The review meeting began at 9:00 a.m. on November 20 and 21 in the Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the Subcommittee are: Drs. Robert Scala (Chairperson), Daniel Longnecker, and Ellen Silbergeld. Members of the Panel of Experts are: Drs. John Ashby, Gary Carlson, Harold Davis, Robert Garman, Lois Gold, David Hayden, Curtis Klaassen, Barbara McKnight and Lauren Zeise. These minutes have been reviewed and approved by all members of the Subcommittee and Panel. 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 Public Information Office, MD B2-04, P. O. Box 12233, Research Triangle Park, NC 27709. Telephone: (919)541-3991; FTS: 629-3991. 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 April 25-26, 1990, 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
November 20-21, 1989

dl-Amphetamine Sulfate. Dr. J. K. Dunnick, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of dl-amphetamine sulfate by reviewing the experimental design, results, and proposed conclusions:

Under the conditions of these 2-year feed studies, there was no evidence of carcinogenic activity of dl-amphetamine sulfate for male or female F344/N rats or male or female B6C3F1 mice fed diets containing 20 or 100 ppm. The administration of dl-amphetamine sulfate was associated with decreased body weight. There were decreased incidences of total neoplasms in dosed rats and mice, of adrenal pheochromocytomas in male rats, of mammary gland fibroadenomas and uterine polyps in female rats, of pituitary gland adenomas in male and female rats and female mice, and of Harderian gland adenomas, liver neoplasms, and lung neoplasms in male and female mice.

Dr. McKnight, a principal reviewer, agreed with the conclusions for female rats and male and female mice. She thought the statistically significant increase in interstitial cell adenomas of the testes in male rats constituted equivocal evidence. Dr. Dunnick explained that the very high historical control incidence (89%) along with no increases in interstitial cell hyperplasias led to the conclusion of no evidence. Further, Dr. McKnight questioned whether decreased weight gain and increased activity in the 13-week studies should be used as a basis for setting doses for 2-year studies in that these are also pharmacologic effects related to amphetamine's use as an appetite suppressant and central stimulant. Therefore, Dr. McKnight said it was likely that rats and mice in the 2-year studies could have tolerated higher doses. Dr. Dunnick replied that in 2-year carcinogenicity studies body weight is an important factor in setting doses, because in order to have a comparable background tumor rate in control and treated animals body weights need to be similar. Decreases in body weight in NTP 2-year studies have been associated with decreased incidences of certain spontaneous tumors in rats and mice.

Dr. Carlson, the second principal reviewer, agreed with the conclusions for carcinogenic activity. He also agreed that decreased incidences of various neoplasms may have been associated with the decrease in body weights; however, a more direct effect of dl-amphetamine could not be ruled out in his opinion. Dr. Dunnick said the data were not sufficient to determine whether decreased tumor incidences were due to decreased body weights or to a direct effect of the chemical. Dr. Carlson commented on the reports of increased activity of dosed animals and stated that the reported activity needed to be better defined and quantitated as a measure of pharmacologic or toxicologic effect. Dr. Silbergleid explained that the 'popcorn effect' and stereotyped behavior are characteristic expressions of amphetamine-induced hypermotility in rodents. Dr. G. Rao, NIEHS, and Dr. Dunnick indicated that these types of activity were observed and were recorded in the Toxicology Data Management System as hyperactivity.
Dr. Zeise, the third principal reviewer, agreed with the conclusions. She commented that the conclusions should also explicitly state that treatment with the drug was associated with decreased tumor incidences and non-neoplastic lesions associated with compound administration might be listed in the conclusions. Dr. Dunnick responded that non-neoplastic effects thought to be biologically as well as statistically significant were usually treated in the discussion and abstract sections of the report. Dr. Zeise questioned the rationale for studying the dl-form instead of the more pharmacologically active and much more frequently prescribed d isomer. Dr. Dunnick reported that at the time the chemical was nominated and the initial studies conceived there were approximately equal numbers of prescriptions written for the dl- and d-isomers of amphetamine.

Subsequent discussion focused on whether or not high enough doses were used in the two-year studies. Dr. Silbergeld said there was a U-shaped curve for some toxic effects observed with amphetamines in animals, and that there was additional information that should be considered in reviewing the appropriateness of the doses in the 2-year studies. Dr. Silbergeld expressed concern that standard pathologic examination might not identify all changes in the nervous system related to amphetamine treatment. Dr. S. Eustis, NIEHS, agreed that standard histopathologic examination might not detect neurochemical or neurophysiologic changes in neural tissues; however, the studies were designed to detect carcinogenic and not neurologic effects of dl-amphetamine. Dr. Scala suggested that the Program needed to be more sensitive to reports in the literature of pharmacologic effects in designing future studies. Dr. D. Rall, NIEHS, agreed that a focus on the toxicity was needed particularly with studies on drugs or therapeutic agents. However, he wanted to point out that there were real decreases in tumor incidences and that these were important and significant findings that needed to be highlighted.

