice provided clear evidence of carcinogenicity at exposure concentrations of 625 or 1,250 ppm. The present inhalation studies - 2-year exposures to 6.25 to 625 ppm or shorter duration exposures to 200,312, or 625 ppm - provide a better characterization of the concentration-dependent responses for 1,3-butadiene-induced neoplasms and nonneoplastic lesions. The present studies confirmed the clear evidence of carcinogenicity for 1,3-butadiene in male B6C3F1 mice based on increased incidences of neoplasms in the hematopoietic system, heart, lung, forestomach, liver, Harderian gland, preputial gland, brain, and kidney. There was clear evidence of carcinogenicity for 1,3-butadiene in female B6C3F1 mice based on increased incidences of neoplasms in the hematopoietic system, heart, lung, forestomach, liver, Harderian gland, ovary, and mammary gland. Low incidences of intestinal carcinomas in male mice, Zymbal's gland carcinomas in male and female mice, and renal tubule adenomas and skin sarcomas in female mice may also be related to chemical administration.

Dr. Goodman, a principal reviewer, agreed with the overall conclusions in male and female mice but disagreed with inclusion of brain and kidney in males and liver in females as support for the level of evidence. He said the last sentence should read 'equivocal evidence of carcinogenicity' instead of 'low incidence of' and omit reference to Zymbal's gland carcinomas in males. Dr. Melnick said that for low numbers of rare tumors he thought 'low incidence' to be meaningful, however, another wording would be considered. Dr. Goodman thought the conclusions for the stop studies should be presented separately from those for the chronic studies. Dr. Melnick noted that the results are presented and analyzed separately but in evaluating the effect of butadiene on an organ the thinking was that all of the evidence should

be brought to bear in drawing conclusions. Dr. Goodman asked that justification be given for the use of sex-linked recessive lethal mutations in Drosophila melanogaster and the micronucleus test. Dr. E. Zeiger, NIEHS, explained that the Drosophila assay is extremely predictive for carcinogenicity as there are very few false positives while the micronucleus test is the only simple measure we have of somatic mutations in vivo.

Dr. Zeise, the second principal reviewer, agreed with the conclusions. Because the study is designed to look at the issue of dose/response, she thought a more extensive analysis of the dose/response data should be included, especially pertaining to the shape of the curve at lower doses. Dr. Melnick said some discussion could be given about the shape of the dose/response curve and the poly 3 test used to provide tumor rates adjusted for intercurrent mortality. Dr. J. Haseman, NIEHS, expressed concern that mathematical modeling of the data might lead to extrapolation and risk assessment calculations, activities that are normally the purview of the regulatory agencies. Dr. Zeise noted that others are already using the NTP data for these purposes. She suggested that NTP not extrapolate, but evaluate the shape of the dose response within the range of observations since the study was designed to explore the dose response and the NTP has the expertise to perform such statistical evaluations.

Dr. van Zwieten, the third principal reviewer, agreed with the conclusions. He said that there should be a statement in the Conclusions to the effect that a carcinogenic response was induced at all exposure levels. Also, a comment about the stop exposure studies regarding duration of exposure necessary for a carcinogenic response would be appropriate here. Dr. Melnick said statements would be brought forward to the Abstract.

Mr. Beliczky reported that the data from these studies had been used by NIOSH recently in conducting a risk assessment the results of which have been provided to the Department of Labor for potential regulatory action by OSHA on allowable exposure levels. Dr. Garman asked whether separate classifications of lymphomas reflect the current recommendation of the NTP. Dr. Eustis said accurate distinctions between types were difficult to make and of little value. Rather, identifying whether the lymphomas originated in the thymus or elsewhere was most useful.

