Board of Scientific Counselors
National Toxicology Program

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

on

December 9-10, 1997

Research Triangle Park, N.C.

The meeting began at 1:30 p.m. on December 9 and 8:30 a.m. on December 10 in the Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the Subcommittee are: Drs. Gary Carlson (Chairperson), John Bailer, Steven Belinsky, James Bus, Linda Chatman, John Cullen, Susan Fischer, Thomas Goldsworthy, and Irma Russo. All members were present. Additionally, there were three *ad hoc* expert consultants present: Dr. Stephen Hecht, University of Minnesota Cancer Centers; Dr. Michele Medinsky, Chemical Industry Institution of Toxicology; and Dr. Jose Russo, Fox Chase Cancer Center. These minutes have been reviewed and approved by the Chairperson. There were written by Dr. Larry G. Hart, Executive Secretary.

When available, a final NTP Technical Report for the studies may be obtained through the Environmental Health Information Service (EHIS). Call 919-541-3841, Fax 919-541-0273, e-mail at ehis@nih.gov, or subscribe on line at ehis@niehs.nih.gov.

The next NTP technical reports peer review meeting will be held March 11, 1998, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, 919-541-3971.

SUMMARY MINUTES
NTP TECHNICAL REPORTS REVIEW SUBCOMMITTEE MEETING
DECEMBER 9-10, 1997

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Presentations

NTP Technical Report Reviews

Diethanolamine. Dr. R.D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of diethanolamine 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 male and female mice and non-neoplastic lesions in male and female rats and mice. Dr. Irwin noted that a major use of diethanolamine is in preparation of diethanolamides of long chain fatty acids. These diethanolamine condensates as they are called are used extensively in preparations such as bath oils, shampoos, and hair dyes, and contain varying amounts of free diethanolamine. The conclusions for the two-year studies in rats and mice were that:

> Under the conditions of these 2-year dermal studies, there was **no evidence of carcinogenic activity** in male F344/N rats administered 16,32, or 64 mg/kg diethanolamine or in female F344/N rats administered 8, 16, or 32 mg/kg. There was **clear evidence of carcinogenic activity** of diethanolamine in male and female B6C3F$_1$ mice based on increased incidences of liver neoplasms in males and females and increased incidences of renal tubule neoplasms in males.
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> Dermal administration of diethanolamine to rats was associated with increased incidences of acanthosis (males only), hyperkeratosis, and exudate of the skin and increased incidences and severities of nephropathy in females. Dermal administration of diethanolamine to mice was associated with increased incidences of cytoplasmic alteration (males only) and syncytial alteration of the liver, thyroid gland follicular cell hyperplasia, and hyperkeratosis of the skin.

Dr. Goldsworthy, a principal reviewer, agreed with the conclusions. He said that since a majority of tumor responses observed in the studies of the condensates were concluded to result from the presence of free diethanolamine, some of his comments would pertain not to just diethanolamine but also the condensates. Dr. Goldsworthy commented that the report should address if and how the distribution and metabolism of diethanolamine would be altered at various tested concentrations and by the potential interactions with the different condensates. He said that besides trying to link diethanolamine levels with neoplastic responses, it would be useful to chart comparative toxicities between the condensates and diethanolamine levels as well as the possibility of nitrosamine formation. Dr. Goldsworthy asked about the significance of the hepatoblastomas in treated male mice. Dr. J.R. Hailey, NIEHS, said they are a neoplasm with a fairly distinct morphology composed of fairly primitive appearing cells, and appear to be part of the spectrum of the progression of liver neoplasms within the mouse, and as such with the higher background rate of liver neoplasms in mice, there is a concomitant increase in the incidence of hepatoblastomas.

Dr. Bailer, the second principal reviewer, agreed in principle with the conclusions. He said the conclusions should be modified to note the significant negative trend in female rat

mammary gland fibroadenomas and the increased survival experienced by rats exposed to diethanolamine. Dr. J. Haseman, NIEHS, said that a decrease in mammary gland tumors is often associated with reduced body weight but not in this case, so more discussion might be merited. Dr. Bailer commented that the high control liver tumor rates in mice (adenomas: 62 % in males and 64 % in females) needed to be addressed, and placed in the context of recent experience. He said that this points up the importance of the concurrent controls in these studies, and even more so because the historical control data base is so small for dermal studies using an ethanol vehicle.

Dr. Chatman, the third principal reviewer, did not agree with the conclusions for mice. She stated that diethanolamine is not a mutagen and is not metabolized to a reactive intermediate but can be converted to a carcinogenic nitrosamine. The possibility of formation of N-nitrosodiethanolamine should have been measured. Dr. Chatman referred to a letter received by the reviewers from the Alkanolamines Panel of the Chemical Manufacturers Association (CMA) which reported that rodent feed during some weeks of the studies was contaminated with high bacterial counts. She thought this could have aided in the formation of N-nitrosodiethanolamine. Dr. Irwin responded that published studies with N-nitrosodiethanolamine given in drinking water show it to be a potent liver carcinogen in Fischer rats while being a noncarcinogen in B6C3F$_1$ mice.

