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

June 23-24, 1992

Research Triangle Park, North Carolina

The review meeting began at 8:30 a.m. on June 23 and 24 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, Jay Goodman, David Hayden, Daniel Longnecker, Barbara McKnight, Ellen Silbergeld, Matthew van Zwieten, Lauren Zeise, and Mr. Louis Beliczky. Dr. Carlson served as Chairperson. Dr. McKnight and Mr. Beliczky were unable to attend but submitted written reviews which were read into the record and discussed. Drs. Klaassen and Longnecker were unable to attend the meeting. These minutes have been reviewed and approved by all members of the Subcommittee who participated. 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. Subsequently, they may be purchased from the National Technical Information Service, U. S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, 703/487-4650.

The next NTP technical reports peer review meeting will be held December 1-2, 1992, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, 919/541-3971.

CONTENTS

SUMMARY MINUTES
TECHNICAL REPORTS REVIEW SUBCOMMITTEE MEETING
June 23-24, 1992

Coumarin. Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of coumarin by discussing the uses and rationale for study, describing the experimental design including additional 2-year stop studies in male rats, reporting on survival and body weight effects, and commenting on compound-related neoplastic and non-neoplastic lesions in rats and mice. She noted that additional step-sections of the kidney were performed in male and female rats. The conclusions were that:

> Under conditions of these 2-year gavage studies there was some evidence of carcinogenic activity of coumarin in male F344/N rats based on increased incidences of renal tubule adenomas. There was equivocal evidence of carcinogenic activity of coumarin in female F344/N rats based on the occurrence of renal tubule adenomas. There was some evidence of carcinogenic activity of coumarin in male B6C3F1 mice based on the increased incidence of alveolar/bronchiolar adenomas. There was clear evidence of carcinogenic activity of coumarin in female B6C3F1 mice based on increased incidences of alveolar/bronchiolar adenomas, alveolar/bronchiolar carcinomas, and hepatocellular adenomas. The marginally increased incidences of squamous cell papillomas of the forestomach in male and female mice receiving 50 mg/kg may have been related to coumarin administration.

The administration of coumarin to rats was also associated with an increased severity of nephropathy in the kidney and of bile duct hyperplasia in the liver, and increased incidences of ulcers of the forestomach, and necrosis, fibrosis, and cytologic alteration of the liver. Administration of coumarin to mice was also associated with centrilobular hypertrophy, syncytial alteration, and eosinophilic focus in the liver.

Dr. Bailey, a principal reviewer, agreed with the conclusions. He said it would be useful to have pharmacokinetic or metabolism data on the fate of coumarin in the Fischer rat and B6C3F1 mouse. Dr. Bailey said he would also like to see included in the report a discussion on the relevancy of these carcinogenicity studies to humans since there are data indicating that humans metabolize coumarin differently than rodents. Dr. Dunnick said the available data on human and rodent metabolism of coumarin had been cited in the Introduction but also would be integrated into the Discussion. Dr. Bailey reported that Rhone Poulenc, Inc. has evaluated the carcinogenic potential of coumarin in CD-1 mice and Sprague Dawley rats, and suggested that the data be obtained and included after NTP review. Dr. Dunnick responded that the FDA has some of this information under review and if available it will be cited in the report.

Dr. Hayden, the second principal reviewer, agreed with the conclusions. He inquired as to the rationale for using male rats only in stop exposure studies rather than males and

females. Dr. Dunnick said the liver lesions reported in the literature were in male rats. Dr. Hayden requested an explanation for such a high incidence of bile duct hyperplasia in the absence of fibrosis in male rat vehicle controls. Dr. Eustis commented that mild bile duct hyperplasia is a common spontaneous degenerative lesion of aging rats. Dr. Hayden said inclusion of a table comparing toxic and carcinogenic effects of coumarin in rats and mice with those reported for the gavage study of 3,4-dihydrocoumarin would be useful.

Dr. Davidson, the third principal reviewer, agreed with the conclusions for male rats and male and female mice but disagreed with the conclusion for female rats. She thought it should be no evidence of carcinogenic activity based on the low incidences of both hyperplasia and renal tubule adenomas in dose groups. Dr. Dunnick said that although the incidence of two adenomas in the high-dose group was not statistically significant from control, these are rare tumors and the rate was above the historical range so an association could not be ruled out. Dr. Davidson inquired as to the rationale for conducting the stop-exposure studies in rats, i.e., was there a relationship between progression and regression of liver lesions and the production of cholangiofibrosis or bile duct carcinomas? Dr. S. Eustis, NIEHS, said the primary purpose was to study the biological behavior of cholangiofibrosis, whether these lesions progressed to cholangiocarcinomas. These lesions were reported in German studies but unfortunately not induced in our studies. He noted that in the NTP studies on furan, stop studies had shown that these lesions progress to carcinomas.

Dr. Bailey moved that the Technical Report on coumarin be accepted with the revisions discussed and with the conclusions as written for male rats and mice, some evidence of carcinogenic activity, for female rats, equivocal evidence of carcinogenic activity, and for female mice, clear evidence of carcinogenic activity. Dr. Hayden seconded the motion. Dr. Davidson offered an amendment that the level of evidence for female rats be changed to no evidence of carcinogenic activity. Dr. Goodman seconded the amendment, which was defeated by two yes (Davidson, Goodman) to six no votes. The original motion by Dr. Bailey was then accepted unanimously with eight votes.

2,3-Dibromo-1-Propanol. Dr. K.M. Abdo, NIEHS, introduced the toxicology and carcinogenesis studies of 2,3-dibromo-1-propanol by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and non-neoplastic lesions in rats and mice. The conclusions were that:

> Under the conditions of these long-term dermal studies, there was clear evidence of carcinogenic activity of 2,3-dibromo-1-propanol in male F344/N rats based on increased incidences of neoplasms of the skin, nose, oral mucosa, esophagus, forestomach, small and large intestine, Zymbal's gland, liver, kidney, tunica vaginalis, and spleen. There was clear evidence of carcinogenic activity of 2,3-dibromo-1-propanol in female F344/N rats based on increased incidences of neoplasms of the skin, nose, oral mucosa, esophagus, forestomach, small and large intestine, Zymbal's gland, kidney, clitoral gland, and mammary gland. There was clear evidence of carcinogenic activity of 2,3-dibromo-1-propanol in male B6C3F1 mice based on increased incidences of neoplasms of the skin, forestomach, liver, and lung. There was clear evidence of carcinogenic activity of 2,3-dibromo-1-propanol in female B6C3F1 mice based on increased incidences of neoplasms of the skin and the forestomach. The increased incidences of alveolar/bronchiolar adenomas in female mice may have been related to chemical administration.
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> In rats, 2,3-dibromo-1-propanol caused increased incidences of hyperkeratosis in the skin, forestomach, and esophagus, epithelial dysplasia in the nose, pleomorphism and basophilic and clear cell changes in the liver, and nuclear enlargement in the kidney. There were also chemical-related increases in the incidences of forestomach ulcers and acanthosis, angiectasis in the liver, and renal hyperplasia in male rats and epithelial dysplasia of the forestomach and bile duct hyperplasia in the liver in female rats. Chemical-related increases occurred in the incidences of hyperplasia in the skin, epithelial dysplasia of the forestomach, and bronchiolar epithelial pleomorphism and hyperplasia in male and female mice and in the incidence of eosinophilic cytoplasmic change in the liver in males.