Dr. Goodman moved that the Technical Report on 1,3-butadiene be accepted but with the conclusions for the chronic studies separated from those for the stop studies by inserting "chronic exposure to" in front of "1,3-butadiene" in the statements for male and female mice. "Brain" and "kidney" would be deleted from the listing for male mice and "liver" from the listing for female mice. Then, a conclusion for the stop study would be added: "There was clear evidence of carcinogenicity in the start/stop study in male B6C3F1 mice based on increased incidences of neoplasms in the hematopoietic system, lung, forestomach, and Harderian gland." Finally, in the last sentence, "Low incidences" would be replaced with "Marginal increases". The motion was tabled for lack of a second. Dr. Zeise moved that the Technical Report on 1,3-butadiene be accepted with the revisions discussed and with the conclusions as written for male and female mice, clear evidence of carcinogenicity. Mr. Beliczky seconded the motion, which was accepted by eight yes to one no votes (Goodman)

with one abstention (Bailey) because of potential conflict of interest due to company affiliation.

p-Nitroaniline. Dr. R.D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of p-nitroaniline by discussing the uses and rationale
for study, describing the experimental design, reporting on survival and body weight effects, and commenting on neoplastic lesions in male mice and nonneoplastic lesions in male and female mice. The conclusions were that:

> Under the conditions of these 2-year gavage studies there was equivocal evidence of carcinogenic activity of p-nitroaniline in male B6C3F1 mice based on the increased incidences of hemangiosarcoma of the liver and hemangioma or hemangiosarcoma (combined) at all sites. There was no evidence of carcinogenic activity of p-nitroaniline in female B6C3F1 mice given doses of 3, 30, or 100 mg/kg.

Dr. van Zwieten, a principal reviewer, agreed with the conclusions. He thought there was insufficient discussion of the results of the 2-year study in rats recently reported in the literature. Dr. Irwin said the discussion of the rat study would be expanded. Dr. van Zwieten suggested that more discussion would be appropriate regarding selection of gavage administration when previous NTP studies of aniline and substituted anilines used the dietary route. Dr. Irwin said the compound was given by gavage because it wasn't stable in feed. Dr. van Zwieten said a brief histomorphological description of the vascular neoplasms observed would be useful in indicating the criteria used to distinguish benign from malignant lesions. Dr. Irwin agreed.

Dr. Bailey, the second principal reviewer, agreed with the conclusions. He questioned why 1000 mg/kg was chosen as a dose level for the 14-day study in view of the oral LD 50 in mice cited as 750 mg/kg. Dr. Irwin commented that the top dose in the 14-day study is chosen to be sufficiently high enough to elicit a toxic response and thus, may in some instances, exceed the LD 50. Dr. Bailey wondered whether dietary administration would have been more akin to actual human exposure to the chemical.

Mr. Beliczky, the third principal reviewer, did not agree with the conclusions in male mice. He said that hemangioma or hemangiosarcoma (combined) at all sites showed a significant positive trend, and although incidences in the dosed groups were not significantly greater than controls by pairwise comparisons, the incidence of these neoplasms in the high dose group (20%) exceeded the NTP historical control range (0 to 12%). Therefore, he thought the level of evidence in male mice should be some evidence of carcinogenic activity. Dr. Irwin said the level chosen was based on the fact that the neoplasms were only marginally increased in incidence and there was no comparable response in female mice. Mr. Beliczky commented that since these studies may have application to specific industries, the Production and Use Section should be expanded to identify which type industries manufacture and use the end products, among which are antioxidants and antiozonants. He believed that since 1978, NIOSH might have additional use and exposure data. Dr. Irwin asked Mr. Beliczky if he could obtain information about industries that produce these products.

Dr. Zeise questioned whether or not the maximum tolerated dose (MTD) had been reached in the 2-year study. Dr. Irwin replied that based on persistent anemia observed in 13-week studies, there was belief that some mortality was likely if 300 mg/kg were the top dose in the 2-year studies. Dr. S. Eustis, NIEHS, acknowledged that a higher top dose probably could have been tolerated, and a statement could be added to that effect.

Dr. van Zwieten moved that the Technical Report on p-nitroaniline be accepted with the revisions discussed and with the conclusions as written for male mice, equivocal evidence of carcinogenic activity, and for female mice, no evidence of carcinogenic activity. Dr. Zeise requested that a statement be added to the Abstract that a higher dose may have been tolerated in male mice. Dr. Bailey seconded the motion, which was accepted by nine yes votes to one no vote (Beliczky).

o-Nitroanisole. Dr. R.D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of o-nitroanisole by discussing the uses and rationale for study, describing the experimental design including additional 2-year stop studies in rats, reporting on survival and body weight effects, and commenting on compound-related neoplastic lesions in rats and mice and non-neoplastic lesions in rats. The conclusions were that:

> Under the conditions of these feed studies, there was clear evidence of carcinogenic activity of o-nitroanisole in male and female F344 rats that received diets containing 6,000 or 18,000 ppm for 6 months based on increased incidences of benign and malignant neoplasms of the urinary bladder, transitional cell neoplasms of the kidney, and benign and malignant neoplasms of the large intestine. There was a chemically related increase in the incidence of mononuclear cell leukemia in male and female rats receiving diets containing 222, 666, or 2,000 ppm o-nitroanisole for 2 years. Marginal increases in uncommon renal tubule cell neoplasms in male rats and forestomach neoplasms in male and female rats were considered uncertain findings. There was clear evidence of carcinogenic activity of o-nitroanisole in male B6C3F1 mice based on increased incidences of benign and malignant hepatocellular neoplasms. There was some evidence of carcinogenic activity of o-nitroanisole in female B6C3F1 mice based on increased incidences of hepatocellular adenomas.
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> Increased severity of nephropathy in male rats and increased incidences of hyperplasia of the renal tubule epithelium and ulcers of the forestomach in male rats, and of transitional cell hyperplasia of the urinary bladder, focal hyperplasia of the forestomach, and hyperplasia of transitional epithelium of the kidney pelvis in male and female rats were associated with exposure to o-nitroanisole.

Dr. Hayden, a principal reviewer, agreed with the conclusions. He thought the rationale for study could be strengthened by adding a statement on consumer exposure as well as occupational exposure, and a sentence added noting that several aromatic amines have been identified as human bladder carcinogens. Dr. Irwin said there were no data on human exposure including the NIOSH National Occupational Exposure Survey.

Dr. McKnight, the second principal reviewer, agreed with the conclusions. She said the rationale section should also include mention of why the stop exposure studies were performed. Dr. Irwin said this would be added. Further, since the conclusions in rats rest heavily on the results of the stop studies, Dr. McKnight suggested that the appendices should contain the same level of detail of reporting on tumor results as that given the usual two-year studies.

Dr. Garman, the third principal reviewer, agreed with the conclusions. He said there was a statement made that absence of renal tubular epithelial cell degeneration in male rats in the high dose group of the stop study may have been due to marked reduction in feed

consumption. This statement should be better elaborated. Dr. Irwin responded that the reduction in feed consumption and body weight was such that the physiology of the animal was altered leading to an interpretation of an association with diminished renal pathology.

Noting that this was one of the first reports to be considered by the Subcommittee that had both conventional two-year and stop study designs, Dr. Klaassen asked for discussion about presentation of design information and results. Dr. Goodman and Mr. Beliczky stated that the results should be considered and reported separately, while Dr. Garman thought they should not be separated as the stop study serves to more or less support or confirm the chronic study. Dr. Irwin observed that the stop study is usually chosen based on the fact of a lesion at a higher dose level in prechronic studies for which there is a question about the biological behavior. In this case, the data from the stop and chronic studies were treated as part of a dose-response and, thus, it was considered appropiate to combine the findings. Dr. S. Eustis, NIEHS, agreed and said the NTP would prefer not to draw separate conclusions in that they could be quite confusing to the public and others who use our data. Dr. Davidson pointed out that the level of evidence in rats would have been less clearcut without the results from the stop studies. Dr. Goodman opined that separation of the statement about mononuclear cell leukemias in rats was appropriate as the incidences of leukemia in male rats were supportive of some evidence and in female rats were supportive of equivocal evidence. Dr. Hayden commented that the evidence was supportive of a positive finding for leukemia in both male and female rats but perhaps not as part of clear evidence. Dr. Klaassen concluded that there was not a consensus on this issue.

Dr. Hayden moved that the Technical Report on o-nitroanisole be accepted with the revisions discussed and with the conclusions as written for male and female rats and male mice, clear evidence of carcinogenic activity, and for female mice, some evidence of carcinogenic activity. Dr. Garman seconded the motion. Dr. McKnight offered an amendment that mononuclear cell leukemia be listed in the first sentence as part of clear evidence in male and female rats. The amendment was tabled for lack of a second. Dr. Hayden's motion was then accepted unanimously with ten votes.

