NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

May 18, 2000
Summary Minutes

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

May 18, 2000
Research Triangle Park, N.C.

The meeting began at 8:30 a.m. on May 18, 2000 in the Rodbell Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the subcommittee are: Drs. John Bailer (Chairperson), James Bus, Linda Chatman, John Cullen, Harold Davis, Norman Drinkwater, Susan Fischer, Stephen Hecht, Michele Medinsky, and Jose Russo. Dr. David Phillips serves as ad hoc consultant to the subcommittee. Drs. Davis, Fischer, Phillips, and Russo were not present. These minutes have been recorded and approved by the Chairperson. They were written by Dr. Larry G. Hart. For information, contact Dr. Mary S. Wolfe, Executive Secretary, at 919-541-3971 or wolfe@niehs.nih.gov.

NTP Technical Report Reviews

Chloral Hydrate. Dr. Frederick Beland, National Center for Toxicological Research (NCTR), introduced the toxicology and carcinogenesis studies of chloral hydrate by noting that the major concern driving initiation of these studies for the FDA was its use as a pediatric sedative. Animal cancer bioassays by other investigators had demonstrated a high incidence of liver tumors in male B6C3F$_1$ mice exposed orally to chloral hydrate (71 to 75 %) contrasted with low incidences of liver tumors in control mice (10 to 15 %). Dr. Beland stated that based on chloral hydrate's use in children and on the tumorigenic effects in mice, the objective of this study was to assess the effect of age and duration of exposure upon the possible tumorigenicity of choral hydrate. He said that the study consisted of both mechanistic studies and a bioassay, and described findings from studies of metabolism and studies of DNA adduct formation. Since there is evidence that choral hydrate does not cause tumors in rats, metabolism was studied in both mice and rats with the thought that differences in metabolism might explain differences in tumor response. After single or multiple doses by gavage, the plasma elimination half-lives in both species for chloral hydrate and two major metabolites, trichloroethanol and its glucuronide, were in minutes, while the metabolite, trichloroacetic acid had a half-life in the order of 8 to 11 hours. Studies looking at formation of a malondialdehyde deoxyguanosine adduct were inconclusive, and like the metabolism studies did not explain the species difference in tumorigenicity. Dr. Beland then described the design of the bioassay in mice which included four different regimens in females ranging from lifetime dosing by gavage in water (Regimen A), to dosing for 3, 6 or 12 months followed by no treatment up to two years (Regimen B), to single doses at 15 or 28 days in females (Regimens C and D), and one regimen in male mice of a single dose at 15 days (Regimen E) followed by no treatment for up to two years. He reported that there were no compound related differences between

sexes in survival or body weights, significant increases in liver weights only at the highest dose and no clinical findings related to chloral hydrate treatment. Dr. Beland commented on compound-related neoplastic and non-neoplastic lesions in mice. The conclusions for carcinogenic activity for the 2-year studies in mice were that:

> Under the conditions of this 2-year gavage study, there was **some evidence of carcinogenic activity** of chloral hydrate in female $B6C3F_1$ mice based on increased incidences of pituitary gland pars distalis adenoma. No increased incidences of neoplasms were seen in female $B6C3F_1$ mice that received a single dose of chloral hydrate at 15 or 28 days of age or in male $B6C3F_1$ mice that received a single dose of chloral hydrate at 15 days of age.
>
> In female $B6C3F_1$mice administered chloral hydrate by gavage for 2 years, there was a significant increase in the severity of hyperplasia in the pituitary gland pars distalis.

Dr. Chatman, a principal reviewer, agreed in principle with the conclusions, noting concerns as to whether dose levels were robust enough to achieve an optimum tumor response, and whether females were the right gender to study. She cited a reference indicating that female mice have a more rapid metabolism and excretion of trichloroacetic acid (TCA) than males. Dr. Joseph Haseman, NIEHS, commented that most chemicals that cause cancer in mice produce it in both sexes but for those chemicals that cause cancer in only one sex, females outnumber males about two to one. Finally, Dr.Chatman commented that since there was not a dose-response for adenomas and the incidence even at the highest dose was similar to the high end of the historical control range, she suggested the appropriate call might be **equivocal evidence of carcinogenic activity**.

Dr. Drinkwater, the second principal reviewer, agreed with the conclusions, but also had concerns about the stated rationale for choosing females for the chronic studies, and a concern in terms of the discussion of the lack of liver tumor development in this study in relation to the Daniel study. He said further discussion of the discrepancy would be appropriate. Dr. Beland agreed he would attempt to better explain why females were chosen and the discrepancies with the Daniel study.

Dr. Gail McCarver, Medical College of Wisconsin, Expert Consultant, the third principal reviewer, agreed in principle with the conclusions, adding that dose needs to be more specifically stated in the Conclusions, and suggested adding to the end of the first sentence the phrase, “in animals treated for two years at the maximum dose of 100 mg/kg.” Dr. McCarver offered some perspective on the clinical value of chloral hydrate in thousands of children in the U.S. daily and the negative impact, its being labelled as causing cancer would have. Thus, the issue of balance is essential in not over interpreting the findings to find an increase in tumors, particularly when there are so much negative data.