Dr. McKnight moved that the conclusions in male and female rats and mice be changed to inadequate study of carcinogenic activity based on the fact that higher doses might have been given. Dr. Zeise seconded the motion. Dr. Carlson disagreed with the motion. He opined that likely the U-shaped curve would not have applied to the toxicity observed in the two-year studies and survival could have been a problem if higher doses had been used. Dr. Garman proposed that the report be tabled so that the NTP could further evaluate the literature and perhaps seek expert advice to aid in clarifying whether or not the high doses were too low. Dr. Scala said a motion to table would take priority over the earlier motion. Accordingly, Dr. Garman moved that the draft Technical Report on dl-amphetamine be tabled until additional information could be obtained as to the adequacy of the doses. Dr. Gold seconded the motion, which was accepted by nine yes votes to two no votes (Ashby, Carlson). Dr. Ashby noted for the record that he voted no because he was concerned that a precedent would be set for bringing back other studies if new knowledge were being brought to bear, i.e., knowledge developed subsequent to the NTP decision to study. Dr. Griesemer said the report would be returned for review at the next meeting.
2-Chloroacetophenone. Dr. R. L. Melnick, NIEHS, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of 2-chloroacetophenone (CN) by reviewing the experimental design, results, and proposed conclusions:

Under the conditions of these 2-year inhalation studies, there was no evidence of carcinogenic activity of 2-chloroacetophenone for male rats exposed to 1 or 2 mg/m^3. There was equivocal evidence of carcinogenic activity for female F344/N rats, based on a marginal increase in fibroadenomas of the mammary gland. There was no evidence of carcinogenic activity for male or female B6C3F1 mice exposed to 2 or 4 mg/m^3 2-chloroacetophenone.

Dr. McKnight, a principal reviewer, agreed with the conclusions for male rats and male and female mice but disagreed with the conclusion for female rats. She thought the dose-related increase in fibroadenomas, adenomas and adenocarcinomas of the mammary gland in female rats was strong enough to support some evidence of carcinogenic activity. Dr. Melnick said that based on findings of a marginal increase of a commonly occurring benign tumor (fibroadenomas) at an incidence that fell well within the historical range for untreated controls, the conclusion of equivocal evidence was judged appropriate. Dr. McKnight inquired as to how zero dose was administered to control animals. Dr. Melnick said the chamber controls were treated the same as the dosed animals except for receiving vapors.

Dr. Longnecker, the second principal reviewer, agreed with the conclusions. He asked whether dosed animals were left in the inhalation chamber following the exposure period. Since the report stated that 10 hours were required for CN concentration to drop to 1% of target concentration, he calculated that if animals remained in the chamber total exposure would have been increased by about 80%. Dr. Melnick responded that animals remained in the chambers but this increased the total exposure to CN by about 5 to 25%; details of the additional exposure would be better explained in the report.

Most of the discussion was concerned with the significance of the mammary gland neoplasms in female rats. Dr. Silberfeld argued that both a significant trend test (P=0.013) and pairwise comparison between control and high dose groups (P=0.017) for fibroadenomas supported some evidence. On the other hand, Dr. Ashby thought the higher incidence in control groups from more recent studies weakened the argument for even equivocal evidence.

Dr. McKnight moved that the Technical Report on 2-chloroacetophenone be accepted with the conclusions as written for male rats and male and female mice, no evidence of carcinogenic activity. Dr. Longnecker seconded the motion, which was accepted unanimously with eleven votes. Dr. McKnight moved that the conclusion for female rats be changed from equivocal evidence of carcinogenic activity to some evidence of carcinogenic activity based on statistically significant, dose-related increases in fibroadenomas or adenomas of the mammary glands, with incidences in both low and high dose groups higher than ever seen in a chamber control group. Dr. Longnecker seconded the motion, which was defeated by six no votes (Ashby, Carlson, Davis, Garman, Gold, Hayden) to five yes votes (Klaassen, Longnecker, McKnight, Silberfeld, Zeise). Dr. Gold moved that the conclusion be accepted as written, equivocal evidence of carcinogenic activity. Dr. Davis seconded the motion, which was accepted by seven yes votes.
(Ashby, Carlson, Davis, Garman, Gold, Hayden, Longnecker) to four no votes (Klaassen, McKnight, Silbergeid, Zeise).
o-Chlorobenzal malononitrile (CS2). In the absence of Dr. K.M. Abdoy NIEHS, NTP Study Scientist, Dr. R.L. Melnick, NIEHS, introduced the toxicology and carcinogenesis studies of o-chlorobenzal malononitrile (CS2) by reviewing the experimental design, results, and proposed conclusions:

Under the conditions of these inhalation studies, there was no evidence of carcinogenic activity of CS2 for male or female F344/N rats exposed to 0.075, 0.25, or 0.75 mg/m³ in air for up to 2 years. There was no evidence of carcinogenic activity for male or female B6C3F1 mice exposed to 0.75 or 1.5 mg/m³ in air for up to 2 years. Concentration-related decreases in the incidences of pituitary gland adenomas and lymphomas were observed in female mice.

Exposure to CS2 caused degeneration and squamous metaplasia of the olfactory epithelium, hyperplasia and metaplasia of the respiratory epithelium, and proliferation of the periosteum of the nasal passage of rats. In mice, exposure to this compound caused suppurative inflammation and hyperplasia and squamous metaplasia of the respiratory epithelium of the nasal passage.

Dr. Klaassen, a principal reviewer, agreed with the conclusions. He commented on the concentration-related decreased incidences of adenomas of the pituitary gland and of lymphomas in female mice and wondered if these decreases might be related to decreases in weight gain and longer life span. Dr. Melnick said there was a suggestion that the decreased incidences of lymphomas might be related to body weight differences in the dosed animals compared to controls. Dr. Klaassen noted the similarity of the nonneoplastic toxic changes in the respiratory epithelium of the nasal passages to those seen with formaldehyde and thought a comparison of the toxicity would be of interest especially in view of the differences in carcinogenicity. Dr. S. Eustis, NIEHS, commented that the most prominent analogous lesion was squamous metaplasia, which was extensive in the formaldehyde studies but was focal and limited in extent in the CS2 studies. However, without actual quantitative data obtained from morphometry or cell turnover studies, more than descriptive comparisons would be difficult.

Dr. Davis, the second principal reviewer, agreed with the conclusions. He asked why a low dose in mice more comparable to the lowest dose in rats was not used. Dr. Melnick reported that in rats there seemed to be a greater chemical-related effect on body weight as well as on lesions within the respiratory tract than in mice in prechronic studies. Since a no-effect-level (NOEL) was not achieved in rats in prechronic studies, a lower dose was used in the two-year studies in an attempt to reach a NOEL. A much lower dose in the two-year mice studies was not necessary since effectively a NOEL had been achieved in the prechronic studies.

There was some discussion about the renal tubular cell adenomas seen in two female rats in the mid-dose exposure group. Dr. Eustis explained that because tumors were not seen in either the low or high exposure groups and there was no supporting hyperplasia, the neoplasms were not considered to be related to exposure to CS2. Drs. Gold, Zeise and Davis questioned the adequacy of the histopathological examination of the kidneys in rats and suggested that the tissues may have been underexamined for tumors.
Dr. Klaassen moved that the Technical Report on o-chlorobenzal malonitrile be accepted with the conclusions as written for male and female rats and mice, no evidence of carcinogenic activity. Dr. Davis seconded the motion, which was accepted unanimously with eleven votes.
1-Epinephrine Hydrochloride. Dr. D. Dietz, NIEHS, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of 1-epinephrine hydrochloride by reviewing the experimental design and results. He noted there were no increases in the incidence of neoplasia that were judged to be exposure-related while non-neoplastic degenerative inflammatory lesions of the nasal mucosa were seen at higher incidences in exposed rats and mice. Dr. S. Eustis, NIEHS, commented on how doses were selected for the two-year studies. Exposure concentrations were selected to represent multiples of human therapeutic levels used in the treatment of asthma, and were arrived at on the basis of consultation with the nominating agency. He acknowledged that this rationale was a departure from usual procedures followed in selecting doses for carcinogenesis studies. He asked for the Panel's guidance on the way the conclusions are stated in the Technical Report and on whether or not the studies constitute a carcinogenicity study or rather a chronic toxicity study.