Pentachloroanisole. Dr. R.D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of pentachloroanisole by discussing the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. He reported on pharmacokinetic studies in rats with pentachloroanisole and a major metabolite, pentachlorophenol, and concluded from the results that sex differences in toxic response to the chemical were not due to differences in absorption or bioavailability. The conclusions were that:

> Under the conditions of these 2-year gavage studies, there was some evidence of carcinogenic activity of pentachloroanisole for male F344/N rats based on increased incidences of benign pheochromocytomas of the adrenal medulla. There was equivocal evidence of carcinogenic activity of pentachloroanisole for female F344/N rats based on marginally increased incidences of benign pheochromocytomas of the adrenal medulla. There was some evidence of carcinogenic activity of pentachloroanisole in male B6C3F1 mice based on increased incidences of benign pheochromocytomas of the adrenal medulla and hemangiosarcomas of the liver. There was no evidence of carcinogenic activity of pentachloroanisole for female B6C3F1 mice given doses of 20 or 40 mg/kg.
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> Pentachloroanisole administration was associated with increased incidences of adrenal medulla hyperplasia in female rats and increased incidences of pigmentation in the renal tubule epithelium, olfactory epithelium, and hepatocytes of male and female rats. In addition, decreased incidences of pancreatic adenomas and focal hyperplasia in male rats and decreased incidences of mammary gland fibroadenomas and uterine stromal polyps and sarcomas (combined) in female rats were observed. Hyperthermia-related lesions in male rats receiving 20 or 40 mg/kg were considered directly related to pentachloroanisole administration.
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> Pentachloroanisole administration was associated with increased incidences of adrenal medulla hyperplasia and hypertrophy, and hepatocellular mixed cell foci in male mice. In male and female mice, nonneoplastic liver lesions associated with pentachloroanisole administration included hepatocellular cytologic alteration, Kupffer cell pigmentation, biliarytract hyperplasia, and subacute inflammation.

Dr. Garman, a principal reviewer, agreed with the conclusions. He asked for clarification of the histomorphologic criteria for diagnostic terminology used in designating malignancy of adrenal medullary lesions.

Dr. Zeise, the second principal reviewer, agreed with the conclusions. However, she asked for discussion on whether the level of evidence in female rats should be raised to some evidence based on an incidence of adrenal tumors in the high dose group that was above the historical control range supported by increased incidences of these tumors in male rats and male mice.

She suggested that a statement be added to the report indicating that the incidence of pheochromocytomas in high dose female rats fell outside that of historical controls. Dr. J. Haseman, NIEHS, noted that the increased incidence of adrenal tumors in dosed female rats was not significant, reflecting in part that survival in the high dose group was increased compared to concurrent and historical control survival rates. Dr. Zeise hoped that information on the pharmacokinetic studies could be added to the report.

Dr. McKnight, the third principal reviewer, agreed with the conclusions for male and female rats and male mice but thought the conclusion for female mice should be changed to equivocal evidence of carcinogenic activity based on the dose-related marginally increased incidence of malignant lymphoma supported by a statistically significant trend test. Dr. Irwin commented that since these are common tumors the historical rates are quite variable such that the high dose rate is well within the range, being slightly higher than the average, and thus, not considered to be chemically-related. Dr. McKnight said that because the 13 "accidental deaths" among male rats were at least indirectly associated with treatment, they should be counted as deaths rather than censored observations in the survival curves or an alternative set of survival curves should be added.Dr. Haseman said that the relatively small number of accidental deaths would likely result in a second set of survival curves that would be almost indistinguishable from the first set. Dr. Irwin said a second set would be generated to see if this was the case.

Dr. Garman moved that the Technical Report on pentachloroanisole be accepted with the revisions discussed and with the conclusions as written for male rats and male mice, some evidence of carcinogenic activity, for female rats, equivocal evidence of carcinogenic activity, and for female mice, no evidence of carcinogenic activity. Dr. Hayden seconded the motion. Dr. McKnight offered an amendment that the level of evidence for female mice be changed to equivocal evidence of carcinogenic activity based on the malignant lymphomas. Dr. Zeise seconded the amendment which was defeated by two yes (McKnight, Zeise) to eight no votes. The original motion by Dr. Garman was then accepted unanimously with ten votes.