There were questions about the possible impact of *Helicobacter hepaticus* on the incidence of liver neoplasms in mice. Dr. Hailey said there were frozen tissues available from about 20 animals, 10 males and 10 females, in which PCR analysis for *H. hepaticus* was negative. Dr. Goldsworthy asked for comment on the impact of increasing liver tumor rates in control mice relative to interpretation of bioassay results. Dr. Hailey replied that in view of higher background incidence, other components have to be assessed, especially progression to a malignant state and increases in numbers or multiplicity, and both were dramatically increased in these studies. Dr. Hecht agreed that formation of nitrosamines was not likely but was disappointed with the lack of detail on analytical methods description so contamination of diethanolamine with N-nitrosodiethanolamine could not be ruled out. Dr. Irwin said he would increase the detail on the analytical methods. Dr. G. N. Rao, NIEHS, stated that standards for the NIH-07 diet used since 1984 are much more stringent than most commercially available diets with regard to allowable bacterial counts.

Public Comment: Dr. William Stott, Dow Chemical Company, representing the Alkanolamines Panel of the CMA, said that their major concerns with the study had to do with lack of early availablity of the draft report, technical problems with the bioassay, and the inconsistency of the findings with regard to genotoxicity and carcinogenicity data bases. Among technical problems which should be better discussed in the report were the route of administration allowing for ingestion of chemical, use of an ethanol vehicle which has potential promotional/cocarcinogenic effects itself, potential for nitrosamine formation *in vivo,* and high liver tumor incidence in control mice. Dr. Stott reported that the

Alkanolamines Panel plans to conduct mechanistic studies to help understand the NTP mouse bioassay results and their relevance to humans.

Dr. Goldsworthy moved that under the conditions of this study the Technical Report on diethanolamine be accepted with revisions discussed and the conclusions as written for male and female rats, **no evidence of carcinogenic activity,** and for male and female mice, **clear evidence of carcinogenic activity**. Dr. Bailer seconded the motion, which was accepted with six yes votes to one no vote (Chatman) and one abstention (Bus). Dr. Bus abstained for reasons of company affiliation.

Coconut Oil Acid Diethanolamine Condensate. Dr. R. D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of coconut oil acid diethanolamine condensate 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 lesions in female rats and male and female mice, and on compound-related non-neoplastic lesions in male and female rats and mice. The conclusions for the two-year studies in rats and mice were that:

> Under the condtions of these 2-year dermal studies, there was **no evidence of carcinogenic activity** of coconut oil acid diethanolamine condensate in male F344/N rats, administered 50 or 100 mg/kg. There was **equivocal evidence of carcinogenic activity** in female F344/N rats based on increased incidences of renal tubule neoplasms in the 50 mg/kg group. There was **clear evidence of carcinogenic activity** in male $B6C3F_1$ mice based on increased incidences of hepatocellular neoplasms. These increases were attributed to the concentration of free diethanolamine present as a contaminant.
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> Exposure of rats to coconut oil diethanolamine condensate by dermal application in ethanol for 2 years resulted in epidermal hyperplasia, sebaceous gland hyperplasia, hyperkeratosis, and parakeratosis in males and females and ulcer in females in the skin at the site of application. There were increases in the incidences of chronic inflammation, epithelial hyperplasia, and epithelial ulcer in the forestomach of female rats.
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> Exposure of mice to coconut oil acid diethanolamine condensate by dermal application for 2 years resulted in increased incidences of eosinophilic foci of the liver in males. Increased incidences of epidermal hyperplasia, sebaceous gland hyperplasia, and hyperkeratosis in males and females, ulcerations in males, and parakeratosis and inflammation in females in the skin at the site of application and of follicular cell hyperplasia in the thyroid gland of males and females were chemical related.

Dr. Irwin discussed a logistic regression model designed to look at the association between hepatocellular neoplasms in female mice and diethanolamine concentration in a quantitative way. He said this tended to strengthen the hypothesis that increased incidences of hepatocellular neoplasms in mice at a minimum are associated with diethanolamine exposures. This model was applied to the other two diethanolamine condensates as well.

Dr. I. Russo, a principal reviewer, agreed with the conclusions.

Dr. Goldsworthy, the second principal reviewer, agreed with the conclusions. However, he argued that the statement wholly attributing tumor responses to free diethanolamine does not appear warranted and needs to be softened. This association is mainly supported by data in female mice, while gaps of information and lack of definitive conclusions exist for

the liver data as well as for other tumor sites. He said correlations were not assessed for an association of liver pathology or liver tumors in male mice. Further, the linkage of hepatic tumor formation with concentrations of free diethanolamine clearly does not associate when evaluating hepatoblastoma occurrence. Dr. Irwin agreed that there may be responses not associated with diethanolamine and we would try to highlight those.

Dr. Hecht, the third principal reviewer, agreed with the conclusions. He said it would have been much more satisfying to have tested the material in the absence of diethanolamine although he realized the strategy was to test the products as they actually are used. Dr. J. Bucher, NIEHS, agreed but because this a safety assessment issue, the materials as they are used in cosmetics was the determining factor. Dr. Hecht asked for some discussion of the significance of the liver tumors in a strain of mouse that already has a considerable spontaneous incidence. This along with the fact that no other substantial tumor responses were observed suggests that this material is a weak carcinogen.