There was some discussion about whether or not there were real differences in severity grades of pituitary hyperplasia between control and treated mice. Dr. Chatman stated that severity grading is subjective. Dr. John Bucher, NIEHS, commented that emphasis should be given to the overall incidence of tumors within the context of the corresponding historical control data, and to focus less on the severity grade differences for hyperplasia. Dr. Beland

agreed that the hyperplasia findings should not be a primary factor in determining level of evidence but rather should be viewed along with the incidence of adenomas.

Dr. Chatman moved that the conclusions be changed for the findings in Regimens A and B from **some evidence of carcinogenic activity** to **equivocal evidence of carcinogenic activity** based on a lack of a dose response for the incidences of pituitary gland pars distalis adenomas, and because the percentage incidence at the high dose was similar to the historical control range. Further, she said there should be a statement added that no hepatocarcinogenicity or chloral hydrate lesions were seen. Finally, she proposed removing the last paragraph discussing the severity of hyperplasia of the pituitary gland pars distalis. Dr. Bus seconded the motion. Dr. Cullen moved to amend the motion by retaining the last sentence pertaining to hyperplasia. Dr. Medinsky seconded the motion. Dr. Chatman asked whether the sentence could include incidence as well as severity of hyperplasia, and Dr. Cullen agreed. Dr. McCarver suggested removing 'chloral hydrate lesions' from the sentence: "No hepatocarcinogenicity or chloral hydrate type lesions were seen" since such lesions have not been defined. Dr. Cullen and Dr. Medinsky agreed. Dr. Cullen's revised amendment was accepted with five yes votes to one no vote (Chatman). Moving to the rest of Dr. Chatman's original motion, Dr. Drinkwater pointed out that the only significant increase in pituitary tumors was in the high dose group of Regimen A. Dr. Chatman proposed adding in the specific dose level (100 mg/kg). Dr. Bucher indicated that this would be precedent setting with regards to the bioassay reports in that when a positive response is seen at any dose, we have indicated that this is a positive response in the study. Dr. Medinsky suggested returning to the original first sentence of the conclusion without Regimen A or B, only with **equivocal** rather than **some evidence**. Dr. Beland suggested adding "in female mice treated for two years". There was agreement by the Members. Dr. Mary Wolfe, NIEHS, read the agreed on revised conclusion: "Under the conditions of this 2-year gavage study, there was **equivocal evidence of carcinogenic activity** of chloral hydrate in female B6C3F$_1$ mice treated continuously for two years based on increased incidences of pituitary gland pars distalis adenomas. No increased incidences of neoplasms were seen in female B6C3F$_1$ mice that received a single dose of chloral hydrate at 15 or 28 days of age and in male B6C3F$_1$ mice that received a single dose of chloral hydrate at 15 days of age. No hepatocarcinogenicity was seen under all dosing conditions." The motion was accepted unanimously with six yes votes. In view of the change in level of evidence, Dr. Cullen moved to change his position on retaining the last sentence on hyperplasia, and Dr.Medinsky seconded the motion to delete the sentence. The motion was accepted unanimously with six votes.

Chloral Hydrate (Feed Restriction). Dr. Julian Leakey, NCTR, introduced the toxicology and carcinogenesis studies of chloral hydrate (feed restriction) which differed from the previous study in that the studies were done in male mice and a dietary control component was included. The FDA has been interested in the effects of dietary restriction on tumor incidences in test animals in that several corporations are using dietary restriction to help control survival in chronic bioassays. He said there was concern that dietary restriction will desensitize bioassays by decreasing the rates of chemically induced tumors, and illustrated this with data on liver tumor risk in low weight animals where weight reduction was effected through dosing with a noncarcinogenic chemical or through dietary restriction. Dr. Leakey described the model wherein body weight can be controlled to give a predicted level of tumor incidences as well as controlling survival. He reported that chloral hydrate had no effect on body weights in either the *ad libitum*-fed (*ad lib)* or

the dietary controlled mice. Dr. Leakey said that the only significant tumor responses obtained in either *ad lib* or dietary control groups were liver tumors.

Dr. Leakey discussed some of the mechanistic studies involving looking at induction of liver enzymes by choral hydrate. Cytochrome P450 4A, a marker enzyme for peroxisome proliferation, was induced by 64 weeks at the same chronic dose that produced a significant tumor response. He said there appeared to be a correlation between peroxisome proliferation and liver tumor incidence. Studies by others had shown that the major metabolite of chloral hydrate, trichloroacetic acid (TCA), was a peroxisome proliferator and also a mouse liver carcinogen. Dr. Leakey reported on pharmacokinetic studies showing TCA to be the major metabolite, with a biphasic plasma elimination curve at most concentrations, probably due to enterohepatic circulation of trichloroethanol glucuronide and metabolism back to TCA. Induction of peroxisome proliferator enzymes was greater in dietary restricted than in *ad lib* animals. The conclusions for carcinogenic activity for the 2-year studies in mice were that:

> Under the conditions used in this 2-year gavage study, there was **some evidence of carcinogenic activity** of chloral hydrate in male B6C3F$_1$ mice based on increased incidences of hepatocellular adenoma or carcinoma (combined) in *ad libitum*-fed mice and on increased incidences of hepatocellular carcinoma in dietary-controlled mice. In the dietary-controlled mice, the dose response for hepatocellular carcinoma was similar to that for the induction of enzymes that are markers for peroxisome proliferation.