Triamterene. Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of triamterene by discussing the use and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. The conclusions were that:

> Under the conditions of these 2-year feed studies, there was equivocal evidence of carcinogenic activity for triamterene in male F344 rats based on a marginal increase in the incidence of hepatocellular adenomas. There was no evidence of carcinogenic activity for triamterene in female rats administered 150, 300, or 600 ppm. There was some evidence of carcinogenic activity for triamterene in male B6C3F1 mice based on a marginal increase in the incidence of hepatocellular carcinomas in the first study and a significantly increased incidence of hepatocellular adenomas in the second study. There was some evidence of carcinogenic activity for triamterene in female B6C3F1 mice based on significantly increased incidences of hepatocellular adenomas and combined incidences of adenomas and carcinomas of the liver.
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> The ingestion of diets containing triamterene was also associated with an increase in the incidence of hepatocellular foci, primarily mixed cell type, and an increase in the severity of nephropathy in rats. In mice, the ingestion of diets containing triamterene was also associated with increased incidences of hepatocellular foci in females and increased incidences of thyroid gland follicular cell hyperplasia in males and females.

Dr. Carlson, a principal reviewer, agreed with the conclusions. He suggested that there should be more relevant toxicology data on triamterene that could be added to the introduction, mainly to help focus on possible target organs such as the liver and kidneys. Dr. Dunnick said this would be done but noted that many of the toxicity studies performed in industry had not been reported in the literature.

Dr. Davidson, the second principal reviewer, agreed with the conclusions. Her agreement for the conclusions in male mice was based primarily on the results from the second study. She suggested that combined incidences of hepatocellular adenomas and carcinomas be added to the statement for both studies in male mice. She stated that the dosing error in the high-dose mice in the two year study was of concern. Dr. Dunnick said the staff considered that both studies supported the conclusion of some evidence in male mice.

Dr. Hayden, the third principal reviewer, agreed with the conclusions. He said there should be some discussion of thyroid changes in dosed male mice since theyare statistically significant and dose-related. Dr. Dunnick said severity grades would be added to the table and more discussion of thyroid follicular cell hyperplasia in treated mice would be included.

Dr. Goodman commented that in view of triamterene being a drug that has been on the

market for 30 years, more information should be included about human toxicity placing it in context with the rodent toxicity data. Dr. Hayden and Dr. McKnight suggested that rats in the 2-year studies might have tolerated higher doses in that mean body weight of dosed rats was within 5 % of controls and terminal survival rates for control and dosed groups were similar. Dr. Dunnick agreed to add a comment to this effect.

Dr. Carlson moved that the Technical Report on triamterene be accepted with the revisions discussed and with the conclusions as written for male rats, equivocal evidence of carcinogenic activity, for female rats, no evidence of carcinogenic activity, and for male and female mice, some evidence of carcinogenic activity. Dr. Bailey seconded the motion. Dr. Goodman offered an amendment that the conclusion in male mice be changed to equivocal evidence of carcinogenic activity. The amendment was tabled for lack of a second. Dr. Davidson offered an amendment that 'adenomas and carcinomas (combined)' be added to the conclusionary statement for male mice. The amendment was tabled for lack of a second. Dr. Carlson's motion was then accepted by eight yes to one no vote (Goodman) with one abstention (van Zwieten) for reason of company affiliation.

SHORT-TERM TOXICITY STUDIES

Diethanolamine. Dr. R. L. Melnick, NIEHS, introduced the short-term toxicity studies of diethanolamine by reviewing the uses, experimental design, and results. Two and 13-week toxicity studies of diethanolamine were conducted in male and female F344/N rats and B6C3F1 mice by oral (drinking water) and dermal administration. In the drinking water studies, concentrations given ranged from 160 to 10,000 ppm in rats and 630 to 10,000 ppm in mice. Toxicologic effects in rats included a poorly regenerative microcytic anemia, renal changes (increased kidney weights, renal tubular necrosis, decreased renal function, nephropathy and mineralization), degeneration of seminiferous tubules of the testis, and demyelination in the brain and spinal cord. In mice, toxicologic effects included cytological alterations and hepatocellular necrosis in the liver, nephropathy and renal tubular necrosis, degeneration of cardiac myocytes, and cytological alterations in the submandibular salivary gland.

