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
and Panel of Experts

on

March 11-12, 1991

Research Triangle Park, North Carolina

The review meeting began at 9:00 a.m. on March 11 in the Conference Center, Building 101, and on March 12 in the Main Conference Room, Building 18, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the Subcommittee are: Drs. Daniel Longnecker (Chairperson), Paul Bailey, Jay Goodman, and Ellen Silbergeld. Members of the Panel of Experts are: Drs. Gary Carlson, Harold Davis, Robert Garman, David Hayden, Curtis Klaassen, Barbara McKnight, Lauren Zeise, and Mr. Louis Beliczky. Dr. Silbergeld was unable to attend the meeting. These minutes have been reviewed and approved by all members of the Subcommittee and Panel 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 July 9-10, 1991, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, (919) 541-3971; FTS 629-3971.

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SUMMARY MINUTES

PEER REVIEW PANEL MEETING

March 11-12, 1991

C.I. Acid Red 114. Dr. J.K. Dunnick, NIEHS, NTP Staff Scientist, introduced the toxicology and carcinogenesis studies of C.I. Acid Red 114 by noting this was one of five chemicals being evaluated as part of the NTP Benzidine Dye Initiative, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related non-neoplastic and neoplastic lesions in male and female rats. The conclusions were that:

> Under the conditions of these 2-year drinking water studies, there was clear evidence of carcinogenic activity of C.I. Acid Red 114 for male F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland and liver. Increased incidences of neoplasms of the oral cavity epithelium, adrenal gland, and lung may have been related to chemical administration. There was clear evidence of carcinogenic activity for female F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, clitoral gland, liver, oral cavity epithelium, small and large intestine, and lung. Increased incidences of mammary gland and adrenal gland tumors may have been related to chemical administration.

Dr. Zeise, a principal reviewer, agreed in principle with the conclusions. However, she proposed that increased incidences of mononuclear cell leukemia and thyroid follicular cell neoplasms in female rats may have been related to chemical administration and should be cited as such in the conclusions.

Dr. McKnight, the second principal reviewer, agreed with the conclusions. She also thought that the increased incidences of mononuclear cell leukemia and thyroid follicular cell neoplasms in female rats may have been related to chemical administration. In response to Drs. Zeise and McKnight, Dr. Dunnick stated that there wasn't enough evidence to support even a marginal finding for thyroid tumors in that there was an increase only in the low-dose group, no increase by the trend test, and no increase in precursor hyperplastic lesions in dosed groups. With regard to mononuclear cell leukemia in female rats, Dr. Dunnick commented that incidences in dosed groups were within the historical control range and high and early mortality in the high dose group was felt to be due to toxicity of the chemical and not leukemia. Dr. McKnight pointed out that by the life table test, the test normally used for leukemia, the pairwise comparison of each of the dose groups with the control group is statistically significant, and the trend test is highly significant.

Dr. Davis, the third principal reviewer, agreed with the conclusions. He wondered why the doses in female rats were double those in males since hematologic data and data on kidney degeneration from the 13-week studies suggested females were more sensitive to toxic effects. Dr. Dunnick said apparent liver toxicity in males in the 13-week studies was the primary reason for the different dose levels used in 2-year studies.

Dr. Zeise moved that the Technical Report on C.I. Acid Red 114 be accepted with the revisions discussed and the conclusions as written for male and female rats, clear evidence of carcinogenic activity, and with the addition of mononuclear cell leukemia to the conclusion for female rats as 'may have been related to chemical administration'. Dr. McKnight seconded the motion, which was accepted unanimously with ten votes.