Dr. Drinkwater, a principal reviewer, agreed with the conclusions. However, he thought that the last sentence of the conclusions, which implies the induction of liver tumors in dietary-controlled mice to be a consequence of peroxisome proliferation, was not solidly based. Dr. Leakey responded that it would have been desirable to have had a higher top dose. He said that further experimentation, such as carcinogenicity bioassays using PPAR knockout mice, would be necessary to confirm such a mechanism.

Dr. Chatman, the second principal reviewer, agreed with the conclusions. She agreed with Dr. Drinkwater that the last sentence of the conclusions was not fully supported and should be removed. Dr. William Allaben, NCTR, suggested such speculation would be more appropriate in the Discussion. Dr. Leakey agreed, noting it was in the Discussion. With regard to the liver changes, Dr.Chatman asked whether there were increased incidences of preneoplastic changes such as foci or hypertrophy. Dr. Thomas Bucci, Pathology Associates International, stated that there were no increases in preneoplastic lesions.

Dr. McCarver, agreed in principle with the conclusions but would include commentary on dosage, i.e., "… increased incidences of hepatocellular adenoma or carcinoma (combined) in *ad libitum*-fed mice in the 25 and 50 mg/kg groups and on increased incidences of hepatocellular carcinoma in dietary-controlled mice treated with 100 mg/kg." Further, she would soften the last sentence by inserting the word "somewhat" in front of "similar".

Dr. Drinkwater wondered whether lower effects seen in the current study compared to the Daniel study might relate to the intermittent dosing used (bolus administration - 5 days/week) contrasted with continuous dosing used (drinking water) in the Daniel study. Dr. Leakey replied that this could certainly be the case because TCA plasma levels would

fall between dosing and peroxisome proliferation is quite reversible. Dr. Bailer suggested that in the dose response model where one is looking at the concentration gradient to also include animal weight as a covariable as part of the analysis. He thought that through the mechanism of modeling, one might end up with some insights into the impact of weight.

Dr. Drinkwater moved that under the conditions of this study the Technical Report on chloral hydrate (feed restriction) be accepted with revisions discussed including removal of the last sentence of the Conclusion pertaining to peroxisome proliferation with the conclusion as written for male mice, stating **some evidence of carcinogenic activity.** Dr. Chatman seconded the motion. Dr. Bus moved to amend the motion by changing the conclusion to **equivocal evidence of carcinogenic activity**. He based this on lack of a dose response for tumors in the *ad lib* groups, and in the dietary-controlled group, where increased incidence was seen only in carcinomas but when combined with adenomas the significance was lost. Dr. Hecht seconded the amendment. After discussion about the definitions of the levels of evidence, the Subcommittee rejected the amended motion by four no votes (Chatman, Cullen, Drinkwater, Medinsky) to two yes votes (Bus, Hecht).

Returning to the original motion by Dr. Drinkwater, Dr. Bus argued for retaining some form of the last sentence in that the issue of peroxisome proliferation in rodents in terms of its relationship to human cancer is a center of scientific debate. Dr. Drinkwater agreed to retain a modified version of the last sentence which reads: "In the dietary controlled mice, induction of enzymes associated with peroxisome proliferation was observed at higher doses." The motion was accepted by five yes votes to one no vote (Hecht).

Indium Phosphide. Dr. J.H. Roycroft, NIEHS, introduced the toxicology and carcinogenesis studies of indium phosphide by discussing the uses and rationale for study, describing the experimental design in rats and mice, reporting on survival and body weight effects, and commenting on compound-related neoplastic and non-neoplastic lesions in male and female mice and rats. Additionally, tissue burden (lung deposition and clearance) studies were conducted in mice and rats from the 14-week and 2-year studies. Based on significant increases in lung weights and severity of lung lesions observed in 14-week studies, and on lung burden estimates, 2-year exposure concentrations for mice and rats were 0, 0.03, 0.1, or 0.3 mg/m^3 . Due to increases in lung weights and spectrum and severity of lung lesions observed at a 13-week interim evaluation, the decision was made to discontinue exposure of mice and rats in the 0.1 and 0.3 mg/m^3 groups at 21 weeks with the groups allowed to continue unexposed for the remainder of the 2-year period. The conclusions for carcinogenic activity for the two-year studies in mice and rats were that:

> Under the conditions of these 2-year inhalation studies, there was **clear evidence of carcinogenic activity** of indium phosphide in male and female F344/N rats based on the increased incidences of benign and malignant neoplasms of the lung. The increased incidences of pheochromocytomas of the adrenal medulla in males and females were also considered to be exposure related. Marginal increases in incidences of mononuclear cell leukemia in males and females, fibroma of the skin in males, and carcinoma of the mammary gland in females may have been related to exposure to indium phosphide. There was **clear evidence of carcinogenic activity** of indium phosphide in maleB6C3F_1 mice based on increase incidences of malignant neoplasms of the lung and benign and malignant neoplasms of the liver. Marginal increases in incidences of adenoma and carcinoma of the small intestine may have been related to

exposure to indium phosphide. There was **clear evidence of carcinogenic activity** of indium phosphide in female B6C3F$_1$ mice based on increased incidences of benign and malignant neoplasms of the lung and liver.

Exposure to indium phosphide by inhalation resulted in nonneoplastic lesions in the lung of male and female rats and mice, the adrenal medulla of female rats, and the liver and heart of male and female mice.

Dr. Medinsky, a principal reviewer, agreed with the conclusions. She stated that this was a very well written report based on a well designed study. In particular, the discussion section of the report does an excellent job of putting the findings in these studies in context with what is known regarding the mechanisms of action of other lung carcinogens. Dr. Medinsky said that the deposition and clearance studies of indium phosphide in the lung and the toxicokinetic model developed from those studies proved to be extremely valuable in relating tumor incidence to the dose of indium phosphide in the lungs.

Dr. Cullen, the second principal reviewer, agreed with the conclusions. However, he thought that **some evidence of carcinogenic activity** might be more appropriate for the findings on liver tumors in male and female mice in view of limited dose-related responses and the fact that tumor incidences were similar to historical control levels (for mice fed other diets). Dr. Haseman said the liver tumors in mice could be dealt with in a manner analogous to pheochromocytomas in rats, i.e., " The increased incidences of liver tumors in males and females were also considered to be exposure related." Dr. Cullen suggested that in view of the fact that the mechanism of injury for indium phosphide is not known, greater discussion of the significance of grouping the animals on the basis of the dose administered or the total lung burden and the effects of the duration of exposure may be useful. Dr. Roycroft responded that he would try to make it as clear as possible in the Results and Discussion sections whether we are talking about continuous exposure vs. stop exposure. Dr. Haseman explained that in terms of the statistical analyses there was no attempt made to rank the continuous versus the stop exposures, and the dose-response trend reported was based strictly on the two stop exposure and control groups.

Dr. Bus, the third principal reviewer, agreed with the conclusions. He commented that the analyses of tissue concentrations of indium phosphide were a valuable component of the study, with the information providing a more accurate assessment of internal dosimetry as well as confirming that the exposure conditions, despite causing pulmonary tumors, likely were not a result of pulmonary particle overload.

In further discussion, Dr. Cullen commented that he had trouble trying to compare the discontinuous and continuous dosed animals as to whether there was a clear dose-related effect. Dr. Roycroft noted that although the external exposure of the two higher dosed groups was only 21 weeks, the tissue clearance of the indium phosphide was extremely slow such that at the end of two years about 25% of the deposited material remained in the lung. Dr. Bailer said he would like to have an idea of the precision associated with area under the curve (AUC) estimates, such as standard errors. Dr. Medinsky speculated that during the 2-year exposure period, the earlier doses might be more important and thought the important dosemetric might be some weighted area under the curve giving more weight to the earlier doses. Dr. Cullen stated that he still had trouble including liver tumors in mice

under **clear evidence** in that there were treatment related but not dose-related effects. Dr. Bus suggested using the wording mentioned by Dr. Haseman.

Dr.Medinsky moved that under the conditons of this study the Technical Report on indium phosphide be accepted with revisions discussed and the conclusions as written for male and female rats, **clear evidence of carcinogenic activity,** and for male and female mice, **clear evidence of carcinogenic activity**, except that in mice, the citation for liver neoplasms would be included in a separate sentence to read: "The increased incidences of benign and malignant neoplasms of the liver in males and females were also considered to be exposure related." Dr.Cullen seconded the motion. In discussion, Dr. Haseman pointed out that the trend test for adenomas in males is quite significant, and carcinomas in males are increased. Dr. Richard Hailey, NIEHS, affirmed that there was a much stronger response in males. Dr. Medinsky asked that her motion be amended to retain the citation for liver neoplasms in male mice under **clear evidence**, while leaving the citation for liver neoplasms in female mice in the separate sentence. Dr. Cullen agreed to this change. The revised motion was accepted unanimously with six yes votes.