In dermal studies, doses of diethanolamine ranged from 32 to 2,000 mg/kg in rats and 80 to 2,500 mg/kg in mice. Rats exhibited dose dependent changes in hematologic parameters and kidney and brain lesions similar to those observed in drinking water studies. Additionally, there were ulcerative skin lesions, inflammation, hyperkeratosis, and acanthosis at the application site. Mice exhibited similar skin lesions as rats, as well as cytologic alterations in the liver, renal tubular necrosis, and cardiac myocyte degeneration. Thus, the chemical was toxic at multiple sites in rats and mice by either route. Target organs identified included bone marrow, kidney, brain, testis, and skin in rats, and liver, kidney, heart, salivary gland, and skin in mice. A no-observed-adverse-effect-level (NOAEL) was not achieved for hematologic changes or nephropathy in rats, or for cytologic alterations of the liver in mice in the drinking water studies. In the dermal studies, a NOAEL was not achieved for hematological changes, nephropathy, or hyperkeratosis of the skin in rats, or for cytologic alterations of the liver or acanthosis of the skin in mice.

Dr. Carlson, a principal reviewer, said this was a well written report. He asked that all doses used be given in the Abstract. Dr. Melnick said they would. Dr. Carlson questioned the statement that a NOAEL was not achieved for female mice in the dermal studies, commenting that 80 mg/kg appeared to be a NOAEL based on lack of cytologic alteration of the liver. Dr. Melnick agreed but pointed out that 80 mg/kg was not a NOAEL for dermal lesions. Dr. Carlson thought that while perhaps statistically correct, it seemed to be stretching a point to say that a NOAEL was not observed in the rat based on hematologic studies when the change was one percent or less. Dr. Melnick noted that these differences at the lowest dose level were part of a clear dose-response.

Dr. Garman, a second principal reviewer, agreed that this was a very well written, thorough, and well documented report. He noted that although there were neurologic signs in rats on the two-week water studies, neuropathologic changes were not noted until 13 weeks and wondered if additional stains might be warranted to be sure the clinical signs were not

indicative of early neuropathologic changes. Dr. J. Mahler, NIEHS, responded that the brain sections from the two-week studies were reviewed with particular attention to the same areas that were affected in the 13-week studies and there were no lesions observed. Dr. Garman asked that more precise neuroanatomic locations be stated in photomicrographs of brain lesions.

Dr. Bailey commented that there had been extensive long-time use of diethanolamine and related amines in industry, primarily in formulations, and corresponding subchronic testing, and he was unaware of findings of neurologic and testicular toxicity. He suggested that the NTP might want to solicit information on these studies, many of which would be unpublished. Dr. J. Bucher, NIEHS, commented that an announcement seeking information about dicthanolamine had already appeared in the Federal Register.

N,N-Dimethylformamide. Mr. D. Lynch, NIOSH, introduced the short-term toxicity studies of N,N-dimethylformamide (DMF) by reviewing the uses and rationale for study, experimental design, and results. Thirteen-week toxicity studies were conducted in male and female F344/N rats and B6C3F1 mice by exposing them to DMF vapors at concentrations of 0, 50, 100, 200, 400 or 800 ppm in whole body inhalation studies. In rats, no mortality was seen. Serum liver enzymes were increased as early as day four, relative liver weights were increased in both sexes, there was minimal to moderate hepatocellular necrosis in both sexes, and prolonged diestrus in females exposed to the highest concentration. No DMF related mortality was seen in mice. Relative liver weights were increased in both sexes at all exposure concentrations. Centrilobular hepatocellular hypertrophy, minimal to mild, was found in all groups of male mice, and in female mice exposed at 100 ppm and higher. Thus, under the conditions of this study, DMF related effects were seen primarily in the liver of both species with rats being more severely affected. For rats of both sexes, the no-observed-adverse-effect level (NOAEL) was 200 ppm, based on the absence of liver histopathology. For mice, a NOAEL could not be determined.