C.I. Pigment Red 23. Dr. K.M. Abdo, NIEHS, NTP Staff Scientist, introduced the toxicology and carcinogenesis studies of C.I. Pigment Red 23 by discussing the uses, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic lesions in male rats and non-neoplastic lesions in male and female rats and mice. The conclusions were that:

> Under the conditions of these 2-year studies, there was equivocal evidence of carcinogenic activity of C.I. Pigment Red 23 for male F344/N rats as evidenced by the marginally increased incidences of renal tubular cell adenomas or carcinomas. There was no evidence of carcinogenic activity of C.I. Pigment Red 23 for female F344/N rats fed diets containing 10,000, 25,000, or 50,000 ppm for up to 2 years. Mononuclear cell leukemia occurred with a decreased incidence in male and female rats receiving C.I. Pigment Red 23. There was no evidence of carcinogenic activity of C.I. Pigment Red 23 for male and female B6C3F1 mice fed diets containing 10,000, 25,000, or 50,000 ppm for up to 2 years.
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> C.I. Pigment Red 23 caused an increase in renal tubular cell hyperplasia in male rats. In mice, C.I. Pigment Red 23 caused an increase in hyperkeratosis and epithelial hyperplasia of the forestomach.

Because of the low numbers of renal neoplasms in male rats observed in this study, there was uncertainty as to whether these tumors were related to chemical exposure. For this reason, step sections from all kidneys from controls and high dose males were evaluated to further characterize the extent of these lesions. Step sections were also done on kidneys from female rats.

Dr. Bailey, a principal reviewer, agreed with the conclusions. He commented on the fact that lots of pigment from one company were used for the 14-day, 13-week, and initial part of the 2-year studies while lots from a different company were used for the rest of the 2-year studies. He said some of the impurities in one company's lot were not found in the other company's lot; these impurities needed to be better identified in the Appendix. Dr. Abdo said the reason for two suppliers was that the first company stopped making the pigment.

Dr. Zeise, the second principal reviewer, agreed in principle with the conclusions. However, she thought the level of evidence for male rats might need to be reconsidered after results of the analysis of step sections from the kidney are presented. Dr. J. Haseman, NIEHS, said the P values obtained after step sectioning were less significant than one might have expected because there were almost twice as many survivors in the high-dose group as in the control group. Also, Dr. Zeise said the finding of three high dose female rats with astrocytomas appears to provide evidence of carcinogenicity. This tumor appears to be uncommon and the incidence falls outside the range of laboratory and overall historical controls. Dr. Zeise said that if the finding cannot be rationalized then the level of evidence should be raised. Dr. G. Boorman, NIEHS, explained that although it appears as though the numbers of astrocytomas reported in this study were statistically significant, astrocytomas and other glial cell tumors are generally combined. The incidences of astrocytomas and other glial cell tumors combined in this study were not statistically significant. Dr. Zeise requested that the combined incidence of glial cell tumors and astrocytomas be given in the report.

Dr. Klaassen, the third principal reviewer, agreed with the conclusions although he felt more emphasis could have been given to the anti-carcinogenic effects of Red 23 in rats. He noted the marked decreased incidences of mononuclear cell leukemia and increased survival in male and female dosed groups compared with control groups. Dr. R. Griesemer, NIEHS, said that the NTP alerts the National Cancer Institute and passes information on to them when it is believed that a chemical has a direct effect in inhibiting cancer formation.

Dr. Zeise noted that as a matter of policy, NTP may choose to limit the top dose in dietary studies to 5% in the diet. However, she thought that the explanation given in the report that doses greater than 5% 'could have led to dietary deficiences as a result of excessive dilution of essential nutrients' overstated the case, and commented that dietary restriction studies indicated otherwise. She asked that the report indicate that animals may have been able to withstand higher doses based on lack of body weight, survival, and toxic effects.

Dr. Bailey moved that the Technical Report on C.I. Pigment Red 23 be accepted with the revisions discussed and the conclusions as written for male rats, equivocal evidence of carcinogenic activity, and for female rats and male and female mice, no evidence of carcinogenic activity. Dr. Zeise seconded the motion, which was accepted unanimously with nine votes. Dr. McKnight was not present for the vote.