Dr. Zeise, a principal reviewer, agreed with the conclusions. She wondered if information including statistics could be given on tumor sites that might have been of borderline significance that weren't discussed. Dr. J. Haseman, NIEHS, responded that because the study was terminated early there were few tumors occurring spontaneously. However, all tumors that occurred in sufficient numbers for meaningful analysis could be included in a table along with P values. Dr. Zeise said she would like to see an indication in the study rationale as to why the dermal route was selected. Dr. Abdo said the most common routes of human exposure were dermal and, to a lesser extent, inhalation. Because Mr. Beliczky, the second principal reviewer, was unable to attend the meeting, Dr. L. Hart, NIEHS, read his review into the record. Mr. Beliczky agreed

with the conclusions. He noted the early termination of the chronic studies because of the presence of antibodies against lymphocytic chorio meningitis virus (LCM) in sentinel animals. Since the LCM virus also puts humans at risk, this action verifies the usefulness of the Sentinel Animal Program and the priority NTP places on the safety of laboratory personnel. Mr. Beliczky stated that since some carcinogenicity data on the chemical has been available since 1983, there should have been efforts by NTP and other Federal agencies to notify the public, industry and workers.

Dr. Hayden asked whether it was usual NTP policy to terminate a long-term study when sentinel animals were diagnosed to be serologically positive for a potential human pathogen. Dr. S. Eustis, NIEHS, said this was the first such instance in his experience at NIEHS; however, in any future situation where there was a viral disease present that could be a hazard to humans the same action would be taken.

Dr. Zeise moved that the Technical Report on 2,3-dibromo-1-propanol be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, clear evidence of carcinogenic activity. Dr. Davis seconded the motion, which was accepted unanimously with seven votes. Dr. Silbergeld was not present.

3,4-Dihydrocoumarin. Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of 3,4-dihydrocoumarin by discussing the uses and rationale for study, the experimental design including additional 2-year stop studies in rats, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. She noted that additional step-sections of the kidney were performed in male and female rats and male mice. The conclusions were that:

> Under the conditions of these 2-year gavage studies, there was some evidence of carcinogenic activity of 3,4-dihydrocoumarin in male F344/N rats based on increased incidences of renal tubule adenomas and focal hyperplasia. The transitional cell carcinomas in two high-dose males may also be chemical related. There was no evidence of carcinogenic activity of 3,4-dihydrocoumarin in female F344/N rats receiving 150, 300, or 600 mg/kg. There was no evidence of carcinogenic activity of 3,4-dihydrocoumarin in male B6C3F1 mice receiving 200, 400, or 800 mg/kg. There was some evidence of carcinogenic activity in female B6C3F1 mice based on increased incidences of hepatocellular adenomas.
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> 3,4-Dihydrocoumarin caused ulcers, hyperplasia, and inflammation of the forestomach, parathyroid gland hyperplasia, and increased severity of nephropathy in male rats.

Dr. Dunnick presented a brief comparison of the toxic effects of coumarin and 3,4-dihydrocoumarin in 2-year studies: (1) Coumarin and 3,4-dihydrocoumarin caused increases in nephropathy and treatment-related kidney neoplasms in male rats; (2) coumarin, but not 3,4-dihydrocoumarin, caused liver toxicity in rats and mice; (3) coumarin and dihydrocoumarin caused treatment-related hepatocellular neoplasms in female mice; and (4) significant treatment-related lung lesions were seen only after coumarin treatment.

Dr. Davidson, a principal reviewer, agreed with the conclusions. She asked why the additional controls used for the stop-exposure study were not used in the terminal evaluation of the study. Dr. Dunnick said additional information would be added to clarify this.

Dr. Hayden, the second principal reviewer, agreed with the conclusions. He thought that mice may have tolerated higher doses. He asked if there was an explanation for the significant reduction in cholinesterase values in male and female rats. Dr. Dunnick said there was not an apparent biological explanation for these reductions.

Dr. Bailey, the third principal reviewer, agreed with the conclusions. He also thought that male and female mice may have tolerated higher doses. Dr. Dunnick said there was treatment-related mortality at 1600 mg/kg in the 13-week studies in mice but not at 800 mg/kg which was the basis for that being the high dose in 2-year studies.

Dr. Zeise noted the occurrence of rare tumors, hepatoblastomas in two male mice, and asked for comment. Dr. S. Eustis, NIEHS, explained that hepatoblastomas are hepatocellular carcinomas with a clonal proliferation of cells which are similar to embryonic hepatoblasts and should not be considered separately from the carcinomas.

Dr. Hayden moved that the Technical Report on 3,4-dihydrocoumarin be accepted with the revisions discussed and with the conclusions as written for male rats and female mice, some evidence of carcinogenic activity, and for female rats and male mice, no evidence of carcinogenic activity. Dr. Bailey seconded the motion, which was accepted unanimously with eight votes.

5,5-Diphenylhydantoin. Dr. R.S. Chhabra, NIEHS, introduced the toxicology and carcinogenesis studies of 5,5-diphenylhydantoin (DPH) by discussing the rationale for incorporating perinatal exposure into the study design. The study designs included conventional two-year exposure of adult animals, perinatal exposure only, and perinatal plus adult exposure. The studies were intended to compare and evaluate the potential values of perinatal exposures in assessing chemical carcinogenicity. Dr. Chhabra described the experimental design, reported on survival and body weight effects, and commented on neoplastic lesions in rats and mice. The conclusions were that:

> Adult-Only Exposure
> Under the conditions of these 2-year, adult-only, dietary exposure studies, there was some evidence of carcinogenic activity in male F344/N rats based on increased incidences of hepatocellular neoplasms. There was no evidence of carcinogenic activity of DPH in female F344/N rats given 240, 800, or 2,400 ppm. There was no evidence of carcinogenic activity of DPH in male B6C3F1 mice given 30, 100, or 300 ppm. There was clear evidence of carcinogenic activity of DPH in female B6C3F1 based on increased incidences of hepatocellular neoplasms.
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> Perinatal-Only Exposure
> Perinatal exposure alone (through dietary administration of 210 ppm DPH during the perinatal period) caused a marginal increase in the incidences of hepatocellular neoplasms in female B6C3F1 mice evaluated 2 years after cessation of exposure. In male and female F344/N rats, exposure to 630 ppm during the perinatal period did not influence the incidences of hepatocellular or other neoplasms. Similarly, exposure of male B6C3F1 mice to dietary levels of 210 ppm DPH during the perinatal period did not affect neoplasm incidences.
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> Combined Perinatal and Adult Exposure
> Combined perinatal and adult dietary exposure to DPH confirmed the findings of the increased incidences of hepatocellular neoplasms for adult-only exposures in male rats and female mice, although combined exposure did not enhance these neoplastic effects. However, in male mice, combined perinatal and adult exposure resulted in increased incidences of hepatocellular neoplasms (hepatocellular carcinomas and multiple adenomas) that were not seen when dietary exposure was limited to the adult exposure period only.