Mr. Beliczky, a principal reviewer, said the report was well written. He noted that extra groups of rats were included for special studies of cardiovascular function and renal function as well as clinical pathology and wondered why this was not done for mice. Mr. Lynch replied that the larger body size and base of experience for these studies in rats were the primary reasons for doing the studies in rats while cost was probably a reason for not doing them in mice. Mr. Beliczky asked whether the study had been initiated because of increased incidences of testicular cancers among aircraft maintenance workers and leather tanners. Mr. Lynch said that was certainly one of the rationales. Mr. Beliczky reported that he had heard that DuPont was conducting a 2-year bioassay and asked whether results were available. Mr. Lynch affirmed this and noted that the bioassay consisted of a 24-month study in rats and an 18-month study in mice, the inlife phase would be completed in December, and a report would be available in 1992.

Dr. Bailey, a second principal reviewer, said this was a good report and the data presented supported the conclusions drawn. He commented that a recent report in the literature indicated that the metabolite AMCC (believed to be in the pathway leading to electrophilic products) was a minor metabolite in rodents but of primary importance in humans. Thus, the risk of toxicity from exposure toDMF would appear to be higher in humans than in rodents. Mr. Lynch said they had not been aware of that reference and would add it.

Dr. Carlson suggested that some of the actual data from the cardiovascular studies be included in the report. Dr. Klaassen noted the increased serum cholesterol levels and wondered whether cholesterol was routinely measured. Dr. M. Thompson, NIEHS, said it wasn't but when measured seemed to be a fairly sensitive indicator of hepatocellular function, and in the current study the increased cholesterol levels would be consistent with the hepatotoxicity observed.

2-Hydroxy-4-Methoxybenzophenone. Dr. J.E. French, NIEHS, introduced the short-term toxicity studies of 2-hydroxy-4-methoxybenzophenone (HMB) by reviewing the natural occurrences and uses of HMB, experimental design, and results. Review of unpublished proprietary information as well as FDA files led to the decision to use a sunscreen lotion base as a dose vehicle and to use both oral and dermal routes of exposure. Additionally, the liver, kidney and male and female reproductive organs were identified as target organs; only the kidney had been indicated in published literature. Two and 13-week toxicity studies of HMB were conducted in male and female F344/N rats and B6C3F1 mice by both dermal and dosed feed routes of exposure. In two and 13-week feed studies in both rats and mice, doses ranged between 3,125 and 50,000 ppm. In rats, liver and kidney weights were increased in both studies, while in 2-week studies, enlarged livers with a marked cytoplasmic vacuolization and renal lesions, consisting of dilated tubules and regeneration of tubular epithelial cells, were found. In 13-week studies, kidney lesions progressed to include papillary degeneration and inflammation, while liver lesions appeared to regress although liver function remained impaired. At the highest dose, epididymal sperm density was lower in males and length of the estrous cycle was increased in females. In 2-week dermal studies in rats, doses were 1.25 to 20 mg/HMB with the only effects being small and variable increases in liver and kidney weights. In 13-week studies, doses ranged from 12.5 to 200 mg/kg/HMB with kidney weights elevated in female rats.

In 2-week feed studies in mice, the only finding was a dose-related increase in liver weight associated with hepatocyte cytoplasmic vacuolization. In 13-week studies, decreased body weights and mild increases in liver weights were seen in both sexes, kidney weights were increased variably in females and there was an increase in estrous cycle length at the highest dose, and at the highest dose in males, there was an increase in epididymal sperm density and microscopic kidney lesions. In 2-week dermal studies, mice received 0.5 to l8 mg/HMB with the only effects noted being minimal, variable increases in liver and kidney weights. In 13-week studies, doses ranged from 22.75 to 364 mg/kg. In males, kidney weights were increased variably while epididymal sperm density was decreased at all dose levels evaluated. In summary, HMB produced generally similar effects following oral and dermal exposure of rats and mice. A no-observed-adverse-effect level (NOAEL) for microscopic lesions was 6,250 ppm HMB in the diet for rats and mice. A NOAEL was not reached for decreased epididymal sperm density in the 13-week dermal study in mice.

Dr. Carlson, a principal reviewer, said this report was really consisted of eight studies and a pretty good job was done of handling a lot of data. He suggested that "topical application" would be more common and correct than "dermal application". Dr. French said the Program historically used "dermal" as specific to skin while "topical" could be other sites, e.g., the eye. Dr. Carlson emphasized that liver function was not determined, and that the enzyme changes determined in these studies were measures of tissue damage.