Dr. I. Russo questioned the use of ethanol as solvent when coconut oil acid diethanolamine condensate is water soluble. Dr. Irwin responded that the primary purpose was to allow comparison of results among all three of the diethanolamides as the other two were not water soluble. Also with water as solvent in dermal studies, the solutions tend to bead up and not spread well.

Public Comment: Dr. Linda Loretz, The Cosmetic, Toiletry, and Fragrance Association (CTFA), said the untimely receipt of the draft reports for all four diethanolamine studies limited CTFA's ability to complete adequate reviews. She said there was inadequate documentation and analysis of the test materials particularly in view of attributing tumor effects of the condensates to diethanolamine. Further, the test animals appear to have ingested some of the material and the use of ethanol as a vehicle was inappropriate and complicates interpretation of study results in that IARC lists consumption of alcoholic beverages as a known human carcinogen. Finally, Dr. Loretz asked that the level of evidence for kidney tumors in male mice be changed from **clear** to **some evidence of carcinogenic activity** as the response was limited to one species, one sex, one dose, and was primarily benign tumors.

Dr. I. Russo moved that the Technical Report on coconut oil acid diethanolamine condensate be accepted with revisions discussed and the conclusions as written for male rats, **no evidence of carcinogenic activity**, for female rats, **equivocal evidence of carcinogenic activity**, and for male and female mice, **clear evidence of carcinogenic activity.** Further, in the sentence pertaining to the evidence in female rats which reads "There was **equivocal evidence of carcinogenic activity** in female F344/N rats based on increased incidences of renal tubule neoplasms,"the word marginal should be inserted in front of "increased". Dr. Fischer seconded the motion. Dr. Goldsworthy moved that the motion be amended such that the last sentence of the conclusions for carcinogenicity be changed from "These increases were attributed to the concentration of free diethanolamine present as a contaminant" to "These increases were associated with the concentration of free diethanolamine present as a contaminant". Dr. Belinsky seconded

the amendment, which was accepted by seven yes votes with one abstention (Bus). The amended motion was then accepted by seven yes votes with one abstention (Bus).

Lauric Acid Diethanolamine Condensate. Dr. R.D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of lauric acid diethanolamine condensate 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 lesions in female mice and non-neoplastic lesions in male and female rats and mice. The conclusions for the two-year studies in rats and mice were that:

> Under the conditions of these 2-year dermal studies, there was **no evidence of carcinogenic activity** of lauric acid diethanolamine condensate in male or female F344/N rats administered 50 or 100 mg/kg or in male $B6C3F_1$ mice administered 100 or 200 mg/kg. There was **some evidence of carcinogenic activity** in female $B6C3F_1$ mice based on increased incidences of hepatocellular neoplasms. These increases were attributed to the presence of free diethanolamine which was present as a contaminant of lauric acid diethanolamine condensate.
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> Dermal administration of lauric acid diethanolamine condensate to rats and mice for 2 years resulted in increased incidences of epidermal and sebaceous gland hyperplasia, hyperkeratosis, chronic inflammation, and parakeratosis at the site of application. Lauric acid diethanolamine condensate administration also resulted in higher incidences of thyroid gland follicular cell hyperplasia in dosed male mice.

Dr. Hecht, a principal reviewer, agreed with the conclusions. His scientific criticisms were similar to those he made for the report on coconut oil acid diethanolamine condensate in that the scientific conclusions are clouded by the use of a mixture with such a large amount of
diethanolamine as a contaminant, the uncertainty as to the actual concentration of the nitrosodiethanolamine impurity, and the confounding issue of the considerable spontaneous incidence of mouse liver tumors.

Dr. Goldsworthy, the second principal reviewer, agreed with the conclusions with the provision that the last sentence of the conclusions for carcinogenicity be amended as was done with the coconut oil acid diethanolamine condensate report. Dr. Bailer, the third principal reviewer, agreed with the conclusions. He said he had some specific questions relating to the logistic model used such as whether the fit had been quantified, why the comparisons were made using survival, and what is the sensitivity of the model to the assumed amounts of diethanolamine in the various condensates. Dr. Haseman said he would consider adding a goodness of fit statistic to the relevant table which would show how well the predicted and observed values agreed. Dr. Bailer noted many organ/tissue sites outlined in the text with trend test P-values of 0.03-0.07, and wondered how it was decided which trends should be highlighted. He noted especially Zymbal gland tumors in male rats, for which he thought the findings might support **equivocal evidence.** Dr. Hailey responded that in the case of Zymbal gland, one of the three tumors reported was determined not to be of Zymbal's gland origin. Dr. Irwin said that biological plausibility or meaningfulness also comes into consideration with border line cases.

Dr. Goldsworthy moved that the Technical Report on lauric acid diethanolamine condensate be accepted with revisions discussed and the conclusions as written for male and female rats and male mice, **no evidence of carcinogenic activity,** and for female mice, **some evidence of carcinogenic activity**. The last sentence of the conclusions for carcinogenicity would be changed, like that for coconut oil acid diethanolamine condensate, to read: "These increases were associated with the presence of free diethanolamine which was present as a contaminant of lauric acid diethanolamine condensate." Dr. Bailer seconded the motion, which was accepted by seven votes with one abstention (Bus).