2,4-Diaminophenol Dihydrochloride. Dr. R.D. Irwin, NIEHS, NTP Staff Scientist, introduced the the toxicology and carcinogenesis studies of 2,4-diaminophenol dihydrochloride 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 mice and rats. The conclusions were that:

> Under the conditions of these 2-year gavage studies, there was no evidence of carcinogenic activity in male or female F344/N rats that received 12.5 or 25 mg/kg 2,4-diaminophenol dihydrochloride. There was some evidence of carcinogenic activity in male B6C3F1 mice based on increased incidences of renal tubular adenomas. There was no evidence of carcinogenic activity in female B6C3F1 mice that received 19 or 38 mg/kg 2,4-diaminophenol dihydrochloride.
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> Pigmentation of the duodenum, forestomach, pancreatic and mesenteric lymph nodes in rats, and of the duodenum, hepatic Kupffer cells, and mesenteric lymph nodes in mice; acanthosis of the forestomach in male mice, and pigmentation and hemosiderin in the renal tubules of both rats and mice, were associated with exposure to 2,4-diaminophenol dihydrochloride.

Mr. Beliczky, a principal reviewer, agreed in principle with the conclusions. By that, he meant that the predominant or overall conclusion for the studies should be some evidence of carcinogenic activity. His rationale was that a single conclusion would be much clearer to a worker reading the NTP conclusion in a Material Safety Data Sheet.

Dr. Carlson, the second principal reviewer, did not agree with the conclusion in male mice. He argued for changing the conclusion to equivocal evidence of carcinogenic activity based on the increased incidence of renal tubular adenomas being only slightly greater then the previous maximum observed in control mice and not statistically significantly different from concurrent controls as well as not being dose-related. Further, he said that treated male mice had a significantly increased survival and the first tumors were observed at 688 days. Dr. Irwin stated that these tumors are very uncommon in mice and a parallel increase in hyperplasias along with additional adenomas found on step sectioning bolstered the proposed conclusion in male mice. Dr. Carlson asked why the water soluble hydrochloride salt was administered in corn oil. Dr. Irwin said a suspension in corn oil was the most stable dose formulation.

Dr. Garman, the third principal reviewer, agreed with the conclusions. He said the special stains used to identify pigment found in lesions in 15-month interim evaluation animals should be characterized. Dr. C. Shackelford, NIEHS, commented that a special stain was used to identify iron.

Mr. Beliczky moved that some evidence of carcinogenic activity be made the predominant conclusion for the Technical Report on 2,4-diaminophenol dihydrochloride. The motion was tabled for lack of a second. Mr. Beliczky then moved that the Technical Report be accepted with the revisions discussed and the conclusions as written for male and female rats and female mice, no evidence of carcinogenic activity and for male mice, some evidence of carcinogenic activity. Dr. Garman seconded the motion, which was accepted by nine yes votes to one no vote (Carlson).

Furan. Dr. R. Irwin, NIEHS, NTP Staff Scientist, introduced the toxicology and carcinogenesis studies of furan by discussing uses of the chemical and rationale for study, describing the experimental design, reporting on survival and body weight effects, and reviewing neoplastic and nonneoplastic lesions in rats and mice. The conclusions were that:

> Under the conditions of these 2-year gavage studies, there was clear evidence of carcinogenic activity of furan for male and female F344/N rats based on increased incidences of cholangiocarcinomas and hepatocellular neoplasms of the liver and of increased incidences of mononuclear cell leukemia. There was clear evidence of carcinogenic activity of furan for male and female B6C3F1 mice based on increased incidences of hepatocellular neoplasms of the liver and pheochromocytomas of the adrenal gland.
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> Fibrosis, hyperplasia, inflammation and proliferation of the biliary tract, and cytomegaly, degeneration, hyperplasia, necrosis, and vacuolization of hepatocytes, in both rats and mice, and the increased severity of nephropathy and associated increase in parathyroid hyperplasia in rats, were associated with exposure to furan.