Dr. Goodman, a second principal reviewer, said the report was well written and the results clearly presented. He asked that a clearer rationale be given as to why the study was

performed in view of the mention in the report that both an FDA Panel and a Cosmetic Ingredient Review Panel concluded that HMB was safe with regard to its current uses. Dr. French responded that HMB was selected from a review of the ether chemical class study and was nominated, primarily, on the basis of human exposure and as a representative benzophenone derivative used as a UV screen and UV stabilizer. Dr. Goodman said it would be useful to indicate how the doses employed and how those that produced toxicity compare with the dose one might anticipate from the "safe" human use of HMB. Dr. French said this would be difficult to do; however, at least in 2-week dermal studies, a lotion vehicle was used at concentrations which represented the maximum amount to be applied in a sunscreen lotion to human skin.

Dr. Carlson commented that there appeared to be too much emphasis placed on lack of a NOAEL for decreased epididymal sperm density in the 13-week dermal study in mice as the effects were clearly not substantiated by the higher doses in the feed study. Dr. Richard Davis, American Cyanamid, said that it might be a matter of consistency noting that for the four studies being reviewed there was a threefold range for control groups alone in sperm density. He suggested adding other measures of male reproductive function such spermatid counts. Dr. B. Schwetz, NIEHS, reported that spermatid head counts were now being collected as a reflection of the activity of the spermatogenesis process.

o-,m-,and p-Nitrotoluenes. Dr. J.K. Dunnick, NIEHS, introduced the short-term toxicity studies of o-,m-,and p-nitrotoluenes by reviewing the uses and rationale for study, the experimental design, and the results. Two and 13-week toxicity studies were conducted by the dosed feed route in male and female F344/N rats and B6C3F1 mice. In 2-week studies, doses to rats and mice ranged from 388 to 20,000 ppm. There were no treatment-related effects although animals showed decreases in body weight at higher doses. In 13-week studies, doses of each isomer to rats and mice ranged from 625 to 10,000 ppm. Histopathologic analysis of rat tissues showed target organ toxicity to the kidney, spleen, and testis from all isomers. Kidney toxicity in males was characterized by the presence of hyaline droplets. There was also hepatic toxicity in male rats receiving the o-isomer characterized by cytoplasmic vacuolization, oval cell hyperplasia, and elevations in serum enzyme levels. Evidence of treatment related liver damage from m- and p-isomers in males and females and also from the o-isomer in females was observed by increases in relative liver weights and elevations in serum bile acids and liver enzymes. There was a mild increase in hematopoiesis and congestion of the spleen in both sexes, and impairment of testicular function and increases in the length of the estrous cycle from all three isomers.

In mice, the only treatment related toxic lesion was observed in the olfactory epithelium of animals dosed with the o-isomer characterized by degeneration and metaplasia. In the 13-week studies, a carcinogenic response to o-nitrotoluene was demonstrated in male rats. Mesotheliomas of the tunica vaginalis were observed in three of ten rats at 5,000 ppm and mesothelial cell hyperplasia was observed in two of ten male rats receiving 10,000 ppm.

Dr. Goodman, a principal reviewer, said the report was well written and the results clearly presented. He stated that the rationale behind the use of each of the genetic toxicology tests employed should be presented and there should be some discussion regarding results. He suggested that a specific subsection of the Discussion could be devoted to genetic toxicology. Dr. Dunnick reported that in collaboration with Dr.E. Zeiger, NIEHS, the genetic toxicology section would be upgraded and expanded.

Dr. Davidson, a second principal reviewer, said the report did a good job of presenting background information and summarizing the results. She commented that although the degree of toxicity of the o-isomer is compared with the other two isomers, the meta and para isomers are not compared with each other regarding relative toxicity. Dr. Dunnick agreed that such a comparison should be added to the Abstract. Dr. Davidson said that considering that the main uses of nitrotoluenes are in the agricultural, rubber and dye industries, how are occupational groups (machine operators, welders and cutters, etc.) exposed. Dr. Janet Haartz, NIOSH, said the only isomer for which occupational data is available is the para. There were no listings for the meta and ortho isomers.