Dr. Gold, a principal reviewer, did not agree with the conclusions as stated in the report, no evidence of carcinogenic activity. She said all four experiments should be termed inadequate study of carcinogenic activity. As it was noted in the text that the high dose in mice was approximately one-eighth and in rats one-fourth of the estimated maximum tolerated dose (MTD), she thought the report should be retitled as something other than a carcinogenesis study. Dr. Gold stated that a statement should be included in the Abstract and elsewhere emphasizing that the studies were conducted at doses considerably lower than the MTD. Dr. Dietz commented that on a μg/kg body weight basis, the high dose in rats and mice ranged from approximately ten to twenty times the human therapeutic dose. He noted that higher concentrations were not used in order to avoid confounding variables associated with the potent pharmacological action of epinephrine. Further, he added that there were microscopic changes in the nasal mucosa in both the 13-week and two-year studies. Dr. Gold asked for discussion as to whether the uterus might be a target organ in female mice. She questioned whether the endometrial stromal polyps had been adequately distinguished from adenomas or adenomatous polyps and noted that there were three adenocarcinomas of the uterus in dosed animals. Dr. Eustis indicated that these tumors would have been examined during quality assessment (QA) and pathology working group (PWG) review and were accurate.

Dr. Silbergeld was concerned that the doses used were insufficient to provide a good toxicology study, and was also concerned as to how thoroughly non-neoplastic effects were examined, particularly in target tissues such as the adrenal glands and the nervous system. Dr. Eustis said the target organs for histopathology quality assessment were considered to be the nasal cavity and lungs although the adrenal glands and other tissues were examined.

Dr. Hayden, the second principal reviewer, thought the experimental design appeared adequate as the dose levels chosen were sufficient to cause damage to the nasal epithelium without affecting growth rate or survival of dosed rats or mice. He commented that no evidence of carcinogenesis was found.

Dr. Zeise moved that the Technical Report on 1-epinephrine be accepted but with the conclusions for male and female rats and mice changed to an inadequate study of carcinogenic activity. Dr. Garman seconded the motion. Dr. Zeise said her motion was based both on the fact of the doses selected being well below the MTD and as well uncertainty as to whether the doses used were adequate to represent
human therapeutic levels. In following discussion, Dr. Silbergeld said she was not convinced that the doses used were even sufficient to elicit pharmacologic signs analogous to those seen in humans given a therapeutic dose. Dr. Klaassen stated that using the current definition of a carcinogenicity study, if negatives are obtained and the doses are below an MTD, the studies are inadequate for detecting whether or not there is carcinogenic activity of the chemical. The motion was accepted by ten yes votes to one no vote (Carlson).
Ethylene Thiourea. Dr. R. S. Chhabra, NIEHS, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of ethylene thiourea (ETU) by reviewing the experimental design, results, and proposed conclusions. The study design included both conventional two-year exposure of adult animals and perinatal exposure. The studies were designed to compare and evaluate the potential value of perinatal exposures in assessing chemical carcinogenicity. The conclusions in the draft report were:

Under the conditions of these 2-year studies, there was clear evidence of carcinogenic activity of ethylene thiourea for male and female F344/N rats, as shown by increased incidences of thyroid follicular cell neoplasms and Zymbal's gland neoplasms. The increased incidences of mononuclear cell leukemia in males and females and renal tubular cell neoplasms in males may have been chemically-related. There was clear evidence of carcinogenic activity for male and female B6C3F1 mice, as shown by increased incidences of thyroid follicular cell neoplasms, hepatocellular neoplasms and adenomas of the pars distalis in the pituitary gland.

Dr. Garman, a principal reviewer, agreed with the conclusions. He said the dosages selected appeared to be appropriate. He thought some comments could be added to the discussion to the effect that the proposed mechanism of thyroid carcinogenicity is probably characterized by a threshold. Dr. R. Griesemer, NIEHS pointed out that while a threshold for development of the thyroid tumors may be a plausible concept, he was not aware of any published evidence supporting it.

Dr. Carlson, the second principal reviewer, agreed with the conclusions. He thought the evidence for the importance of the perinatal exposure to be not very strong. Dr. Chhabra stated that perinatal plus adult exposure at the highest concentrations significantly increased the incidence of thyroid gland neoplasms in male and female rats, relative to adult exposure only.

Dr. McKnight, the third principal reviewer, agreed with the conclusions. She said that if interest centered on comparing the effects of perinatal (F0) exposure and standard adult feeding (F1) exposure, rather than trends in tumor incidence as concentrations of both F0 and F1 exposure increased, it was not clear what purpose the treatment groups with lowest F0 and F1 exposure served. Some of the reviewers expressed difficulties in fully understanding statistical comparisons reported in the tables.