Dr. Goodman, a principal reviewer, agreed with the conclusions. He commented on the four widely used in vitro tests for genetic toxicity noting the inability of three of the assays, mutagenesis in mouse lymphoma cells and chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells, to improve on the ability of mutagenesis in Salmonella typhimurium for predicting carcinogenicity of chemicals in long-term rodent studies and thought presentation of data from these assays should be very limited in the report. Further, he said the possibility should be considered that sister chromatid exchanges and chromosome aberrations might be artifacts resulting from lysosome breakdown secondary to cytotoxicity and not direct chemical action on DNA.

Mr. Beliczky, the second principal reviewer, agreed with the conclusions. He said the discussion and comparison of furan and furan compounds was excellent, and the presence of uncommon mutations in cellular genes suggested they were exposure related.

Dr. Hayden, the third principal reviewer, agreed with the conclusions. He criticized the lack of photomicrographs of the pertinent lesions, particularly to show the differentiation between cholangiofibrosis and cholangiocellular carcinoma. Dr. Irwin said this was an oversight and photomicrographs would be added. Dr. Hayden commented on the frequent discrepancies in furan dose formulations between the study laboratory and the analytical chemistry laboratory and asked whether the animals had received the proper doses. Dr. Irwin responded that this was an analytical problem because of the high volatility of furan. Considerable care was taken with the dosing solutions in the animal rooms to minimize potential loss.

Dr. Davis commented on the variable incidence of mononuclear cell leukemia with time. Dr. J. Haseman, NIEHS, said the NTP database is updated at least yearly with data from older studies being dropped and newer studies added so as to maintain about a five-year window. Dr. Hayden and Dr. Zeise thought increased incidences of urinary bladder papillomas in female rats and squamous cell papillomas of the forestomach in male mice deserved more discussion. Dr. Irwin

agreed. Mr. Beliczky said that based on the carcinogenicity of furan and the wide industrial use there should be information included on potential worker exposure. Dr. Janet Haartz, NIOSH, said the most recent exposure data indicated only 14 plants with 35 workers potentially exposed. She said she would follow-up to see if there are better data.

Dr. Goodman moved that the Technical Report on furan be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, clear evidence of carcinogenic activity. Mr. Beliczky seconded the motion, which was accepted unanimously with ten votes.

Naphthalene. Dr. K. M. Abdo, NIEHS, NTP Staff Scientist, introduced the toxicology and carcinogenesis studies of naphthalene 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 mice. The conclusions were that:

> Under the conditions of these 2-year studies, there was no evidence of carcinogenic activity of naphthalene in male B6C3F1 mice exposed by inhalation to concentrations of 10 to 30 ppm for 6 hours daily, 5 days per week, for 103 weeks. There was some evidence of carcinogenic activity of naphthalene in female B6C3F1 mice, as indicated by the increased incidences of pulmonary alveolar/bronchiolar adenomas.
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> In both male and female mice, naphthalene caused increased incidences and severity of chronic inflammation, metaplasia of the olfactory epithelium, and hyperplasia of the respiratory epithelium in the nose and chronic inflammation in the lungs.

Dr. Carlson, a principal reviewer, agreed with the conclusions. He asked for clarification of the incidence of inflammation. Dr. M. McDonald, NIEHS, explained that there were animals with inflammation and animals characterized histologically with a more extensive inflammatory response called granulomatous inflammation, and there was no real overlap. Dr. Carlson suggested that the extensive work of Alan Buckpitt and coworkers on naphthalene metabolism and toxicity should be reviewed and mentioned since these studies may help in understanding any tie between metabolism and inflammation in the lungs. Dr. Abdo said he was familiar with some of this work and would cite it.

Dr. Davis, the second principal reviewer, agreed with the conclusions. He wondered why the conclusion in female mice was not clear evidence and thought the reason needed to be better explained. Dr. Abdo said the level of some evidence was chosen in part because all but one of the lung tumors were benign. Dr. Davis asked whether there was information on metabolites of naphthalene in humans, and, if so, it would be useful to add to the table comparing metabolites across species. Dr. Abdo said he would follow up on this and cite information on human metabolism in the report.