Naphthalene. Dr. K.M. Abdo, NIEHS, introduced the toxicology and carcinogenesis studies of naphthalene by discussing the uses and rationale for study, describing the experimental design in rats, reporting on survival and body weight effects, and commenting on compound-related neoplastic and non-neoplastic lesions in male and female rats. Dr. Abdo reported on a NTP 2-year inhalation study in $B6C3F_1$ mice reported in 1992 which found that naphthalene was carcinogenic in female mice resulting in an increased incidence of alveolar/bronchiolar adenomas. He noted that the CDC had analyzed urine samples from nearly 1,000 adults for the metabolites of naphthalene, 1-naphthol and 2-naphthol, and found metabolites in over 80% of the samples, suggesting a widespread human exposure. The conclusions for carcinogenic activity for the 2-year studies in rats were that:

> Under the conditions of this 2-year inhalation study, there was **clear evidence of carcinogenic activity** in male and female F344/N rats exposed to 10, 30, or 60 ppm
> of naphthalene based on increased incidences of respiratory epithelial adenoma and olfactory epithelial neuroblastoma of the nose. In male and female rats, exposure to naphthalene caused significant increases in the incidences of nonneoplastic lesions of the nose.

Dr. Ronald Melnick, NIEHS, presented information on toxicokinetic modeling efforts aimed at estimating amounts of naphthalene inhaled by rats and mice at exposure concentrations used in the 2-year studies, estimating amounts of inhaled dose metabolized during the 6-hour exposure and an 18-hour post exposure period, estimating steady state concentrations of naphthalene in lung and liver during exposure, and estimating rates of metabolism in lung and liver at steady state. For rats there were also multiple exposures in that animals were examined after two weeks or 3, 6, 12, or 18 months to see whether kinetic parameters determined from the single exposure changed over time. Dr. Melnick stated conclusions drawn: (1) due to its low vapor pressure and high blood-to-air partition coefficient, most of absorbed naphthalane (internalized dose) is eliminated via metabolism; (2) at 30 ppm, steady state naphthalene concentration is slightly greater in the mouse lung than in the rat lung, but concentration is less in 30 ppm mice than in 60 ppm rats; (3) rate of naphthalene metabolism is higher in the mouse lung than in rat lung; and (4) data are insufficient to

adequately estimate tissue concentrations of naphthalene oxide, the putative carcinogenic intermediate.

Dr. Cullen, a principal reviewer, agreed with the conclusions. He said that as nasal adenomas are uncommon tumors, their discussion needs to be supplemented as well as their likelihood to progress. Further, given the significant background on nasal inflammation and limited evidence of genetic toxicity, the role of inflammation in genesis of these lesions needs to be considered. Dr. Ronald Herbert, NIEHS, said the discussion on nasal adenomas would be expanded. Dr. Hailey reported that he and Dr. Haseman had looked at the 10 NTP studies where there had been an increase in nasal carcinogenesis, and in the two studies where there was the most severe degree of inflammation, there had been the fewest numbers of tumor. Dr. Cullen noted that neuroblastomas are uncommon in humans as well as rats, and said discussion of biological relevance to human health risk is warranted.

Dr. Medinsky, the second principal reviewer, agreed with the conclusions. She said that her major criticism was that the pharmacokinetic model for naphthalene disposition in rats didn't include a nasal compartment, which in view of the only carcinogenic effect being seen in the nose, provided a disconnect for her. Dr. Medinsky noted there are data suggesting the particular isozyme that metabolizes naphthalene is present in the nose, and naphthalene's high partition coefficient suggests likely nasal deposition. Dr. Melnick responded that we would like to include a nasal compartment, and as she suggested a way may be to combine the PBPK model to a fluid dynamic model. A difficulty lies in not having data such as naphthalene deposition in nasal mucosa and mucus/air partition coefficients.

Dr. Hecht, the third principal reviewer, agreed with the conclusions.

Dr. McCarver asked if there was information on human levels of naphthalene or metabolites and how these would compare with levels in the toxicokinetic studies. Dr. George Lucier, NIEHS, surmised that they would be two orders of magnitude lower for average individuals, not necessarily occupationally exposed.

Public Comments. Mr. Ron Landis, Landis and Associates, representing the Naphthalene Panel of the Chemical Manufacturers Association (CMA) commented that for completeness statements should be added to the Results section that there was an apparent decrease in thyroid tumors with treatment, that the incidences of tumors in all organs were not increased with naphthalene exposure, and that the findings of toxicokinetic analysis indicate that lung metabolism is saturated over the dose range studied, a finding perhaps of importance to risk assessment. Finally, Mr. Landis thought the lack of causation of significant lung pathology from lifetime exposure to naphthalene should be included in the Discussion section

Dr. Cullen moved that under the conditions of this study, the Technical Report on naphthalene be accepted with revisions discussed and the conclusions as written for male and female rats, **clear evidence of carcinogenic activity**. Dr. Drinkwater seconded the motion, which was accepted unanimously with six yes votes.