Oleic Acid Diethanolamine Condensate. Dr. R.D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of oleic acid diethanolamine condensate by discussing the uses and rationale for study, describing the experimental design, reporting on any survival or body weight effects, and commenting on compound-related non-neoplastic lesions in male and female rats and mice. The conclusions for the two-year studies in rats and mice were that:

> Under the conditions of these 2-year dermal studies, there was **no evidence of carcinogenic activity** of oleic acid diethanolamine condensate in male or female F344/N rats administered 50 or 100 mg/kg or in male or female B6C3F$_1$ mice administered 15 or 30 mg/kg.
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> Dermal administration of oleic acid diethanolamine condensate to male and female rats was associated with epidermal hyperplasia, sebaceous gland hyperplasia, hyperkeratosis, parakeratosis, chronic active inflammation of the dermis, and ulcer of the skin at the site of application. Dermal administration of oleic acid diethanolamine condensate to mice was associated with epidermal hyperplasia, sebaceous gland hyperplasia, hyperkeratosis, chronic active inflammation of the dermis, and exudate of the skin at the site of application in males and females and parakeratosis and ulcer of the skin at the site of application in males.

Dr. Goldsworthy, a principal reviewer, agreed in principle with the conclusions. He asked for discussion about whether **equivocal evidence** was considered for the occurrence of interstitial cell adenomas of the testis in male rats. He noted that this response appears to be increased with respect to the two most suitable controls, the concurrent controls and the controls from the other diethanolamine studies. Dr. Haseman said that of the two studies that were in the historical database by this route of administration, one had a control rate of testicular tumors higher than the high dose rate in the current study. Also, in none of the other three concurrent studies was there an apparent effect on these tumors.

Dr. I. Russo, a second principal reviewer, agreed with the conclusions. She wondered whether the tumor responses would have been similar to those in the other two condensate studies if the content of free diethanolamine had been similar instead of lower, and suggested that the graph used in the coconut oil acid condensate report showing the content of diethanolamine for each of the condensates be added to this report.

Dr. Carlson and others expressed concerns about the large number of impurities in the test material. Dr. C. Smith, NIEHS, noted that the results of the purity analysis were in the appendix and the impurities were mainly other fatty acids, free diethanolamines, or unidentifiable organic impurities. Dr. Bucher said that we would determine if there is a particular purity grade material designation for these diethanolamides and then would put that in the titles of each.

Dr. Goldsworthy moved that the Technical Report on oleic acid diethanolamine condensate be accepted with revisions discussed andwith the conclusions as written for male and female rats and mice, **no evidence of carcinogenic activity**. Dr. Russo seconded the motion, which was accepted by seven votes with one abstention (Bus).

Pentachlorophenol, Purified. Dr. R. Chhabra, NIEHS, introduced the toxicology and carcinogenesis studies of pentachlorophenol 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 lesions in male rats. Dr. Chhabra reported the findings of the earlier bioassay of two technical grades of pentachlorophenol in B6C3F$_1$ mice. The conclusions for the two-year studies in rats were that:

> Under the conditions of this 2-year feed study, there was **some evidence of carcinogenic activity** in male F344/N rats based on increased incidences of mesothelioma and nasal squamous cell carcinoma. There was **no evidence of carcinogenic activity** of pentachlorophenol in female F344/N rats exposed to feed containing 200, 400, or 600 ppm for 2 years or 1,000 ppm for 1 year.

Dr. Belinsky, a principal reviewer, did not agree with the conclusion for male rats. He found the level of evidence for nasal lesions hard to justify, noting the incidence of tumors jumped all around with none reported at the high dose in the 2-year studies. He wondered what effects an ongoing infection would have on facilitating that type of lesion. Dr. R. Maronpot, NIEHS, said that the highest rate of fungal infection was in the controls and, further, in the animals that had squamous cell carcinomas, only one had evidence of fungal infection so there appears to be little or no relation between fungal infection and tumors. Dr. Belinsky asked for clarification on the rationale for the stop study chosen. Dr. Chhabra said he would try to explain the thought process in the design. First, the chemical had already been shown to be a carcinogen in mice with liver as the target organ, and the 28-day toxicity studies supported that so 2-year studies were designed on that basis. The reason for the stop exposure study was to see if there were preneoplastic liver lesions at six months, and then stop exposure and follow progression or regression of lesions for six months. Since there was only mild liver toxicity shown with an interim sacrifice, the decision was made to continue exposure to one year and stop, maintaining animals on control diet for the second year.

Dr. Chatman, the second principal reviewer, did not agree with the conclusions in male rats. She said that incidences of nasal tumors in two-year studies were not statistically significant and did not show a dose-response relationship, and exposed groups did not show increases in defined preneoplastic lesions. Dr. Haseman said internal staff discussions explored these same issues but because the nasal tumors are so uncommon, the conclusion was warranted. Further, Dr. Chatman thought the frequency of fungal nasal infections was an additional variable interfering with interpretation of the findings. She wondered if the animals were immunocompromised.

Dr. Fischer, the third principal reviewer, agreed with the conclusions. She said the active fungal infection might be a confounding variable. Dr. Fischer asked about levels of pentachlorophenol in the food supply to give some comparison with levels fed to the animals. Dr. Chhabra responded that there is information on levels in food and this would be added to the report. Dr. Fischer noted the numbers of metastatic lesions were elevated in all of the treatment groups and said this should be addressed in the discussion. Dr.