Dr. Bailey, the third principal reviewer, agreed with the conclusions. He said that an explanation should be given for the absence of cataractogenesis in view of background information indicating such effects in mice. Dr. G. Rao, NIEHS, said B6C3F1 mice were responsive at the Ah locus. [A report in the literature with nine inbred mouse strains exposed to naphthalene indicated cataracts developed only in responsive strains.] Dr. Rao commented that in the current studies exposure may not have been adequate for a cataractogenic effect since the animals often closed their eyes during exposure periods.

Dr. Hayden said inhalation exposure seemed to be appropriate based on how humans are often exposed. Thus, since previous studies with naphthalene in rats have been by other routes of administration, he proposed that a future study in rats by inhalation could be useful. Dr. Zeise said she disagreed with discounting the relationship of hemangiosarcomas in females to naphthalene exposure because they occurred at various sites. Dr. J. Haseman, NIEHS, noted that the main reason for discounting them was that the incidence in the high dose group (4%)

was similar to the mean historical control value. There were concerns expressed about the poor survival in the male mice control group.

Dr. Carlson moved that the Technical Report on naphthalene be accepted with the revisions discussed and the conclusions as written for male mice, no evidence of carcinogenic activity, and for female mice, some evidence of carcinogenic activity. Dr. Davis seconded the motion, which was accepted unanimously with ten votes.

Quercetin. Dr. J.K. Dunnick, NIEHS, NTP Staff Scientist, introduced the toxicology and carcinogenesis studies of quercetin by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on nonneoplastic and neoplastic lesions of the kidneys in male and female rats. The proposed conclusions were that:

> Under the conditions of these 2-year studies, there was some evidence of carcinogenic activity of quercetin for male F344/N rats based on an increased incidence of renal tubular cell adenomas in the 40,000 ppm group. There was no evidence of carcinogenic activity for female F344/N rats fed diets containing 1,000, 10,000, or 40,000 ppm of quercetin. Hyperplasia of the renal tubular epithelium and the severity of nephropathy were increased in dosed male rats.

Dr. Dunnick added that because of the low but slightly increased number of renal neoplasms in male rats, additional step sections of residual kidneys from all control and high-dose rats were cut and evaluated.

Dr. Garman, a principal reviewer, agreed with the conclusions. He thought the conclusions in male rats were quite reasonable based both on the frequencies of hyperplasia and of benign and malignant renal tubular epithelial tumors and on the morphology of these tumors. Dr. Garman asked whether the induction of tumors was related to hyalin droplet nephropathy, and if so, he opined, this might imply a decreased level of concern with regard to human exposure to quercetin. Dr. R. Hailey, NIEHS, said there was no evidence for the hyalin droplet nephropathy in this study, and also no evidence of kidney lesions from interim evaluations done at six and 15 months. Dr. Garman asked for clarification of the identity and tissue location of the pigment found in the gastrointestinal tract. Dr. Hailey said the identity was not determined.

Dr. Goodman, the second principal reviewer, agreed with the conclusions. With regard to step sectioning of the kidneys, since this is a fairly new approach, he thought it would be useful to present a detailed table (based upon data from this and previous NTP reports in which the approach was used) indicating the effect this more detailed analysis has on the incidence of kidney tumors in control and treated animals. Dr. J. Haseman, NIEHS, said that for the eight studies where step sections have been done and evaluated, the rate of renal tubular neoplasms in male rats is 3.7%, which is slightly more than double the rate in the current historical control database of 1.6%. Dr. Dunnick reported that the findings from the step sections in other studies have been supportive of the original diagnosis. Dr. Goodman suggested that specific references of studies on chemically-induced alpha-2u-globulin nephropathy in male rats should be considered for inclusion in the discussion. Dr. Dunnick said they would be added.

Mr. Beliczky, the third principal reviewer, agreed with the conclusions. He commented that the increased sensitivity of detection for renal tumors and preneoplasic lesions resulting from step sectioning was impressive. He asked whether studies on quercetin had been done in mice. Dr. Dunnick responded that several previous studies by others in mice had shown no evidence of carcinogenic effects.