Dr. Goodman, a principal reviewer, agreed in principle with the conclusions. However, he proposed that the conclusion for male rats under "Adult-Only" be changed from some evidence to equivocal evidence of carcinogenic activity based on decreases in weight exceeding ten percent in high dose animals and the fact that the liver tumor incidence was within the historical control range. Dr. Chhabra responded that in the three perinatal studies done in the same laboratory and at the same time, there was only one

liver tumor observed in male rat controls (1/150). Further, of the four liver tumors at highest exposure levels in adult-only and five in combination exposure, four of the five adenomas were multiple adenomas, supporting the level of evidence chosen. Dr. Goodman suggested omitting from consideration of possible carcinogenicity of DPH certain treatment groups of female rats and mice in which the maximum tolerated dose (MTD) appears to have been exceeded. Dr. Goodman said the speculation about the possible role of arene oxide metabolite binding in the toxicity and carcinogenicity of DPH was appropriate in the Discussion but mention should be made of the negative genotoxicity results. Dr. Chhabra agreed to do this.

Dr. Hayden, the second principal reviewer, agreed with the conclusions. He noted that since DPH is commonly used clinically in combination with other anticonvulsants, such as phenobarbital, it might be of interest to see if such drug combinations enhance or alter the toxicity/carcinogenicity of DPH. Dr. Chhabra explained that since the primary rationale for the study was to evaluate the value of perinatal exposure in assessing chemical carcinogenicity and not DPH per se, the pure drug itself was preferred. Dr. Hayden asked that the rationale for selecting DPH for study be made more specific. Dr. Hayden suggested that the schematic diagram of the experimental design for the chronic studies used by Dr. Chhabra in his opening remarks be in the report. Dr. Chhabra agreed to include more discussion of the rationale and, also, to add the schematic of the design to the final report.

Because Dr. McKnight, the third principal reviewer, was unable to attend the meeting, Dr. L. Hart, NIEHS, read her review into the record. She agreed with the conclusions. Dr. McKnight thought that the experimental design did not make optimum use of the animals, and a better choice would have been to replace the low F1-low F0 group with a high F1-medium F0 combination. Dr. Chhabra said that the ideal design would have been 16 dose groups but for practical reasons only eight were used. Dr. McKnight said the statistical analyses for the combined perinatal and adult exposures should be presented in the Appendices. Dr. Haseman said that this would be done.

Dr. Silbergeld stated that this study failed to detect toxicity of a chemical that is known to be toxic to other systems; i.e., DPH is a known teratogen in humans and in rodents within the dose range used here. She suggested removing "Toxicology" from the title of the report. Dr. Chhabra replied that dose levels were chosen which would not have teratogenic effects as this could confound the assessment of carcinogenicity. Dr. J. Bucher, NIEHS, added that a complete necropsy was done on perinatally exposed animals at the end of two years and any malformations or defects would have been detected. Dr. Chhabra said that for the Perinatal-Only Exposure and Combined Perinatal and Adult Exposure it would be noted in the conclusions that no fetal toxicity or teratogenicity was observed under the conditions of these studies. Dr. Zeise inquired as to why the drug was administered in the feed rather than by gavage. Dr. Chhabra said that using feed allowed a maximum systemic exposure of the drug to animals and this mode of oral administration minimized the loss of animals that might have occurred

if the gavage route had been chosen.

Dr. Hayden moved that the Technical Report on 5,5-diphenylhydantoin be accepted with the conclusions as written for male and female rats and mice under the three combinations of Adult-Only Exposure, Perinatal-Only Exposure, and Combined Perinatal and Adult Exposure. Dr. Davis seconded the motion. Dr. Goodman offered an amendment that for Adult-Only Exposure, the conclusion for male rats be changed from some evidence to equivocal evidence of carcinogenic activity based on a trend test that was only marginally positive; tumor incidence within the historical range, and a decrease in weight gain exceeding ten percent. Dr. Silbergeld seconded the amendment. The amendment was accepted by six yes votes to one no vote (Zeise) with one abstention (van Zwieten) for reasons of company affiliation. Dr. Goodman then offered a second amendment: The MTD was deemed to have been exceeded in female rats in the 0:2,400 ppm and 630:2,400 ppm dose groups, and in female mice in the 0:600 ppm and 210:600 ppm dose groups based on an excessive (i.e., 20-43 %) decrease in body weight gain. Therefore, the carcinogenicity data obtained from these dose groups were not considered in this assessment of the carcinogenicity of 5,5-diphenylhydantoin. The amendment was tabled for lack of a second. Dr. Hayden's original motion as amended by Dr. Goodman was accepted by seven yes votes with one abstention (van Zwieten) for reasons of company affiliation.

Manganese (II) Sulfate Monohydrate. Mr. J.D. Cirvello, NIEHS, introduced the toxicology and carcinogenesis studies of manganese (II) sulfate monohydrate by discussing the occurrence, uses and rationale for study of manganese, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic lesions in mice and nonneoplastic lesions in rats and mice. The conclusions were that:

> Under the conditions of these 2-year feed studies, there was no evidence of carcinogenic activity of manganese (II) sulfate monohydrate in male and female F344/N rats given 1,500, 5,000, or 15,000 ppm. There was equivocal evidence of carcinogenic activity of manganese (II) sulfate monohydrate in male and female B6C3F1 mice, based on the marginally increased incidence of thyroid gland follicular cell adenomas and the significantly increased incidences of follicular cell hyperplasia.
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> The ingestion of diets containing manganese (II) sulfate monohydrate was associated with increased severity of nephropathy in male rats. Focal squamous hyperplasia of the forestomach in male and female mice and ulcers and inflammation of the forestomach in male mice were associated with ingestion of diets containing manganese (II) sulfate monohydrate.

Dr. van Zwieten, a principal reviewer, agreed with the conclusions. He suggested that photomicrographs illustrating some of the thyroid lesions seen in mice would be a useful addition to the report.

Dr. Zeise, the second principal reviewer, agreed in principle with the conclusions adding that it appeared that the maximum tolerated dose (MTD) was not reached for female rats and male mice. Dr. S. Eustis, NIEHS, said a statement would be added that female rats and male mice could have possibly tolerated slightly higher doses.