Haseman said the numbers of metastatic lesions were misleading because, first, most of the metastatic lesions had a different cell of origin, and, secondly, many tumors metastasized to a number of different sites, with each counted as a different metastatic tumor.

Dr. Bus contended that the findings on reduction of body weight gain in the 28-day studies as well as information from the previous rat bioassay suggested that 600 ppm was close to an MTD, and thus the 1000 ppm dose was far in excess of an MTD. So the classification described for tumors does not seem to be in the right place. Dr. Chatman said the 1000 ppm group was really a different study. Dr. J. Russo commented that the mesothelioma findings are quite important and should not be minimized. Dr. Goldsworthy asked whether there was any reason males would be more sensitive than females with regard to the two tumor sites. Dr. Maronpot said he would not expect a difference in the nasal carcinomas; however, with regard to mesotheliomas, male Fischer rats spontaneously have more tumors at that site than females but for unexplained reasons.

Public Comment: Dr. Po-shiung Lin, University of North Carolina at Chapel Hill, presented data from research studying macromolecular binding and genotoxic effects of pentachlorophenol in tissues obtained from the interim sacrifice at 27-weeks in the 2-year NTP study. He said that research with some mineral fibers indicated that free radical formation is thought to be involved in development of mesotheliomas. Their findings show that in male rats exposed to 1,000 ppm pentachlorophenol, there is a two-fold increase in DNA lesions in the kidney. If free radicals are involved in pentachlorophenol carcinogenesis, this could explain the strange dose-response relationship observed for mesothelioma in male rats.

Dr. Bruce Bernard, SRA International, representing the United States Pentachlorophenol Task Force, said he was going to focus on the mesotheliomas in the epididymis and nasal neoplasms. He criticized the dose setting, noting doses were set on the basis of 28-day studies and not on the basis of 90-day studies as is normally done. Dr. Bernard stated that the 1000 ppm dose groups were originally intended to be carried forward for two-years but exposure was stopped after one-year when it became obvious that survival would be a problem. He concluded that for various reasons including lack of a dose-response and no tumors at the highest cumulative dose, the data support **equivocal evidence of carcinogenic activity** at best. Dr. Bernard said that if the Subcommittee cannot reach that conclusion, he requested deferral until the next meeting to allow more scientific debate.

Dr. Bucher said he wished to clarify that the stop study was exactly that; dosing was to be terminated after one year.

Dr. Belinsky moved that the Technical Report on pentachlorophenol, purified, be accepted with revisions discussed and the conclusions as written for female rats, **no evidence of carcinogenic activity**, and with the conclusions for male rats changed to **equivocal evidence of carcinogenic activity**. Neoplastic effects were seen only in male rats in the

stop study at a dose that caused a significant decrease in body weight gain. Dr. Chatman seconded the motion. After some discussion suggesting that the standard 2-year study at 200, 400, or 600 ppm and the stop study at 1,000 ppm be treated separately, Dr. Belinsky made a substitute motion that "under the conditions, there was **no evidence of carcinogenic activity** in male and female rats exposed to feed containing 200, 400, or 600 ppm pentachlorophenol for 2 years, and there was **some evidence of carcinogenic activity** in male rats and **no evidence of carcinogenic activity** in female rats exposed to feed containing 1,000 ppm pentachlorophenol for 1 year followed by control diet for 1 year." Dr. Chatman seconded the substitute motion. Dr. Bus moved to amend the second part of the motion to designate the stop study as **inadequate study of carcinogenic activity** based on there being significant toxicity well beyond classical MTD definitions. There being no second, the amendment was tabled. Dr. Belinsky's substitute motion was then accepted unanimously with eight votes.

Isobutene. Dr. J. Roycroft, NIEHS, introduced the toxicology and carcinogenesis studies of isobutene by discussing the uses and rationale for study, describing the experimental design, reporting on any survival or 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 for the two-year studies in rats and mice were that:

> Under the conditions of these 2-year inhalation studies, there was **some evidence of carcinogenic activity** of isobutene in male F344/N rats based on an increased incidence of follicular cell carcinoma of the thyroid gland. There was **no evidence of carcinogenic activity** of isobutene in female F344/N rats or male or female $B6C3F_1$ mice exposed to 500, 2,000, or 8,000 ppm.
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> Exposure to isobutene by inhalation for 2 years resulted in icreased incidences and/or severities of nasal lesions including hyaline degeneration of the olfactory epithelium in male and female rats and mice and hyaline degeneration of the respiratory epithelium in male and female mice.

Dr. Bailer, a principal reviewer, agreed in principle with the conclusions. He said that as there were tumors only in the high dose and in only one site and one species, he would be comfortable as well with **equivocal evidence of carcinogenic activity** in male rats. Dr. Bailer said that one type of information he would like to see, and in other reports as well, would be comment on typical levels of human exposure, and in this case for the 7,000 employees potentially exposed. Dr. Roycroft responded that there was no human exposure data, and there is only a limited amount of data in the literature that gives even a hint of what persons might be exposed to with isobutene, e.g., one percent of gasoline may contain isobutene. Dr. Lucier reported that the NIEHS/NTP has recently established an interagency agreement with the Centers for Disease Control and Prevention tapping into their outstanding analytic capabilities to provide information on exposure assessment for chemicals of interest to the NTP including those evaluated by this Subcommittee.