Dr. Carlson said he was not convinced that two squamous cell carcinomas of the tongue in high-dose female rats were unrelated to chemical administration. Dr.

Dunnick said the number was within the historical control range and microscopic analysis indicated no supporting preneoplastic lesions.

Dr. Garman moved that the Technical Report on quercetin be accepted with the revisions discussed and the conclusions as written for male rats, some evidence of carcinogenic activity, and for female rats, no evidence of carcinogenic activity. Dr. Goodman seconded the motion, which was accepted unanimously with ten votes.

Resorcinol. Dr. M. Jokinen, NIEHS, NTP Staff Scientist, introduced the toxicology and carcinogenesis studies of resorcinol by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting that there were no treatment-related non-neoplastic or neoplastic lesions in rats or mice. The conclusions were that:

> Under the conditions of these 2-year studies, there was no evidence of carcinogenic activity of resorcinol in male F344/N rats given 112 or 225 mg/kg or female F344/N rats given 50, 100, or 150 mg/kg in water by oral gavage 5 days a week for 104 weeks. There was no evidence of carcinogenic activity of resorcinol in male or female B6C3F1 mice given 112 or 225 mg/kg in water by oral gavage 5 days a week for 104 weeks.

Dr. Klaassen, a principal reviewer, agreed with the conclusions. He proposed that NTP measure blood levels of this chemical and others at various time points. Dr. R. Irwin, NIEHS, responded that NTP is now incorporating routinely in 2-year studies and many short-term toxicity studies the evaluation of blood levels as well as some basic pharmacokinetic parameters.

Dr. Hayden, the second principal reviewer, agreed with the conclusions. However, he questioned the adequacy of the study in male rats by quoting a statement from the conclusions that: "Because of the significant early reduced survival of male rats administered 225 mg/kg, this group was not considered suitable for the evaluation of carcinogenic potential". Dr. J. Haseman, NIEHS, said that survival in this group was probably sufficient to detect a strong carcinogenic effect, and that survival in the 112 mg/kg group was unaffected, supporting the adequacy of the study for evaluating carcinogenicity. Dr. Hayden commented that he was struck by the apparent and, perhaps, cumulative neurotoxicity, and suggested that a statement be added to the conclusions noting that clinical findings indicative of chemical-related toxicity to the central nervous system were observed in dosed animals of both sexes, and that a number of deaths attributed to chemical toxicity occurred in dosed rats and male mice. Dr. Jokinen responded that high dose rats in the 2-year study seemed subjectively to have exaggerated clinical signs by the end of the five-day dosing period each week that might suggest effects on the central nervous system although there were no morphologic lesions observed to support this. Dr. Hayden said this does not negate the possibility of interference with neurotransmitters. As to possible cumulative toxicity, Dr. Jokinen said chemical disposition studies indicated resorcinol was rapidly cleared from the blood and about 90% was excreted within 24 hours primarily as a conjugate in the urine.

Further discussion ensued as to whether resorcinol could be considered to be a neurotoxin. Dr. Irwin said the observations were primarily empirical such that he would have reservations about calling it a neurotoxin. Dr. Carlson cautioned that since the Technical Reports were used by many people for different purposes, one has to be careful about calling resorcinol a neurotoxin unless there is good evidence including a dose-response relationship. Mr. Beliczky reported that there is considerable exposure of workers in the rubber products industry where resorcinol has been used as part of an adhesive system, and in view of the reported clinical signs in rodents such as hyperexcitability and tremors, the apparent neurotoxicity should be noted in the Conclusions.