Sodium Nitrite. Dr. Po Chan, NIEHS, introduced the toxicology and carcinogenesis studies of sodium nitrite by discussing the uses and rationale for study, describing the

experimental design in mice and rats, reporting on survival and body weight effects, and commenting on compound-related neoplastic lesions in female mice, possible compound-related neoplastic lesions in female rats, and compound-related non-neoplastic lesions in male and female rats, and male mice. The conclusions for carcinogenic activity for the 2-year studies in rats and mice were that:

> Under the conditions of this 2-year drinking water study, there was **no evidence of carcinogenic activity** of sodium nitrite in male F344/N rats exposed to 750, 1,500, or 3,000 ppm. There was **equivocal evidence of carcinogenic activity** in female F344/N rats based on increased incidences of fibroadenoma and multiple fibroadenoma of the mammary gland. There was **no evidence of carcinogenic activity** of sodium nitrite in male B6C3F$_1$ mice exposed to 750, 1,500, or 3,000 ppm. There was **some evidence of carcinogenic activity** of sodium nitrite in female B6C3F$_1$ mice based on an exposure-related increase in the incidences of squamous cell papilloma or carcinoma (combined) of the forestomach.
>
> Exposure to sodium nitrite in drinking water resulted in increased incidences of epithelial hyperplasia in the forestomach of male and female rats and in the glandular stomach of male mice.
>
> Decreased incidences of mononuclear cell leukemia occurred in male and female rats.

Dr. Frank Ye, NIEHS, presented statistical analyses and information on the toxicokinetic modeling of nitrite absorption and elimination and methemoglobinemia in rats and mice. Objectives were (1) to study the relationship between nitrite and methemoglobinemia by developing a toxicokinetic model to characterize the nitrite induced methemoglobin process, and (2) to compare net absorption and elimination rates of nitrite from plasma. Dr. Ye displayed a simple model which takes advantage of simultaneous observations of nitrite, hemoglobin, and methemoglobin, to characterize the pattern of relationship between the nitrite in plasma and the hemoglobin. The studies found that nitrite is rapidly absorbed after oral exposure, and it may depend on dose level. Overall clearance of nitrite may depend on species, route, and dose, and based on the model, it can be said that initially, nitrite is rapidly cleared from plasma due to binding to hemoglobin causing oxidation to methemoglobin. Finally, reduction of hemoglobin to its ferrous form is sensitive to (1) basal methemoglobin reductase activity, (2) strong binding of nitrite to methemoglobin, and (3) sensitivity to the autocatalytic cycle.

Dr. Hecht, a principal reviewer, agreed with the conclusions in rats and male mice but did not agree with the evaluation in female mice for which he thought the data only supported **equivocal evidence of carcinogenic activity.** Dr. Hecht's other major criticism was that the NTP had ignored a lot of the literature on endogenous formation of nitrosamines and this needed to be updated and expanded.

Dr. Bus, the second principal reviewer, agreed with the conclusions in male rats and mice but disagreed with the conclusions in female rats and mice. For female rats, he argued that the non-dose dependent response in mammary gland (increased fibroadenoma only at the mid-dose) occurred in the presence of high concurrent and historical control incidences of this lesion, and with no increases in carcinomas, thus supporting **no evidence**. Dr. Bucher said the incidence in the mid-dose group was the highest we had seen so did not feel we

could dismiss the finding. For female mice, Dr. Bus said it should be emphasized that the elevated incidence of forestomach tumors in the high dose was not statistically significant from control, and statistical significance for forestomach tumors (combined) was observed only in the trend test, thus supporting only **equivocal evidence.** Dr. Chan agreed that the level of evidence in female mice could be debated; however, the positive trend for papillomas and carcinomas combined and the incidence in high dose females exceeding the historical range for NTP controls on NTP-2000 diet lent support to the conclusion of **some evidence**. Further, he found it hard to ignore the two carcinomas.

Dr. Chatman, the third principal reviewer, disagreed with the conclusions for female rats and mice, agreeing with Dr. Bus that the level of evidence in female rats should be **no evidence**, and in female mice, **equivocal evidence.** She wondered why drinking water was chosen rather than dosed feed as the route of administration for the 2-year study.

Dr. Hailey addressed a comment by Dr. Bus and by Dr. Gary Williams earlier concerning incidence of gastrointestinal neoplasms in mice on the NTP-2000 diet compared with the NIH-07 diet. He said there was an increase noted in intestinal neoplasms but not in forestomach neoplasms. Dr. Bailer suggested that significant negative trends for tumors such as for mononuclear cell leukemias in male and female rats warranted more attention in the report.

Public Comments. Dr. Larry L. Borchert, Director of Research (Retired), Oscar Mayer Foods Corporation and Adjunct Professor, Meat Science and Muscle Biology, University of Wisconsin-Madison, representing the American Meat Science Association provided historical background on the food industry's need to use sodium nitrite and the safety of sodium nitrite as a food additive. He said the U.S. Department of Agriculture and its prececessors have permitted use of sodium nitrite in processed meat products since 1925, when it was proven safe for human consumption. The industry welcomed this because it accelerated the curing process from days and weeks to hours. To meet safety concerns arising in the 70s, Dr. Borchert stated that the industry (1) eliminated sodium nitrate, (2) reduced usage levels of sodium nitrite, (3) incorporated sodium ascorbate into all processed meat products, and (4) monitored nitrosamine formation in fried bacon. A national survey conducted in 1996 showed that processed meats in the U.S. contain about 10 ppm of sodium nitrite and no sodium nitrate.