Dr. R. Griesemer, NIEHS, contrasted the lack of neurologic effects of manganese in rodents with characteristic neurotoxicity in humans by noting that effects found in humans are related to the neuromelanin-containing parts of the brain while rats and mice don't have neuromelanin. Dr. Silbergeld stated that since manganese is a known neurotoxin, the Program should have incorporated specific measures of neurobehavioral assessment into the experimental design. Dr. Hayden commented that the only indication of a carcinogenic effect in male rats was in pancreatic islet cells where the incidences of hyperplasia and adenomas were slightly higher in treated groups. Since manganese preferentially accumulates in tissues rich in mitochondria with islet cells among the richest, he thought there might be a correlation. Dr. Eustis said some discussion could be added. Dr. J. Haartz, NIOSH, asked whether it was known what the actual oxidation state of manganese was in the animal diet since manganese is rather easily oxidized and oxidation state plays a role in the carcinogenicity of certain metals. Dr. T. Goehl, NIEHS, said the oxidation state would be confirmed or corrected.

Dr. van Zwieten moved that the Technical Report on manganese (II) sulfate monohydrate be accepted with the revisions discussed and with the conclusions as written for male and female rats, no evidence of carcinogenic activity, and for male and female mice, equivocal evidence of carcinogenic activity. Dr. Zeise seconded the motion. Dr. Zeise then offered an amendment to add a statement that female rats and male mice might have tolerated higher dose levels. Dr. Bailey seconded the motion, which was defeated by two yes votes (Bailey, Zeise) to five no votes with one abstention (Silbergeld). Dr. Silbergeld said she abstained because the study was wholly inadequate to assess toxicity, so it was impossible to determine where one was overall on a dose/response. Dr. Silbergeld offered an amendment that the following sentence be added to the end of the second paragraph of the conclusions: "The study was inadequate to detect or assess any neurotoxicity that would have been expected to be associated with chronic manganese exposure." Dr. Davis seconded the motion, which was accepted by six yes to two no votes (Davidson, Goodman). After some discussion, Dr. Eustis agreed to review the literature and add information on neurotoxicity in rodents. The original motion by Dr. van Zwieten as amended by Dr. Silbergeld was accepted unanimously with eight votes.

Polybrominated Biphenyls (Firemaster FF-1). Dr. R.S. Chhabra, NIEHS, introduced the toxicology and carcinogenesis studies of polybrominated biphenyls (PBBs) by discussing the rationale for incorporating perinatal exposure into the study design. The study designs included conventional two-year exposure of adult animals, perinatal exposure only, and perinatal plus adult exposure. The studies were intended to compare and evaluate the potential values of perinatal exposures in assessing chemical carcinogenicity. Dr. Chhabra described the experimental design, reported on survival and body weight effects, and commented on neoplastic and non-neoplastic lesions in rats and mice. The conclusions were:

Adult-Only Exposure

Under the conditions of these 2-year, adult-only, dietary exposure studies, there was clear evidence of carcinogenic activity for polybrominated biphenyls (PBBs) in male and female F344/N rats and male and female B6C3F1 mice based on increased incidences of hepatocellular neoplasms.

Perinatal-Only Exposure

Perinatal exposure alone (through dietary administration of 10 ppm PBBs to the dams) had no effect on the incidences of neoplasms in female F344/N rats, but in male rats, perinatal exposure was associated with a marginally increased incidence of hepatocellular adenomas that may have been related to chemical administration. In male and female B6C3F1 mice, perinatal exposure to 30 ppm PBBs resulted in significantly increased incidences of hepatocellular neoplasms.

Combined Perinatal and Adult Exposure

Combined perinatal and adult dietary exposure to PBBs confirmed the findings of the adult-only exposures for the increased incidences of hepatocellular neoplasms in rats and mice. In male rats, there were no enhancing effects of combined perinatal and adult exposure. However, perinatal exposure enhanced the susceptibility of female rats receiving adult exposure of 10 or 30 ppm to the induction of liver neoplasms. For male and female rats, a combined analysis of the incidences of leukemia in the adult-only, perinatal-only, and combined perinatal and adult exposure groups revealed an apparent association between increasing incidences of mononuclear cell leukemia and exposure to PBBs. In male and female mice, it was not possible to adequately assess the enhancing effects of combined perinatal and adult exposure on hepatocellular tumors, because adult-only exposure to 10 or 30 ppm PBBs resulted in high incidences (84-98%) of liver neoplasms. However, with increased perinatal exposure, there were increases in the numbers of mice with hepatocellular carcinomas and in the numbers of mice with multiple hepatocellular adenomas, which suggests an enhancement of PBB-related hepatocellular carcinogenicity associated with perinatal exposure.

Dr. Goodman, a principal reviewer, agreed in principle with the conclusions. However, he thought that for male rats under "Perinatal-Only Exposure", the phrase "a marginally

increased incidence of" should be changed to "an equivocal increase in the incidence of" which better characterizes the questionable nature of the increase. Also, under the heading of "Combined Perinatal and Adult Exposure", he suggested that all reference to mononuclear cell leukemia be omitted. Dr. Chhabra noted that life table analyses of data from all eight experimental groups indicated that significant increases in incidences of leukemia were associated with increasing concentration levels of PBBs in adult rats. Dr. Goodman proposed omitting from consideration of possible carcinogenicity of PBBs certain treatment groups in which the maximum tolerated dose (MTD) appears to have been exceeded noting that this would not change the overall level of evidence. Dr. J. Haseman, NIEHS, commented that the reduced weight gain in these groups may have resulted from the hepatocellular carcinogenicity. Dr. M. Elwell, NIEHS, thought the high incidence of leukemia also could have been a contributing factor. Dr. Goodman said the argument that PBBs were genotoxic was weak and should be deleted. Dr. Chhabra agreed.

Dr. Zeise, the second principal reviewer, also agreed in principle with the conclusions, but said they should note that the power of the study in rats to distinguish the impact of perinatal exposure may have been compromised by the apparent inadvertent exposure of control rats to PBBs. This issue should be discussed and clarified. Dr. Chhabra said there was certainty that control animals did not receive PBBs in the diet and stated that it is common to find PBBs in control animal tissues because PBBs are a ubiquitous environmental contaminant. Dr. Zeise said the decreases in neoplastic and nonneoplastic lesions noted for mice were difficult to interpret without results of statistical comparisons which account for the poor survival of treated mice.

Because Dr. McKnight, the third principal reviewer, was unable to attend the meeting, Dr. L. Hart, NIEHS, read her review into the record. Dr. McKnight agreed in principle with the conclusions while asking whether increases in thyroid follicular cell adenomas should be mentioned as part of the effects for male mice under combined perinatal and adult exposure. Dr. Haseman said the presence of an adenoma in an untreated control male mouse resulted in lack of statistical significance. Dr. McKnight questioned the statement in the results that for female rats there was significantly increased incidence of liver neoplasms from perinatal exposure alone, and suggested that it would be better to say that there was an enhancing effect of perinatal dosing on the incidence of liver neoplasms in animals exposed as adults. Dr. McKnight said the statistical analyses for the combined perinatal and adult exposures should be presented in the Appendices. Dr. Haseman said that this would be done.