Dr. Klaassen moved that the Technical Report on resorcinol be accepted with the revisions discussed and the conclusions as written for male and female rats and mice, no evidence of carcinogenic activity. Dr. Garman seconded the motion. Mr. Beliczky offered an amendment that the concerns expressed by Dr. Hayden about neurotoxic effects of resorcinol be addressed in the Conclusions. Dr. Hayden seconded the amendment. Dr. J. Haseman, NIEHS, suggested that a statement in the Abstract spoke to these concerns and could be added to the Conclusions to provide the emphasis requested. As follows: "Clinical signs suggestive of a chemical-related effect on the central nervous system, including ataxia, recumbency, and tremors, were observed in rats and mice in the 2-year studies." Dr. Hayden agreed. The amendment was accepted by seven yes to three no votes (Bailey, Goodman, Klaassen). The original motion by Dr. Klaassen was then accepted unanimously with ten votes.

TOXICITY STUDIES.

t-Butyl Perbenzoate. Dr. H.B. Matthews, NIEHS, NTP Staff Scientist, introduced the short-term toxicity studies of t-butyl perbenzoate (t-BP) by reviewing the uses of t-BP and rationale for study, findings from chemical disposition studies, experimental design, and results. Studies by the dermal route indicated limited (16%) absorption through the skin of rodents while by the oral gavage route, there was rapid and complete absorption. The chemical was administered by the gavage route to groups of F344/N rats and B6C3F1 mice of both sexes for 14-days and 13-weeks. In 14-day studies, t-BP doses ranging from 70 to 1112 mg/kg failed to produce marked toxicity other than epithelial hyperplasia, ulceration, and acute inflammation of the forestomach in animals receiving the highest dose. In 14-day studies with metabolites of t-BP, benzoic acid and tertiary butanol, there was minimal toxicity observed. In 13-week studies, there was depressed weight gain in animals receiving the highest dose, 500 mg/kg. Other toxicity was limited to dose-dependent hyperplasia of the forestomach which was observed in most dosed animals. Hyperplasia was characterized by increased cellularity and basophilia of the squamous epithelium with variable degrees of hyperkeratosis. Based on results of this study, it was concluded that the no-observed-adverse-effect-level (NOAEL) for t-BP given orally was approximately 30 mg/kg for both rats and mice.

Dr. Hayden, a principal reviewer, said this was a well documented and clearly written report indicating that t-BP had little or no toxicity to rodents other than changes in the stomach. He cautioned that on a long-term study there might be problems with stomach ulcers or perforating ulcers leading to excessive mortality.

Dr. Bailey, a second principal reviewer, also thought this to be a well-performed study and well written report. He commented that there were reports cited of workers exposed by inhaling vapors while the text states that t-BP is not "normally inhaled", so somewhat of a contradiction exists.

Dr. Davis asked whether the forestomach lesions were focused primarily around the limiting ridge as in some recent studies. Dr. M. Elwell, NIEHS, said that at the higher doses lesions were seen over large areas of the forestomach. Dr. J. Haartz, NIOSH, commented on the worker exposure numbers in the text and said the most recent NIOSH survey indicates about four times that many workers are exposed. She said she would supply the data to Dr. Matthews.

p-Chloro-alpha,alpha,alpha-trifluorotoluene. Dr. C.W. Jameson, NIEHS, NTP Staff Scientist, introduced the short-term toxicity studies of p-chloro-alpha, alpha, alpha-trifluorotoluene (CTFT) by reviewing the study rationale, experimental design, and results. CTFT (97% pure) was administered to groups of F344/N rats and B6C3F1 mice of both sexes for 14 consecutive days by gavage once a day in either corn oil or an experimental molecular encapsulation vehicle, alpha-cyclodextrin. Toxicokinetic studies were performed to compare the bioavailability of CTFT between the two vehicles. Although absorption from the alpha-cyclodextrin vehicle was somewhat faster, the biological half-life of CTFT was the same with both vehicles, and bioavailability was shown to be complete for both vehicles. Rats were found to be somewhat more sensitive to toxic effects of CTFT than mice, likely due to more rapid elimination from the bodies of mice. CTFT was found to accumulate in the kidneys of male rats, and there was a linear relationship between the kidney CTFT concentrations and the kidney content of alpha-2u-globulin. Microscopic changes included a dose-related toxic nephropathy consistent with that previously described as "hyaline droplet nephropathy". Dosed male and female rats had hepatocyte hypertrophy and cytoplasmic vacuolization of the adrenal cortex. Hepatocellular hypertrophy and clinical pathology findings consistent with cholestasis and mild liver injury were noted in mice in the two highest dose groups. The toxicity results were similar with either vehicle, suggesting that alpha-cyclodextrin may be an appropriate vehicle for toxicity studies with other chemicals.