There was considerable discussion about the nature of the association between chemical exposure and the proliferative lesions in the thyroid gland. Dr. Silbergeld had prepared a table comparing incidences of these lesions across dose groups in rats with blood levels of thyroid hormones in animals sacrificed at nine months. She discussed the possibility of there being an indirect effect of ETU on the thyroid although the data do not allow a conclusion to be drawn. Dr. Scala said a more robust statistical treatment was needed. However, Dr. McKnight cautioned against overemphasis on P values as biochemical values such as hormone levels are not available for many time points where data on proliferative lesions are available. Dr. Carlson said the Panel should not overlook the other tumors that were increased. Dr. Ashby opined that the evidence supported ETU as being mutagenic. Dr. Scala suggested that the enhanced effects on the thyroid might be due only to the longer exposure period.
and not necessarily to a different mechanism. Dr. Klaassen commented that a significant point about this study was that one would have expected in utero exposure to be a much more sensitive way to detect carcinogens and at least in this case, it was not.

Dr. Zeise commented on the occurrence of the uncommon tubular cell neoplasms in rats. She observed that recent NTP experience has shown that additional animals with renal tumors may have been missed in the histopathological examination (single sections), and suggested further histopathological examination (step-sections) to determine whether the observed neoplasms are related to ETU exposure. Dr. Eustis indicated that NTP would consider the recommendation.

The consensus of the Panel was that there was not an overwhelming effect of perinatal exposure to ETU on increased incidences of neoplastic lesions and that better data presentation would help highlight what effects there were of treatment for the reader of the report. Dr. Gold suggested that the results should be reorganized and reported in terms of three questions addressed by the experimental design: (1) were there effects of perinatal exposure?; (2) were there carcinogenic effects in a typical two-year bioassay?; and (3) did perinatal exposure enhance or potentiate carcinogenic effects seen in a subsequent two-year bioassay?

Dr. Garman moved that the Technical Report on ethylene thiourea be deferred for further consideration so that the questions raised could be addressed in a revision of the report. Dr. Hayden seconded the motion, which was accepted unanimously with eleven votes.
Furfural. Dr. R.D. Irwin, NIEHS, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of furfural by reviewing the experimental design, results, and proposed conclusions:

Under the conditions of these 2-year gavage studies, there was some evidence of carcinogenic activity of furfural for male F344/N rats, based on increased incidences of cholangiocarcinomas and bile duct dysplasia and fibrosis. There was no evidence of carcinogenic activity for female F344/N rats that received doses of 0, 30, or 60 mg/kg furfural. There was clear evidence of carcinogenic activity for male B6C3F1 mice, based on increased incidences of hepatocellular adenomas and hepatocellular carcinomas. There was some evidence of carcinogenic activity in female B6C3F1 mice, based on increased incidences of hepatocellular adenomas. Renal cortical adenomas or carcinomas in male mice and squamous cell papillomas of the forestomach in female mice may have been related to exposure to furfural.

Dr. Ashby, a principal reviewer, agreed with the conclusions for male rats and male and female mice although he commented that the basis for the levels of evidence should be more precisely defined. Dr. Irwin commented that the difference in levels of evidence for mice derived from there being significant dose-related increases in both hepatocellular adenomas and carcinomas in males as opposed to increases only in adenomas in female mice. Dr. Ashby thought the conclusion in female rats could be inadequate study of carcinogenic activity due to the reduced survival in the high dose group.

Dr. Hayden, the second principal reviewer, agreed with the conclusions for male rats and male and female mice. For female rats, due to poor survival, he wondered whether there were adequate numbers to make a valid assessment of carcinogenicity. Dr. Irwin said overall survival in high dose female rats was considered to be adequate for evaluation particularly since there was no indication of lesions in animals surviving to the end of the study. He said that two sets of survival curves will be shown in the report—censored and uncensored. Dr. Hayden commented that based on likely human occupational exposure dermal or inhalation routes of administration would have been more appropriate.

Dr. Gold, the third principal reviewer, agreed in principle with the conclusions. She commented that the most widespread exposure to furfural is in the diet of the general population and it should be stated in the introduction that the chemical is a naturally occurring constituent of many common foods. With respect to the liver tumors in mice, Dr. Gold said the text should say that the tumors were significantly increased only in the high dose of each sex and that the maximum tolerated dose (MTD) was not reached. She questioned the evaluation of some evidence based solely on hepatocellular adenomas because the evidence was less strong for the combined incidences of hepatocellular adenomas and carcinomas. She noted that 4/50 female controls had hepatocellular carcinomas. Dr. R. Griesemer, NIEHS, said only the responses of dose-related increases in adenomas contributed to the level of evidence and not the incidences of carcinomas. Dr. J. Haseman, NIEHS, said the incidences of carcinomas could be included and discussed in the Abstract. This was agreeable to Dr. Gold.