Dr. Silbergeld asked whether there had been contamination of the test mixture with brominated dibenzofurans, compounds for which there had been recent toxicity studies reported. Dr. T. Goehl, NIEHS, said a mass spectral analysis had been done for dibenzofurans. The fragmentation patterns would indicate the number of bromines but not their position on the furan ring. Dr. Silbergeld commented that some of the brominated biphenyls were known to induce anorexia, a property which might have

contributed to the reduced weight gain noted by Dr. Goodman.

Dr. Silbergeld moved that the Technical Report on polybrominated biphenyls be accepted with the conclusions as written for male and female rats and mice under the three combinations of Adult-Only Exposure, Perinatal-Only Exposure, and Combined Perinatal and Adult Exposure. Dr. Zeise seconded the motion. Dr. Goodman offered an amendment: "The MTD was deemed to have been exceeded in male and female rats in the 0:30 ppm and 10:30 ppm dose groups, based on an excessive (i.e., 20-29 %) decrease in body weight gain. Therefore, the carcinogenicity data obtained from the dose groups in question were not considered in this assessment of the carcinogenicity of the PBBs". The amendment was tabled for lack of a second. Dr. Silbergeld's motion was then accepted unanimously with eight votes.

Talc. Dr. K.M. Abdo, NIEHS, introduced the toxicology and carcinogenesis studies of talc by discussing the rationale for study, describing the experimental design, reporting on survival and body weight effects, describing effects on respiratory function, and commenting on compound-related neoplastic lesions in rats and non-neoplastic lesions in rats and mice. The conclusions were that:

> Under the conditions of these inhalation studies, there was some evidence of carcinogenic activity of talc in male F344/N rats based on an increased incidence of benign and malignant pheochromocytomas of the adrenal gland. There was clear evidence of carcinogenic activity of talc in female F344/N rats based on increased incidences of alveolar/bronchiolar adenomas and carcinomas of the lung and benign and malignant pheochromocytomas of the adrenal gland. There was no evidence of carcinogenic activity of talc in male or female B6C3F1 mice exposed to 6 or 18 mg/m^3.
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> The principal toxic lesions associated with inhalation exposure to talc in rats included chronic granulomatous inflammation, alveolar epithelial hyperplasia, squamous metaplasia and squamous cysts, and interstitial fibrosis of the lung. These lesions were accompanied by impaired pulmonary function characterized primarily by reduced lung volumes, reduced quasistatic lung compliance, reduced gas exchange efficiency, and nonuniform intrapulmonary gas distribution. In mice, inhalation exposure to talc produced chronic inflammation of the lung with the accumulation of alveolar macrophages.

Dr. van Zwieten, a principal reviewer, agreed with the conclusions. He said that if available, information should be added to the Introduction regarding particle sizes of talc to which humans are exposed during various industrial and cosmetic uses. This would allow a comparison with the aerosol particle size distribution of talc in the animal studies. Dr. Abdo said such information was not available but since the material used was cosmetic grade he assumed humans were exposed to similar particle sizes. Dr. van Zwieten stated that the section dealing with the histological description of pulmonary neoplasms in rats leaves the impression that uncertainty existed regarding diagnosis of hyperplasia and benign and malignant neoplasia and asked for clarification. Dr. S. Eustis, NIEHS, said the pathologists were confident of the lesions diagnosed as tumors but there was a small number of lesions of inflammatory or hyperplastic nature for which it was difficult to tell if they were early tumors.

Dr. Goodman, the second principal reviewer, said his initial position was to disagree with the conclusions. However, he would defer a recommendation pending discussion of whether or not the maximum tolerated dose (MTD) was exceeded in female rats and consideration of the data concerning the trend towards an increased incidence of spontaneous pheochromocytomas in rats. Dr. Eustis argued that in this particular study, the appearance of lung tumors together with impaired pulmonary function is relevant to what might occur in humans with dust overload. Thus, he said that even though the

MTD may have been exceeded, the study is valid. Dr. Goodman believed that lung tumors produced in female rats following exposure to talc might have been secondary to chronic toxicity. He noted that the recommended time-weighted average (TWA) human exposure level for talc containing no asbestos fibers is 2 mg/m^3 and thought that this dose should have been used in the current study. Dr. Abdo agreed.

Because Dr. McKnight, the third principal reviewer, was unable to attend the meeting, Dr. L. Hart, NIEHS, read her review into the record. Dr. McKnight agreed in principle with the conclusions with the exception that consideration should be given to raising the level of evidence in male rats to clear evidence, since one of the arguments for the level chosen, i.e., no supporting hyperplasia in the adrenal gland, was not warranted. Further, there was strong supporting evidence from the increases in malignant pheochromocytomas and benign and malignant pheochromocytomas combined among female rats. Dr. Eustis said because of the high incidence of bilateral pheochromocytomas there was not enough tissue present to find hyperplasias. When considering all the evidence including there being a preponderance of benign tumors in male rats, the level of evidence seemed appropriate. Dr. McKnight had commented that evidence from humans suggests that direct effects on the adrenal gland may be possible. Dr. Eustis said that although the possibility cannot be ruled out that talc may reach the adrenal gland, its lack of solubility in aqueous fluids and the way the substance is cleared by the lungs make it very unlikely there would be a direct effect on the adrenal. Dr. McKnight thought that a sentence should be added to the conclusions stating that male and female mice might have tolerated higher doses. Dr. Eustis noted that as reported in the conclusions, exposure to talc produced chronic inflammation of the lungs in mice which supported an MTD being reached.

Dr. Goodman asked if the conclusion for female rats could be worded "clear evidence of carcinogenic activity only under those circumstances in which there was an indication of chronic toxicity". Dr. Eustis replied that in the discussion the appearance of tumors is clearly placed in the context of the chronic toxicity. Dr. Silbergeld said she was increasingly concerned about a kind of rigid criterion whereby evidence of carcinogenicity is discounted if toxicity is present. Dr. Eustis commented that the degree of chronic disease, based on fibrosis and inflammation, was quite similar between male and female rats so it would be difficult to argue that the MTD was exceeded in one sex and not the other. Dr. J. Haseman, NIEHS, pointed out that after the levels of evidence there is a paragraph in the conclusion that delineates all toxic lesions associated with chemical exposure in the lung.

Dr. J. Haartz, NIOSH, asked that more details be given on the spatial distribution of the talc in the chambers, and analyses of contaminants such as metals from impurities in the compressed air used. During the public comment period, Dr. Carlson read from a letter from Dr. Frank Mirer, Health and Safety Department, United Auto Workers. Dr. Mirer said the dose selection should be considered in light of current enforceable Permissible Exposure Limits, which are 5 mg/m^3 respirable fraction and 15 mg/m^3 for

total dust. Thus, the low dose selected for this experiment is below the OSHA limit when time weighted averaging is considered. Dr. Mirer concluded that the studies in male rats and mice of both sexes should be considered inadequate for determination of carcinogenicity of talc.