Dr. Medinsky, the second principal reviewer, agreed with the conclusions. She complimented the NTP for the use of pharmacokinetic data, in this case, urinary metabolites. Dr. Medinsky said it would be useful to have a metabolic scheme for isobutene included so the reader can readily see where the biomarker, 3-hydroxyisobutyric acid (HIBA), fits in the metabolic fate of isobutene relative in fact to what might be the putative toxic metabolite for this chemical. Dr. Roycroft said that a metabolic scheme could be included, and further, the monoepoxide is supposedly a putative metabolite and a modeling effort has been started on isobutene to predict some blood concentrations of the epoxide.

Dr. Belinsky, the third principal reviewer, agreed with the conclusions. He thought it interesting that there was no apparent precursor lesion, no increases in hyperplasia for the thyroid neoplasms. Dr. R. Herbert, NIEHS, commented that hyperplasias can be considered a preneoplastic lesion for thyroid tumors and this has been seen with other studies; however, in this study there were not any.

Dr. Bailer moved that the Technical Report on isobutene be accepted with revisions discussed and the conclusions as written for male rats, **some evidence of carcinogenic activity**, and for female rats and male and female mice, **no evidence of carcinogenic activity**. Dr. Medinsky seconded the motion, which was accepted unanimously with eight votes.

Isoprene. Dr. R. Melnick, NIEHS, introduced the toxicology and carcinogenesis studies of isoprene 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 lesions in male and female rats and non-neoplastic lesions in male rats. A previous NTP 26-week inhalation exposure plus 26-week recovery study had provided clear evidence of the multiple-site carcinogenicity of isoprene in male $B6C3F_1$ mice. The conclusions for the two-year studies in rats were that:

> Under the conditions of this 2-year inhalation study, there was **clear evidence of carcinogenic activity** of isoprene in male F344/N rats based on increased incidences of mammary gland neoplasms, renal tubule adenomas, and testicular adenomas. There was **some evidence of carcinogenic activity** of isoprene in female F344/N rats based on increased incidences and multiplicity of mammary gland fibroadenoma. A low incidence of rare brain neoplasms in exposed female rats may have been due to exposure to isoprene.
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> Exposure to isoprene by inhalation for 2 years resulted in increased incidences of renal tubule hyperplasia and splenic fibrosis in male rats.

Dr. Melnick also discussed the metabolism of isoprene and compared the tumor responses and metabolisms of isoprene with two close structural analogs, 1,3-butadiene and chloroprene, that had been studied previously in 2-year studies by the NTP. Dr. Melnick then described a physiologically based pharmacokinetic model that was developed for isoprene and how it was used to evaluate dose-response relationships for tumor formation at the different sites.

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

Dr. Medinsky, the second principal reviewer, agreed with the conclusions. She said the structure-activity comparisons made with the analogs maximized the usefulness of the data collected on the three chemicals. Dr. Medinsky was most impressed by the use of pharmacokinetic data to obtain a more refined dose metric and a more refined demonstration of the changes in tumor response with respect to dose. She wondered why the authors believed the parent, isoprene, may be involved directly in tumor formation. Dr. Melnick said the relationship in the kidney seems to be driven by the mono- or diepoxide intermediate but in the mammary gland of male rats there may be some contribution from the parent because of the greater response at the highest exposure.

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

Public Comment. Dr. A. Philip Leber, Goodyear Tire and Rubber Company, representing the International Institute for Synthetic Rubber Producers, said that he had specific comments on the tumors reported. With regard to male rat renal tubule tumors, he said that the dimer of isoprene is limonene which is associated with α2μ-microglobulin accumulation and renal tumors in rats. [ED. NOTE: Protein droplet accumulation was

not observed in the male rat kidney in the current study.] In his view, the low incidence of carcinomas and lack of indication of progression of male mammary tumors to malignancy supports only **some evidence**. This would also be the case with the testicular tumors, and the mammary tumors in females as well, where **equivocal evidence** would be appropriate. Finally, because of inadequate time for public review of the report, he suggested deferral be considered.

Dr. Belinsky moved that the Technical Report on isoprene be accepted with revisions discussed and the conclusions as written for male rats, **clear evidence of carcinogenic activity**, and for female rats, **some evidence of carcinogenic activity**. Dr. Medinsky seconded the motion, which was accepted by six yes votes to one no vote (Goldsworthy) with one abstention (Bus). Dr. Bus abstained for reasons of company affiliation.

Furfuryl Alcohol. Dr. R. D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of furfuryl alcohol 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 lesions in male and female rats and male mice and on compound-related non-neoplastic lesions in male and female rats and mice. The conclusions for the two-year studies in rats and mice were that:

> Under the conditions of these 2-year inhalation studies, there was **some evidence of carcinogenic activity** of furfuryl alcohol in male F344/N rats based on increased incidences of combined neoplasms of the nose. There was **equivocal evidence of carcinogenic activity** of furfuryl alcohol in female F344/N rats based on marginally increased incidences of neoplasms of the nose and renal tubule neoplasms. There was **some evidence of carcinogenic activity** of furfuryl alcohol in male $B6C3F_1$ mice based on increased incidences of renal tubule neoplasms. There was **no evidence of carcinogenic activity** of furfuryl alcohol in female $B6C3F_1$ mice exposed to 2, 8. or 32 ppm.
>
> Exposure of male and female rats and male mice to furfuryl alcohol was associated with increased incidences of nonneeoplastic lesions of the nose and increased severities of nephropathy. Exposure of female mice to furfuryl alcohol was associated with increased incidences of nonneoplastic lesions of the nose and corneal degeneration.