With respect to bile duct neoplasms, Dr. Gold also asked that the conclusions state that two dosed male rats developed neoplastic, and two non-neoplastic.
bile duct lesions. She thought that the level of some evidence would be supported even if the MTD had not been reached, if bile duct dysplasia and fibrosis were uncommon, and if the animals with these non-neoplastic lesions died early from other causes and therefore did not have time to develop neoplasms. Dr. Irwin said the bile duct lesions were histologically similar to the cholangiocarcinomas, would progress, and thus they did support some evidence. He said that based on liver toxicity and mortality in the 90-day studies higher doses likely would not have been tolerated.

In further discussion, Dr. Zeise questioned why the increases in uncommon squamous papillomas and mesenteric tumors in treated rats were not given more weight in the evaluation, given the occurrence of squamous cell papillomas of the forestomach in female mice. Dr. Eustis indicated that it was difficult to find a biological reason for them being related to treatment.

Dr. Ashby moved that the Technical Report on furfural be accepted with the conclusions as written but with the provision that the levels of evidence were less than clearcut. As part of the motion, he said the following clarifying language should be included in the report: (1) a justification for the dose levels used and that MTDs were achieved; (2) in male rats, an explanation of how the non-neoplastic bile duct lesions were essential to the level of evidence; (3) in female rats, clear justification for why the study was not inadequate; (4) in male mice, an explanation for the level of evidence selected; and (5) in female mice, an explanation for why the forestomach papillomas were not part of the level of evidence. Dr. Hayden seconded the motion, which was accepted by nine yes votes with two abstentions. Dr. Garman abstained for reasons of company affiliation while Dr. Zeise abstained because she thought that two other tumor types, mesenteric tumors in male rats and forestomach tumors in female rats and mice, should have been considered in the evaluation.
Tetranitromethane. Dr. J. R. Bucher, NIEHS, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of tetranitromethane by reviewing the experimental design, results, and proposed conclusions:

Under the conditions of these 2-year inhalation studies, there was clear evidence of carcinogenic activity of tetranitromethane for male and female F344/N rats and male and female B6C3F1 mice, based on increased incidences of alveolar/bronchiolar neoplasms in both species and squamous cell carcinomas of the lung in rats.

Chronic inflammation of the nasal mucosa was related to exposure in rats and mice, and hyperplasia and squamous metaplasia of the respiratory epithelium were increased in exposed rats.

Dr. Gold, a principal reviewer, agreed with the conclusions for carcinogenic activity but said there were some inaccuracies with the nonneoplastic lesions listed which needed to be corrected. Dr. Bucher agreed. Dr. Gold urged emphasizing in the conclusions the markedly increased incidences of carcinomas alone and that there were many metastases. She stated that the OSHA allowable worker exposure level (PEL) is close to the concentrations of tetranitromethane that induced tumors in rodents and that this should be pointed out in the discussion. Dr. Bucher concurred.

Dr. Ashby, the second principal reviewer, agreed with the conclusions. He said the carcinogenic response was qualitatively predictable by chemical structure and mutagenicity data but the potency of the response was not predictable. He commented on the high levels of alveolar/bronchiolar neoplasms in control animals, particularly in male mice. Dr. Bucher replied that the control incidences of tumors in all the sex/species combinations were approximately equal to historical control rates.

Dr. Zeise, the third principal reviewer, agreed with the conclusions. She said the possibility should be considered that tetranitromethane exposure may have resulted in sarcomas of the lung in female rats. Dr. Bucher said the staff was not convinced of an association of these tumors with chemical exposure. Dr. Zeise asked if an epidemiologic study was planned considering that the exposure concentrations were not much higher than those to which workers are exposed. Dr. Bucher noted that both EPA and NIOSH are interested in doing such a study if an appropriate worker group can be identified.

Discussion centered around the issue of including data on nonneoplastic lesions in the abstract and conclusions; in this case, lesions of the nasal passages. Dr. S. Eustis, NIEHS, commented that incidence rates alone were not very informative without measures of severity of the irritation or injury. All nonneoplastic lesions are graded by the original study pathologist and information is added in the text when considered relevant to interpretation of effects. Severity grades could be included for all nonneoplastic lesions where increases are statistically significant and are also considered biologically significant.