Dr. Goodman moved that the conclusion be modified to state that in light of lung toxicity previously noted, the MTD was exceeded in female rats. Dr. Bailey seconded the motion which was defeated by two yes (Bailey, Goodman) to five no votes with one abstention (Silbergeld). Dr. Silbergeld abstained because she thought the motion as framed was not informative given the complexities known about the MTD for these types of compounds. Dr. van Zwieten moved that the Technical Report on talc be accepted with the revisions discussed and with the conclusions as written for male rats, some evidence of carcinogenic activity, for female rats, clear evidence of carcinogenic activity, and for male and female mice, no evidence of carcinogenic activity. Dr. Hayden seconded the motion. Dr. Goodman offered an amendment to insert a clause in the second sentence of the conclusions between "rats" and "based" as follows: ",under conditions in which there was evidence of chronic lung toxicity,". The amendment was tabled for lack of a second. Dr. Silbergeld offered an amendment to insert "these same doses of" between "to" and "talc" in the first sentence of the second paragraph of the conclusions. Dr. Zeise seconded the motion, which was accepted by six yes votes to two no votes (Davis, Goodman). Dr. Zeise offered an amendment that a sentence be added to the effect that mice may have been able to tolerate higher doses. The amendment was tabled for lack of a second. Dr. van Zwieten's original motion as amended by Dr. Silbergeld was then accepted by seven yes votes to one no vote (Goodman).

Tumeric Oleoresin (Curcumin). Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of turmeric oleoresin (major component - curcumin) 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 no evidence of carcinogenic activity of turmeric oleoresin in male F344/N rats administered 2,000, 10,000, or 50,000 ppm. There was equivocal evidence of carcinogenic activity of turmeric oleoresin in female rats based on increased incidence of clitoral gland adenomas. There was equivocal evidence of carcinogenic activity of turmeric oleoresin in male B6C3F1 mice based on a marginally increased incidence of hepatocellular adenomas at the 10,000 ppm dose, and the occurrence of carcinomas of the small intestine in the 2,000 and 10,000 ppm dose groups. There was equivocal evidence of carcinogenic activity of turmeric oleoresin in female B6C3F1 mice based on an increased incidence of hepatocellular adenomas in the 10,000 ppm dose group.
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> Turmeric oleoresin ingestion was also associated with increased incidences of ulcers, hyperplasia, and inflammation of the forestomach, cecum, and colon in male rats and of the cecum in female rats. In female mice ingestion of diets containing turmeric oleoresin was also associated with an increased incidence of thyroid gland follicular cell hyperplasia.

Dr. Davis, a principal reviewer, agreed in principle with the conclusions although the lack of toxicity seen at 13 weeks and minimal body weight changes present after two years indicate higher doses could have been tolerated in mice. Dr. Dunnick agreed while explaining that at the time the experiments were designed the Program used the rationale that non-nutrient materials should not exceed 5% of the diet; i.e., 50,000 ppm. Dr. Davis noted that turmeric oleoresin was used since pure curcumin was not available and wondered why. Further, since twenty one percent of the test material were compounds other than curcumin, he asked for comment on the biological activity of these compounds. Dr. Dunnick said that pure curcumin is simply not available while turmeric oleoresin has been used for centuries as a spice. There are no reports in the literature on the biological activities of the other components.

Dr. Silbergeld, the second principal reviewer, agreed with the conclusions in male and female rats but considered that the data supported raising the levels of evidence in male and female mice to some evidence of carcinogenic activity. Dr. Silbergeld said it would be useful to have more information on the comparative metabolism and disposition of turmeric which might help to explain a lack of dose-response as well as differing sites of toxicity/carcinogenicity among sexes and species. Dr. S. Eustis, NIEHS, said there was information over a range of gavage doses in Wistar rats that 60-65% of the dose was absorbed

Dr. Davis moved that the Technical Report on turmeric oleoresin be accepted with the revisions discussed and with the conclusions as written for male rats, no evidence of carcinogenic activity, and for female rats and male and female mice, equivocal evidence of carcinogenic activity. Dr. Goodman seconded the motion. Dr. Silbergeld offered an amendment that given the lack of effect of the highest dose used in mice on body weight, food consumption, and other parameters, it was not clear that the maximum tolerated dose was achieved. There was some discussion as to whether the decreased final weight gain in high-dose female mice (12%) was significant. Dr. J. Haseman, NIEHS, said he would do the statistical analysis for the final report. Dr. Davis seconded the amendment, which was defeated by two yes votes (Davis, Silbergeld) to five no votes with one abstention (Zeise). There was agreement by the staff that a statement could be added to the Discussion that it appeared that mice could have tolerated higher doses. Dr. Davis's original motion then was accepted unanimously with eight votes.

SHORT-TERM TOXICITY STUDIES

Glutaraldehyde. Dr. F. W. Kari, NIEHS, introduced the short-term toxicity studies of glutaraldehyde by reviewing the use and rationale for study, experimental design, and results. Two and 13-week toxicity studies were conducted in male and female F344/N rats and B6C3F1 mice by whole-body inhalation exposure. In 2-week studies, exposure concentrations ranged from 0 to 16 ppm with all rats and mice exposed to 5 or 16 ppm dying before the end of the studies, and all mice exposed to 1.6 ppm dying also. Deaths were attributed to severe respiratory distress with the greater sensitivity of mice due to more severe nasal occlusion apparently because smaller airways of the nasal passage in mice were more easily blocked by cell debris and keratin.

In 13-week studies, exposure concentrations ranged from 0 to 1000 ppb. There were no exposure-related deaths in rats but all mice exposed to 1000 ppb and two female mice exposed to 500 ppb died before study's end. There was no clear evidence of systemic toxicity in rats or mice by histopathologic or clinical pathologic assessments. In rats, lesions were most severe in the anterior portions of the nasal passages, and involved both the respiratory and olfactory epitheliums. In mice, histologic lesions consisted of minimal to mild squamous metaplasia of the laryngeal epithelium, suppurative inflammation in anterior parts of the nasal cavity, and minimal squamous metaplasia on the tips of the nasoturinates. The no-observed-adverse-effect level (NOAEL) was 125 ppb for respiratory lesions in rats. A NOAEL was not reached for mice, as inflammation was found in the anterior nasal passage at concentrations as low as 62.5 ppb. A collaborative effort was undertaken with scientists from the Chemical Industry Institute of Toxicology to characterize the acute and subchronic respiratory tract proliferative responses to glutaraldehyde. There was shown to be clear concentration-related increases in cell replication in all four sex-species combinations.

Dr. Bailey, a principal reviewer, said this was a well performed study. He questioned how there could be severe tissue damage in the anterior nares of high-dose (1000 ppb) mice at 13-weeks but no inflammation reported. Dr. M. Elwell, NIEHS, explained that with the damage, squamous exfoliation, inflammation was present, especially in the nasal vestibule, but as more of a diffuse inflammation and exudate in the lumen of the nasal cavity.