Dr. Klaassen, a principal reviewer, thought this was a good study and report and welcomed the inclusion of pharmacokinetic data. He suggested that the conclusion should emphasize that the plasma concentration of CTFT increased more rapidly with the alpha-cyclodextrin vehicle. He also suggested that the NTP consider measuring levels of cytochrome P-450 isozymes, especially with chemicals that increase liver weight as was the case here. Dr. Jameson said this would be considered especially for chemicals where liver is expected to be a target organ.

Dr. Goodman, a second principal reviewer, said that since one of the unique features of the study was the comparison of the two dose vehicles this fact should be highlighted in the title. He said molecular encapsulation should be better defined. Further, he questioned whether the alpha-cyclodextrin was preferable to corn oil as the gavage vehicle. Dr. Jameson said that the term molecular complexation might be more appropriate and that there was a good description of molecular encapsulation in an earlier draft and then deleted. This would be returned as part of the revisions. He noted that because of the chemical's presence in ground water, a drinking water study would have been desirable but limited water solubility was a problem. The more rapid availability for absorption from the aqueous alpha-cyclodextrin vehicle than from corn oil was felt to better mimic availability from water.

Trinitrofluorenone. Dr. F. Kari, NIEHS, NTP Staff Scientist, introduced the short-term toxicity studies of trinitrofluorenone (TNF) by reviewing the rationale for study, experimental design, and results. Because the principal route of exposure of humans to TNF would likely be dermal, studies were conducted to compare chemical absorption, distribution, excretion, and tissue retention, as well as toxicity in 14-day studies in rats and mice by the oral and dermal routes of exposure. The results of dermal studies indicated absorption was insufficient to allow a full evaluation of the systemic toxicity of TNF; therefore, 13-week toxicity studies were conducted by incorporating TNF into the feed of F344/N rats and B6F3F1 mice of each sex. In both species, there was a widespread occurrence of a dark brown pigment in dosed animals with little evidence of toxicity related to the pigment accumulation. Treatment-related effects in male rats included mesenteric vascular inflammation, renal inflammation, testicular degeneration with reduced sperm count and motility, splenic hematopoiesis, and oval cell hyperplasia and mixed cell foci in the liver. Top dose female rats had centrilobular hepatocyte cytoplasmic alteration and splenic hematopoiesis. Dosed mice of both sexes showed cystic degeneration of the thyroid gland, hepatic hypertrophy, and splenic hematopoiesis. The no-observed-adverse-effect-level (NOAEL) for microscopic changes other than pigment accumulation was 1000 ppm for rats. A NOAEL could not be determined for mice from this study. Limited dermal absorption would likely prevent significant systemic toxicity resulting from contact of TNF with the skin.

Dr. Carlson, a principal reviewer, said this was a well written report. He noted that the preferred route of exposure, based on human exposure, would have been dermal, yet dosed feed was chosen because 14-day chemical disposition studies showed that less than 10% of a dermal dose was absorbed. Therefore, he thought the extensive disposition studies should be summarized in the Abstract, and currently only those for the dermal route are. Dr. Kari said additional information about the disposition studies would be added to the Abstract.

Dr. Garman, a second principal reviewer, said the report presented a large body of information in a well organized and succinct manner. He found the pigment accumulation in the large neurons in the brain stem to be particularly unusual. Since ultrastructural studies were performed, he asked that consideration be given to including an electron micrograph in the text showing the membrane-bound pigment particles. Dr. Davis seconded this request, and Dr. Kari agreed to add a plate.