Dr. Gold moved that the Technical Report on tetranitromethane be accepted with the conclusions as written for male and female rats and mice, clear evidence of carcinogenic activity, and that the sentence on nonneoplastic lesions be changed.
to reflect more precisely the results. Dr. Ashby seconded the motion. Dr. Zeise offered an amendment that the lung sarcomas and mixed malignant tumor in female rats be mentioned under neoplastic effects in the summary table in the Abstract. Dr. Silbergeld seconded the amendment, which was accepted by eight yes votes to three no votes (Gold, Hayden, Klaassen). The Panel then accepted the original motion by Dr. Gold unanimously with eleven yes votes.
Vinyl Toluene. Dr. G. A. Boorman, NIEHS, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of vinyl toluene by reviewing the experimental design, results, and proposed conclusions:

Under the conclusions of these 2-year inhalation studies, there was no evidence of carcinogenic activity for male or female F344/N rats exposed to 100 or 300 ppm vinyl toluene and no evidence of carcinogenic activity for male or female B6C3F1 mice exposed to 10 or 25 ppm.

There was evidence of chemical-related toxicity to the nasal passage in both rats and mice.

Dr. Ashby, a principal reviewer, agreed with the conclusions. He commented on the decreased incidences of three tumor types in exposed groups of mice -- lymphomas and pulmonary neoplasms in males and liver neoplasms in females. He said the report's classifying vinyl toluene as a mutagen was based on slim evidence.

Dr. Garman, the second principal reviewer, agreed with the conclusions. With regard to the technical aspects of the study, he asked for an explanation as to why liquid vinyl toluene entered the inhalation chambers. He said this raised questions about the technical conduct of the study. Dr. Boorman explained that there was a dosing accident with one chamber 21 weeks into the study whereby six mice were exposed to the liquid chemical. The animals were removed from the study and he did not think the incident reflected on the overall conduct of the study.

Dr. Ashby moved that the Technical Report on vinyl toluene be accepted with the conclusions as written for male and female rats and mice, no evidence of carcinogenic activity. Dr. Garman seconded the motion, which was accepted unanimously with eleven yes votes.
TOXICITY STUDIES

Pentachlorobenzene. Dr. M. M. McDonald, NIEHS, NTP Study Scientist, introduced the short-term toxicity studies of pentachlorobenzene by reviewing the rationale, experimental design, and results. Pentachlorobenzene was administered by the dosed feed route to groups of F344/N rats and B6C3F1 mice of both sexes for 15-days or 13-weeks. In 15-day studies, all rats exposed to the highest dose, 10,000 ppm, and all mice exposed to 3,300 or 10,000 ppm died. Of exposed rats that survived, males had an accumulation of abnormal hyaline droplets in renal cortical epithelium and females had centrilobular hepatocellular hypertrophy. Chemical-related histologic lesions were not observed in exposed mice. In 13-week studies, no compound-related deaths occurred. The most prominent histologic lesion in male rats was hyaline droplet nephropathy. Centrilobular hepatocellular hypertrophy was observed in exposed male and female rats. The only exposure-related histologic lesion in mice of either sex was centrilobular hepatocellular hypertrophy. Increased liver porphyrin levels were noted in rats and mice exposed to higher concentrations of pentachlorobenzene. Minimal-to-mild thyroid follicular cell hypertrophy was present in male and female rats at the two highest doses. A minimal-to-mild microcytic, mildly hypochromic, poorly regenerative anemia was observed in dosed rats. The no-observed effect levels (NOELs) for histologic lesions were 33 ppm for male rats and 330 ppm for female rats. The NOEL for histologic lesions in female mice was 100 ppm. NOEL was not reached for male mice.

Dr. Klaassen, a principal reviewer, said this represented a good toxicity study with the main effects observed on the liver, thyroid and male rat kidney. He said a table for organ weights and more consistent presentation would be helpful. Dr. Klaassen asked for more discussion of chemical effects on the thyroid and whether or not the effects were direct or indirect.

Dr. M. Thompson, NIEHS, said the data did not support a direct effect.

Dr. Davis, a second principal reviewer, said that an explanation for the ventral body swelling in mice at the highest dose would be of interest. Dr. McDonald said the increase in liver weight and size would probably account for the swelling. Dr. Davis asked for more precise definitions of the severity code for lesions, i.e., what do minimal, mild and marked mean?