Dr. Silbergeld, a second principal reviewer, thought these were excellently conducted experiments and information in the report was clearly presented. However, she was disappointed that the opportunity was not taken to study effects on organ systems other than the respiratory tract, particularly the brain and peripheral nervous system and the immune system. She questioned the basis for the conclusion that changes in heart and liver weights were not biologically significant. Dr. J. Bucher, NIEHS, noted that small and variable changes, especially in organ weights are difficult to interpret. Dr. Silbergeld said a number of changes observed in immune system parameters were considered "secondary to generalized stress", and wondered whether the possibility had

been considered that glutaraldehyde, like formaldehyde, may have immunotoxic effects. Dr. M. Thompson, NIEHS, said decreases in lymphocytes in male rats were mild and not seen in females so not considered to be very significant findings. Dr. Silbergeld commented that toxic effects were seen at levels well below the OSHA PEL of 200 ppb.

Dr. Davidson asked why the exposure concentrations were so high in the 14-day studies considering that significant mortality was seen at lower doses. Dr. Kari replied that because of the reactivity of the chemical, literature values for LC50 and other measures of toxicity varied widely so a wide range of doses was necessary to be sure to see the spectrum of toxicity in our studies. Dr. J. Haartz, NIOSH, recommended that further details of the exposure system including configuration and components be added to the report for the use of other inhalation toxicologists. Dr. Kari agreed to add this information. Dr. Silbergeld and Dr. Hayden asked for inclusion of more discussion comparing the similarities and differences in toxicity between glutaraldehyde and formaldehyde.

1,6-Hexanediamine Dihydrochloride. Dr. C.D. Hebert, NIEHS, introduced the short-term toxicity studies of 1,6-hexanediamine dihydrochloride (HDDC) by reviewing the uses and rationale for study, experimental design, and results. Toxicity studies were conducted in male and female F344/N rats and B6C3F1 mice by the drinking water (2-week studies only) and whole body inhalation routes (2- and 13-week studies). In 2-week drinking water studies, all animals survived, and no changes in body weight, no gross microscopic pathological changes, and no clinical signs were observed. Consequently, 13-week drinking water studies were not done. In 2-week inhalation studies, animals were exposed to between 10 and 800 mg/cubic meter/day. At the highest dose, all rats and female mice died, as well as 40% of male mice. Clinical signs related to irritation of the upper respiratory tract of both species were seen only at 800 mg/m^3. Treatment-related histopathologic lesions at the three highest exposure levels in rats and the two highest exposure levels in mice included inflammation and necrosis of the laryngeal and tracheal epithelium, as well as focal inflammation and ulceration of both the respiratory and olfactory nasal mucosa.

In 13-week inhalation studies, animals were exposed to concentrations of HDDC ranging between 1.6 and 160 mg/m^3. With the exception of a slight increase in liver weights in male mice from the two highest exposure groups, no treatment-related changes in absolute or relative organ weights and in clinical signs or gross lesions were seen in either species. Microscopic lesions were limited to the upper respiratory tract in the two highest exposure groups, and were similar in rats and mice. These lesions included minimal to mild focal erosion/ulceration, inflammation, and hyperplasia of the laryngeal epithelium as well as degeneration of the olfactory and respiratory nasal epithelium. HHDC had no adverse effect on reproduction of either species and was not genotoxic. In the 13-week studies, the no-observed-adverse-effect-level (NOAEL) for respiratory damage was 5 mg/m^3 for rats and mice.

Dr. Silbergeld, a principal reviewer, said the test chemical is a derivative of the widely used high production volume chemical hexanediamine (HDA). Her concern with the study had to do with the decision to test HDDC rather than HDA. She said the rationale seemed to be based on producing a relatively stable compound that could be handled under test conditions; however, public health concerns relate to HDA. HDA is reportedly toxic to humans, and ingestion/inhalation studies have been conducted in rodents with HDA. Dr. Hebert said that we don't have information on the form HDA is in the environment or, more specifically, the airway epithelium but based on its chemical properties we would suspect it would be in or form the monochloride or dihydrochloride salt. Practically speaking, the parent compound, HDA, rapidly precipitates out on walls of inhalation exposure chambers.

Because Mr. Beliczky, the second principal reviewer, was unable to attend the meeting, Dr. L. Hart, NIEHS, read his review into the record. Mr. Beliczky had some of the same concerns about the use of HDDC rather than HDA and the form to which humans would be exposed. He thought that adequate toxicity data were available so the study

reestablished already existing information. Dr. Hebert commented that much of the data referred to were not available at the time the current studies were being designed and conducted. Mr. Beliczky felt the study did not provide adequate information to help decide the need for a chronic study. Dr. Hebert said that due to its low toxicity, other than irritant effects, there would be a low priority for chronic studies with HDDC.

Dr. J. Haartz, NIOSH, requested that more information be given in the report about inhalation aerosol generation and monitoring. Dr. Carlson seconded this request adding a request for characterization of the aerosol used.

Methyl Ethyl Ketone Peroxide. Dr. E. Zeiger, NIEHS, introduced the short-term toxicity studies of methyl ethyl ketone peroxide (45% solution in dimethyl phthalate) (MEKP) by reviewing the uses, experimental design, and results. Two-week and 13-week toxicity studies were conducted in male and female F344/N rats and B6C3F1 mice by topical application of MEKP in dimethyl phthalate. In 2-week studies, doses ranged from 51 to 810 mg/kg in rats and from 112 to 1800 mg/kg in mice. No rats died during the studies but several mice in each dose group died. Body weight gains of rats decreased with increasing doses, while body weight gains of mice were not treatment-related. Primary effects seen in both species were extensive coagulative necrosis of the epidermis and dermis, adnexa with variable degrees of inflammation, and epidermal regeneration at the application site. Effects considered secondary to dermal lesions included increased hematopoiesis in the spleen of rats and mice and increased myeloid hyperplasia of sternebral bone marrow in mice. Mice showed a marked, dose-related increase in liver weight.

In 13-week studies, rats received topical doses ranging from 1 to 107 mg/kg and mice doses ranging from 0.3 to 36 mg/kg. The top two dose groups of rats and mice were terminated early because of the severity of skin lesions induced. All animals in the remaining dose groups survived to the end of the study and weight gains were generally lower with increasing doses. Skin lesions at the site of application in the top dose groups of remaining animals included necrosis, inflammation, and hyperplasia. Lesions in lower dose groups were limited to acanthosis and hyperplasia in rats, and acanthosis in mice. Splenic and bone marrow effects similar to those seen in 2-week studies were seen in animals that showed ulcerative or necrotic injury, and were considered secondary responses. MEKP was not mutagenic in Salmonella, but a positive response was obtained in the mouse lymphoma test. Sister chromatid exchanges and chromosomal aberrations were induced in Chinese hamster ovary cells, but no increases in micronuclei were seen in mouse peripheral blood. A no-observed-adverse-effect level (NOAEL) for the skin lesions could not be determined for either species.