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

Dr. Bus, the second principal reviewer, agreed with the conclusions. He noted that part of the conclusions for female rats and entirely for male mice are based on the observation of renal tubule neoplasms , particularly when they were observed in extended evaluation step sections. For the reader, it is unclear whether the newly observed lesion(s) from the step section are in an animal for which lesions are already present or are truly a unique new lesion in an animal with no lesions in the initial evaluation. He said this needs to be clarified. Dr. Irwin agreed. Dr. Bus commented that more attention should have been paid to the 13-week study results in setting doses for the chronic studies in rats, in that the top dose, 32 ppm, appeared to exceed an MTD, and the bottom dose, 2 ppm, was still above a no effect level. Dr. Irwin said that in setting dose levels for the 2-year study, mean body weights at 32 ppm were within 10 % and the shape of the growth curve indicated this would probably not change much or these animals might even recover. In fact, in the 2-year study body weights of 32 ppm female rats were about the same as those of controls.

Dr. J. Russo, the third principal reviewer, agreed with the conclusions.

Dr. Carlson commented on the rationale for studying furfuryl alcohol as part of a class study with furan and furfural, noting that cholangiocarcinomas and other hepatocellular neoplasms were significant neoplastic findings with furan and furfural. He thought the fact that a liver response was not seen with furfuryl alcohol contrasted with the other two

analogs should be pointed out in the discussion. Dr. Irwin agreed. Dr. Bailer said he thought the level of evidence in male rats should have been **clear evidence** and not **some evidence** based on a dose-response and four malignant tumors in the high dose group. Dr. Haseman said that although there were no squamous cell carcinomas of the nose in the control group for this study there have been one or two in some of our other control groups. Dr. Irwin said that lack of supporting data in female rats also entered into the decision to go with **some evidence.**

Dr. Bus moved that the Technical Report on furfuryl alcohol be accepted with revisions discussed and the conclusions as written for male rats and mice, **some evidence of carcinogenic activity**, for female rats, **equivocal evidence of carcinogenic activity**, and for female mice, **no evidence of carcinogenic activity**. Dr. Cullen seconded the motion, which was accepted by six yes votes to two no votes (Bailer, Goldsworthy).

Pyridine. Dr. J. K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of pyridine by discussing the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic lesions in male F344/N rats and male and female $B6C3F_1$ mice,uncertain neoplastic findings in female F344/N rats and male Wistar rats, and compound-related non-neoplastic lesions in male and female F344/N rats and male Wistar rats. The conclusions for the 2-year studies in rats and mice were that:

> Under the conditions of these 2-year drinking water studie, there was **some evidence of carcinogenic activity** of pyridine in male F344/N rats based on increased incidences of renal tubule neoplasms. There was **equivocal evidence of carcinogenic activity** of pyridine in female F344/N rats based on increased incidences of mononuclear cell leukemia. There was **equivocal evidence of carcinogenic activity** in male Wistar rats based on an increased incidence of interstitial cell adenoma of the testis. There was **clear evidence of carcinogenic activity** of pyridine in male and female $B6C3F_1$ mice based on increased incidences of malignant hepatocellular neoplasms.
>
> In F344/N rats, exposure to pyridine resulted in increased incidences of centrilobular cytomegaly and degeneration, cytoplasmic vacuolization, and pigmentation in the liver of males and females; periportal fibrosis, fibrosis, and centrilobular necrosis in the liver of males; and bile duct hyperplasia in females. In male Wistar rats, pyridine exposure resulted in increased incidences of centrilobular degeneration and necrosis, fibrosis, periportal fibrosis, and pigmentation in the liver, and secondary to kidney disease, mineralization in the glandular stomach and parathyroid gland hyperplasia.

Dr. Cullen, a principal reviewer, agreedwith the conclusions. He noted the large amount of inflammation in mouse livers and asked whether they had been screened for the possible presence of *Helicobacter hepaticus* infection. Dr. Hailey said there was no frozen tissue available to perform PCR-based assays for identification of *H. hepaticus*;. However, the liver lesions observed were not consistent with those typically associated with *H. hepaticus* infection.

Dr. Fischer, the second principal reviewer, agreed with the conclusions. She said the discussion should include comments on increased incidences of metastatic neoplasms in mice compared to rats. Dr. Dunnick agreed. Dr. Fischer expressed concern that the Wistar rats exposed to 400 ppm did not live long enough to produce tumors, and, thus, this experiment was not informative.