Dr. Silbergeld commented that monitoring of defects on porphyrin metabolism might be a sensitive indicator of toxic response yet the method used to measure porphyrins was an insensitive one. She noted the observation of severe neurotoxicity in high dose mice yet no attempt to quantify these observations. Dr. Scala said that since many of these short-term toxicity studies were stand alone experiments, it behooved the NTP to characterize toxic signs as completely as possible. Dr. R. Yang, NIEHS, said this study was designed as part of the Superfund program with emphasis on filling data gaps, and since there were not any good chronic studies on pentachlorobenzene, the emphasis had been more on range finding for further chronic evaluations.
1,2,4,5-Tetrachlorobenzene. Dr. M. M. McDonald, NIEHS, NTP Study Scientist, introduced the short-term toxicity studies of 1,2,4,5-tetrachlorobenzene by reviewing the rationale, experimental design, and results. 1,2,4,5-Tetrachlorobenzene was administered by the dosed feed route to groups of F344/N rats and B6C3F1 mice of both sexes for 14-days and 13-weeks. In the 14-days studies, all rats survived but all mice in the top dose group (3000 ppm) died. The only significant histologic lesions were accumulations of abnormal hyaline droplets in renal cortical epithelium of male rats. In 13-week studies, all rats survived; two female mice in the top-dose group were killed in a moribund condition. Hyaline droplet nephropathy was observed in treated male rats. Centrilobular hepatocellular hypertrophy was observed in the livers of exposed male and female rats. Minimal to mild centrilobular hepatocellular hypertrophy and hepatocyte degeneration were seen in mice. Thyroid follicular cell hypertrophy was present in male and female rats. Decreased values for hemoglobin concentration, mean corpuscular hemoglobin, hematocist, and mean cell volume indicated a moderate, poorly regenerative microcytic anemia in both species. The NOEL for histologic lesions was 30 ppm for female rats; a NOEL was not observed for male rats. The NOEL for histologic lesions in male and female mice was 300 ppm.

Dr. Klaassen, a principal reviewer, stated that some of the more important data should be available in the text; in particular, he thought the thyroxin data should be in a table in the text.

Dr. Longnecker, a second principal reviewer, said a concise integrative summary of previous studies at the front of the report would be helpful. He said the ratio of corn oil to diet should be stated.
D & C Yellow No. 11. Dr. W. C. Eastin, NIEHS, NTP Study Scientist, introduced the short-term toxicity studies of D & C Yellow No. 11 by reviewing the rationale, experimental design, and results. D & C Yellow No. 11 was administered in feed at concentrations up to 50,000 ppm to groups of F344/N rats and B6C3F1 mice of each sex for 14-days or 13-weeks. A separate study was done to determine effects on female rats during a reproductive cycle and on their offspring. Results of 14-day and 13-week studies were similar for both species although dietary intake of chemical in mice was more than twice that in rats. There was no mortality. Liver weights were increased in dosed rats and mice and a dose-related yellow-brown pigment was observed in hepatocytes, Kupffer cells, and biliary epithelium in both sexes of both species, and in tubular epithelium of the kidney in both sexes of rats. Hepatocellular degeneration progressed slightly in severity with time of exposure in rats. In male rats, all dosed groups had increased number and size of hyaline droplets in tubular epithelium. In the perinatal toxicity study, fertility, gestation length, litter size, and pup birth weights were unaffected by treatment. Microscopic evaluation showed lesions in pups in all dose groups similar to those described in liver and kidney of rats in 14-day and 13-week studies including male rat kidney cytoplasmic alterations.

Dr. Garman, a principal reviewer, said the draft report was well written and thorough. He thought the concluding sentence of the Abstract was too general. He recommended that in the concluding sentences the special situation of the hyaline droplet nephropathy in the male rat should be discussed separately from the other toxic effects.

Dr. Silbergeld, a second principal reviewer, said the study was acceptable within its own design. Her major criticism, however, was with the study design itself rather than study conduct. Given that the major clinical observations reported in the literature with D&C Yellow No. 11 relate to its allergenic properties, she found it puzzling that the NTP studies were not done by the dermal route, and that immunotoxicology and dermatotoxicity studies were not done. Dr. Eastin said the nomination to study this color additive specifically requested the oral route because of the potential for ingestion. With regard to dermal exposure, D&C Yellow No. 11 is approved by FDA for external applications and is a component of many topical cosmetic formulations. Dr. Silbergeld thought that the pathology was inadequate for the nervous and female reproductive systems.

Dr. Eastin said current short-term studies are most often conducted as preliminary to chronic studies, but more of the future prechronic studies will likely be designed to address toxicity as a separate question, and Dr. Silbergeld's comments will be helpful in review of those designs.