Dr. Davis, a principal reviewer, said this was a very well written and concise report. He asked for clarification on contradictory statements in the Abstract that say in one statement that in the 13-week studies all high-dose mice died while in the next sentence there is indication that some mice in the two highest dose groups were terminated early because of severe skin lesions. Dr. Zeiger said all animals in the high-dose group died and all surviving mice in the next highest dose were sacrificed during week 6 due to the early onset and severity of skin lesions. This would be corrected in the Abstract. Dr. Davis asked for discussion on what constitutes significant human exposure. Dr. Zeiger said there was clearly potential human exposure and because MEKP is one of the most highly reactive of the peroxides, it was considered a good candidate for study.

Dr. Hayden, a second principal reviewer, thought the study appeared to have been well done although the two highest doses selected in mice were excessive and this was alluded to in the report.

Dr. Silbergeld said some of these compounds have been demonstrated to be neurotoxic and wondered whether clinical observations of the animals would have been sufficient to rule out any behavioral toxicity. Dr. R. Griesemer, NIEHS, said the Program uses a three-tiered approach in neurotoxicity testing. In these studies, only the first tier; i.e., gross clinical exam and histologic examination of nervous tissue, would have been used.

Riddelliine. Dr. P.C. Chan, NIEHS, introduced the short-term toxicity studies of riddelliine by reviewing the rationale for study, experimental design, and results. Two-week and 13-week toxicity studies were conducted in male and female F344/N rats and B6C3F1 mice by administering riddelliine by oral gavage in phosphate buffer. In 2-week studies, where doses ranged from 0.33 to 25 mg/kg, four of five male rats in the highest dose group died before terminal sacrifice, while there were no deaths or body weight effects in female rats and mice. In 13-week studies, rats received doses up to 10 mg/kg while mice were given doses up to 25 mg/kg. After 13 weeks, half of the animals were sacrificed while the other half in each group were further divided into two groups with half observed during a seven-week recovery period and half observed during a 14-week recovery period before sacrifice of survivors. In rats, all animals except males in the highest dose group survived the 13-week treatment period. During the seven and 14-week recovery period, no male rats died while there was some mortality in the highest dose group of females. In rats, the most significant treatment-related histopathologic lesions occurred in the liver, and included hepatocyte cytomegaly and karyomegaly, cytoplasmic vacuolization, centrilobular necrosis, mixed inflammatory cell infiltration, and bile duct hyperplasia. Vascular lesions in kidney and lung were observed in most high-dose rats, and additional lesions were found in heart, spleen, kidney and pancreas. After the 14-week recovery period, hepatocyte karyomegaly, cytomegaly, and cytoplasmic vacuolization persisted. Bile duct hyperplasia was markedly increased in female rats, and foci of altered or hyperplastic hepatocytes were observed in rats allowed to recover for up to 14 weeks. Adenomas of the liver occurred in 2 of 10 females in the high-dose group at 13 weeks and in 1 of 5 females after 14 weeks recovery; no hepatic adenomas were found in controls.

In the 13-week study in mice, no deaths related to riddelliine treatment occurred. Centrilobular cytomegaly in the liver was noted at 13 weeks in high-dose male and female mice, and persisted in females through the 14-week recovery period. At the end of the 14-week recovery period, bile duct hyperplasia was seen in high-dose female mice. Some reproductive and developmental toxicity was observed in female rats and mice. Riddelliine produced genotoxic effects in most test systems studied. The spectrum of neoplastic and nonneoplastic effects produced were similar to those previously described for other pyrrolizidine alkaloids. The no-observed-adverse-effect level (NOAEL) for histopathologic changes in the 13-week studies was 3.3 mg/kg for mice and 0.1 mg/kg for rats.

Dr. Goodman, a principal reviewer, said this was a thorough series of studies and the report was clear, well written, and to the point. However, in light of what is already known about the toxicity of the pyrrolizidine alkaloids, he did not see an adequate rationale presented as to why the study was performed, i.e., why was riddelliine selected for study by the FDA. Dr. Chan reported that at the time the FDA was concerned about contamination of meat products, milk, honey and herbal teas, as it is the most common pyrrolizidine alkaloid found in range plants in the west. Also, Dr. Goodman said that in light of the fact that in both humans and animals lung as opposed

to liver lesions occur following exposure to these alkaloids should lower exposure levels have been used and for longer than 90-days. Dr. J. Bucher, NIEHS, agreed that lower doses for a longer time might have shown lung lesions. Some discussion concerning the lack of pulmonary effects could be included.

Dr. Davidson, a second principal reviewer, said the report was well written and the background information on the pyrrolizidine alkaloids was helpful. She asked why gavage was chosen as the route of administration rather than diet in view of the fact that the alkaloids contaminate human food sources. Dr. Chan said gavage was chosen because the supply of test chemical was limited as the FDA has to collect plants and extract the material.

Dr. Bailey said that although NOAELs are given for the test animals it would be useful to readers if information could be added on levels of riddelliine in various foods. Dr. W. Allaben, NCTR/FDA, said that kind of information could be provided.

Tetrachlorophthalic Anhydride. Dr. J. Mahler, NIEHS, introduced the short-term toxicity studies of tetrachlorophthalic anhydride (TCPA) by reviewing the use, experimental design, and results. Thirteen-week toxicity studies were conducted in male and female F344/N rats and B6C3F1 mice by administering TCPA by oral gavage (corn oil) at doses ranging from 94 to 1500 mg/kg. Compound-related effects occurred primarily in rats with deaths considered due to chemical toxicity seen in five males and one female at the highest dose. The kidney was the primary target organ with extensive and severe necrosis of renal tubules in animals dying prior to study termination. In rats surviving to study's end, there were dose-dependent increases in relative kidney weight in both sexes that were associated microscopically with tubular dilatation localized to the outer medulla. A no-observed-adverse-effect level for histologic lesions in the kidney was not reached. No adverse effects were seen in mice. Decreases in red blood cell parameters consistent with a mild, poorly regenerative anemia were the only evidence of possible chemical effect in this species.

Dr. van Zwieten, a principal reviewer, said the report was generally well-written and reflected the results obtained. He thought there needed to be more interpretation of some of the organ weight changes as to what they meant, especially increased heart weights in high-dose male rats and kidney weights in female rats. Dr. Mahler said the heart weight change was an increase in the relative weight and in the absence of any histologic lesion probably was a reflection of the marked body weight decrease in the high-dose group.

Dr. Davis, a second principal reviewer, commented that the downward trend in the use of TCPA, the limited clear-cut toxicity reported in the literature, and the fact that all significant human exposure occurs by inhalation led him to question why the chemical was tested, and then, why by the gavage route. With regard to the rationale for study, Dr. Mahler surmised the National Cancer Institute was interested in studying TCPA as a representative of the multi-ring anhydride class of chemicals supported by its structural similarity to carcinogenic aromatic halides. As to the exposure route, Dr. Mahler said a practical reason was that preliminary data from an inhalation study conducted by the manufacturer suggested that a systemic body burden could not be achieved by that route. Dr. J. Haartz, NIOSH, noted that the National Occupational Exposure Survey (NOES) only assesses potential exposure, and further, due to cost tends to focus on the larger companies making or using a chemical so that the actual numbers of people being exposed may be greater than indicated.