Dr. Douglas Archer, Food Science and Human Nutrition Department, University of Florida, representing the Food Safety Advisory Committee of the American Meat Institute Foundation, said that he intended to highlight some of the positive aspects of having sodium nitrite in our food supply by covering four points. First, he stressed the positive physiological role of nitric oxide and its metabolites, including nitrite. Second, that nitrite is physiologically an endogenous compound. Third, he stressed the profound effect of sodium nitrite in preventing growth and toxin production by *Clostridium botulinum*. Fourth, he stressed the growth retardant role that sodium nitrite plays on food pathogens as a bactericidal agent in conjunction with stomach acid. Dr. Archer closed by emphasizing that nitrite is an endogenous compound that at physiological levels has no toxic effects but contributes significantly to human well being, and food safety is enhanced by the protective action of sodium nitrite in processed meat and meat products.

Dr. Gary Williams, New York Medical College, representing the American Meat Institute Foundation, stated that the small incidence of forestomach tumors seen in high dose female mice at the end of the study is not attributable to sodium nitrite, for several reasons. One, the high pHs of the female mouse forestomach and sodium nitrite solutions used were not conducive to formation of DNA-deaminating nitrous acid or of carcinogenic N-nitroso compounds, and there was no evidence for this reaction. Second, the genetic toxicology database for sodium nitrite supports the view that it is not an *in vivo* genotoxic agent. Third, the new NTP-2000 diet appears to facilitate development of more spontaneous gastrointestinal neoplasms than does the NIH-07 diet, so there is no adequate historical database for forestomach tumors with the new diet. Fourth, sodium nitrite has not been shown to be a forestomach carcinogen in rats in this bioassay or the scientific literature.

Dr. Hecht moved that under the conditions of this study the Technical Report on sodium nitrite be accepted with revisions discussed including changes in the conclusions for female rats and female mice. The proposed revised conclusions were for male and female rats and male mice, **no evidence of carcinogenic activity,** and for female mice, **equivocal evidence of carcinogenic activity**. Dr. Bus seconded the motion. The staff agreed to put more discussion in the text on interpretation of the increased incidences of fibroadenomas in the 1500 ppm exposure group of female rats. The motion was accepted unanimously with six yes votes.

***p,p'*-Dichlorodiphenyl Sulfone**. Dr. Raj Chhabra, NIEHS, introduced the toxicology and carcinogenesis studies of *p,p'*-dichlorodiphenyl sulfone (DDS) by discussing the uses and rationale for study, describing the experimental design in mice and rats, reporting on survival and body weight effects, and commenting on compound-related non-neoplastic lesions in male and female rats and mice. The conclusions for carcinogenic activity for the two-year studies in mice and rats were that:

> Under the conditions of these 2-year feed studies, there was **no evidence of carcinogenic activity** of *p,p'*-dichlorodipheny sulfone in male F344/N rats exposed 10, 30, or 100 ppm or in female F344/N rats exposed to 30, 100, or 300 ppm. There was **no evidence of carcinogenic activity** of *p,p;*-dichlorodiphenyl sulfone in male or female B6C3F$_1$ mice exposed to 30, 100, or 300 ppm.
>
> Exposure to *p,p'*-dichlorodiphenyl sulfone for 2 years caused increased incidences of nonneoplastic lesions of the liver in male and female rats and mice.

Dr. Fred Parham, NIEHS, said the objective of the toxicokinetic study was to characterize the absorption, distribution, metabolism, and elimination of DDS in rats and mice under the conditions of the 2-year bioassay. Data sources were time-course data for radiolabeled DDS in tissues and excreta after intravenous injection, similar data after single gavage doses, and plasma concentrations of DDS in rats and mice after 2 weeks, and 3, 12, and 18 months of exposure in feed. He said the pharmacokinetic model used was similar to the one used in the naphthalene study reported earlier, and demonstrated a nonlinear metabolism of DDS by a Michaelis-Menten mechanism in the liver. Conclusions from the toxicokinetic studies were that absorption of DDS was very rapid, first pass liver extraction was very low while the amount metabolized within the first day is higher. DDS induces enzymes involved in its metabolism, and elimination half-lives were higher in rats (147-321 hours) than in mice (55-80 hours), Noting the toxicokinetic presentations made for three of the day's

studies. Dr. Medinsky said it would be helpful if the NTP could standardize the presentations. Dr. Christopher Portier, NIEHS, agreed, and asked for her input in moving toward developing a more standard format.

Dr. Hecht, a principal reviewer, agreed with the conclusions.

Dr. Bus, the second principal reviewer, agreed with the conclusions. He questioned the designation of a positive response for the mouse micronucleus test, perhaps because of a low control value in the second replicate, and thought equivocal might be more appropriate. Dr.Chhabra responded that he discussed this with a genetic toxicologist and concluded that the finding was inconclusive.

Dr. Drinkwater, the third principal reviewer, agreed with the conclusions. He commented that a dose-dependent increase in eosinophilic foci in the liver of female mice along with the ability of DPS to cause microsomal enzyme induction and hepatomegaly are consistent with activity of the chemical as a weak hepatic tumor promoter, and this should be discussed. Dr. Chhabra agreed and said he would clarify this in the discussion.

Dr. Drinkwater moved that under the conditions of this study the Technical Report on *p,p'*-dichlorodiphenyl sulfone be accepted with revisions discussed and the conclusions as written for male and female rats and mice, **no evidence of carcinogenic activity**. Dr. Cullen seconded the motion, which was accepted unanimously with five votes (Dr. Hecht was absent for the vote).

Presentation: **Effect of NTP-2000 Diet on Body Weight, Survival and Lesions in Rodents of the NTP Toxicology and Carcinogenesis Studies --** Dr. G.N. Rao, NIEHS, began by discribing the characteristics of the NIH-07 diet which had been used since the inception of the NTP in 1979 until 1994. He noted that the protein composition (23 %) was almost double that recommended by the National Research Council (NRC) Subcommittee on Animal Nutrition, which probably was associated with a high incidence of nephropathy in most strains of rats in long-term studies, especially in males. Other concerns were with the calcium/phosphorous ratio and high levels of vitamin D. Dr. Rao reported that beginning in 1987 various modified diets were evaluated to meet these concerns. Coincident with these evaluations, it was noticed that corn oil gavage used in NTP studies resulted in decreased leukemia and increased survival in Fischer 344 rats. This led to development of the NTP-90 diet which resulted in marked improvement in health of rats and a decreased tumor load in mice. Dr. Rao said that following a workshop at NIEHS in 1993 to review all the information and revision of rodent diet guidelines by the NRC Subcommittee and the American Institute of Nutrition Committee on Animal Nutrition, the final formulation of the NTP-2000 diet was prepared. Dr. Rao compared nutrient composition of the new diet with the NIH-07, noting for the new diet a higher fat and fiber content, lower protein, and much lower Vitamin D content. During the last half of 1994, project managers were given the option of using the new or old diets, and studies begun on the NIH-07 diet were completed on that diet. Dr. Rao compared studies completed in the 1990s in rats, both non-inhalation and inhalation studies, with NIH-07 and NTP-2000 diets for growth, final body weight, and survival over 2 years. The most striking differences were increased survival in male rats and to a lesser extent in females on the NTP-2000 diet. Dr. Rao then compared animals on the two diets for degree and severity grade of two common nonneoplastic lesions in rats, cardiomyopathy and nephropathy. For animals on NTP-2000, there was a shift to

lower severity grades for both lesions compared with animals on NIH-07. Further, water consumption in NTP-2000 rats was decreased. Dr. Rao reported on decreases in certain tumors in rats on NTP-2000 compared with NIH-07, especially pheochromocytoma, mammary tumors, and leukemias in males, and mammary tumors, anterior pituitary tumors, and leukemias in females. Dr. Rao briefly discussed the effects of NTP-2000 compared with NIH-07 diet in mice, noting the findings were similar to those seen in rats. He said there was no real change in body weight in males, maybe a slight decrease in females in non-inhalation studies, a significant increase in survival especially in females and a marked decrease in liver tumors in females. Dr. Rao concluded by addressing concerns expressed about irradiation of the diet to minimize bacterial or viral infection and mold since it is not practical to autoclave the diet. He stated that irradiation is not contributing to any significant loss of nutrients or increase in toxic contaminants such as peroxides.

Public Comment: Dr. Gary Williams, New York Medical College, representing the American Meat Institute Foundation, presented some concerns with the NTP-2000 diet, particularly with regard to the Technical Report for the sodium nitrite bioassay to be reviewed today, and for which the only positive finding is in mice, a species for which there is much less experience with the NTP-2000 diet than in rats. He noted that in three NTP bioassays to be peer reviewed in the afternoon, there are forestomach tumors and small intestine tumors in controls for two (sodium nitrite, indium phosphide), events that would be much less likely in control mice on the NIH-07 diet. Dr. Williams raised the questions of whether there is a possibility of formation of lipid peroxides due to the higher lipid content of the new diet, and increased retention time in the stomach due to higher lipid and reduced water consumption. He concluded by suggesting that there is not yet a good historical control database for comparing tumors with tumors seen in mice on the NIH-07 diet. Dr. Rao rebutted that NTP-2000 was not a high fat diet, rather the percentage was only 2.8 % more than in the NIH-07 diet. With regard to increased stomach retention time, he commented that the increased fiber should offset the increased lipid resulting in little change in retention times.