Dr. Bus, the third principal reviewer, did not agree with the conclusions for female rats and mice and for male Wistar rats. He said the conclusion of **equivocal evidence** in female rats was not warranted based on lack of dose-response, incidence values only very slightly exceeding recent NTP historical control values, and excessive body weight depressions which make interpretation of chemically-associated tumors very confounded. Dr. Dunnick

responded that by definition, the increases in leukemias were uncertain findings. With regard to male Wistar rats, Dr. Bus stated that the severe toxicity associated with markedly decreased survival and effects on body weight gain especially at the top two doses compromised interpretation of the increased incidence of testicular tumors in the high dose group. Finally, he thought it difficult to understand a conclusion of **clear evidence** in female mice in view of the profound body weight loss over the last 25 weeks of the study, and even though there was a dose-related increase in malignant liver tumors, liver adenomas and total tumors were not altered. Dr. Dunnick said the level of **clear evidence** was justified by the large dose-related increased incidences of malignant neoplasms. The body weight loss was due in part to the development of liver tumors. Dr. Haseman interjected that while the control incidence of liver tumors in female mice may have been one of the highest ever seen in the Program, almost all were adenomas. On the other hand, almost every dosed animal that lived one year or more developed a liver tumor, often multiple tumors, and often carcinomas or hepatoblastoma, with many metastasizing to the lung, constituting one of the strongest carcinogenic effects at this site ever seen in his experience. Dr. Bus said this certainly changed his perspective on the tumors in female mice.

There ensued further discussion about whether hepatoblastomas should be viewed and weighted separately from hepatocellular carcinomas. Dr. Hailey thought they should be viewed as part of a natural progression and that with chemicals having tumor promoter activity there is almost always an associated increase with hepatoblastomas. There was discussion about the appropriateness in general of combining benign and malignant neoplasms. Dr. J. Russo argued that combining can be misleading. Dr. Hailey commented that with some tumor types combining might be controversial but with, for example, the liver (mice) and the kidney (rats), which are at issue here, there is a spectrum of lesions going from foci or hyperplasia to adenoma to carcinoma that represents a morphological and biological continuum, so combining seems appropriate. Dr. Bailer said that based on the data in the report, he would have considered **clear evidence** as the conclusion for male F344 rats. Dr. Bucher observed that we are using our combined experience to delineate between **some evidence** and **clear evidence** based on a historical perspective within the Program.

Dr. Bus moved that the Technical Report on pyridine be accepted with revisions discussed and the conclusions as written for male F344 rats, **some evidence of carcinogenic activity**, and for male and female mice, **clear evidence of carcinogenic activity**. The conclusions for female F344 rats and male Wistar rats would be changed from **equivocal evidence of carcinogenic activity** to **inadequate study of carcinogenic activity**. Dr. Cullen seconded the motion. Dr. Haseman said that **inadequate study** is infrequently used and usually when there is some major flaw that makes the study uninterpretable. Dr. Bailer moved to amend the motion to change the level of evidence for female rats and male Wistar rats back to **equivocal evidence of carcinogenic activity**. Dr. Cullen seconded the amendment, which was accepted by six yes votes to one no vote (Bus). Dr. Bus's motion as amended by Dr. Bailer was accepted unanimously with seven votes.

1-Chloro-2-propanol, Technical. Dr. J. K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of 1-chloro-2-propanol by discussing the rationale for study, describing the experimental design, and reporting on any survival or body weight effects. The conclusions for the two-year studies in mice and rats were that:

> Under the conditions of these 2-year drinking water studies, there was **no evidence of carcinogenic activity** of 1-chloro-2-propanol (75% to 76% 1-chloro-2-propanol; 24% to 25% 2-chloro-1-propanol) in male or female F344/N rats exposed to 150, 325, or 650 ppm. There was **no evidence of carcinogenic activity** of technical grade 1-chloro-2-propanol (75% to 76 % 1-chloro-2-propanol; 24% to 25% 2-chloro-1-propanol) in male or female B6C3F$_1$ mice exposed to 250, 500, or 1,000 ppm.

Dr. Chatman, a principal reviewer, agreed with the conclusions. She noted that pancreas was a target tissue in prechronic studies, and in light of epidemiologic reports of higher rates of pancreatic cancer in humans working in the chlorohydrin industry, she wondered if the top dose should have been higher in the 2-year study. Dr. Chatman asked why inhalation was not the preferred route of exposure. Dr. Dunnick responded that the chemical was administered orally because of concern about its being present in fumigated foods.

Dr. J. Russo, the second principal reviewer, agreed with the conclusions. He commented on the significant decrease in drinking water consumption in dosed animals in the prechronic studies and asked whether this could be due to some type of hypothalamic damage. Dr. Dunnick said water consumption was decreased more in the 14-day than in the 13-week studies, and by 13-weeks decreases in water consumption were much less as animals adapted to the taste of the chemical. Dr. Chatman inquired whether exposure level was monitored in view of the decrease in water consumption. Dr. Dunnick said chemistry is done on the drinking water bottles and on the dose preparations, and in general, levels were within targeted exposure levels.

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

Dr. Goldsworthy also thought that the top dose may have been below a Maximal Tolerated Dose (MTD). Dr. Hailey said that the 2-year study doses were based on the significant pancreatic lesions in the 13-week studies. Dr. Bus commented that proper use of the subchronic study results to choose the chronic doses was followed and thats all you can do. Dr. Cullen asked whether the pancreatic lesions in the 13-week studies were metaplastic and the size of the pancreas changed. Dr. Hailey replied that there was no change in size although there was apoptosis of acinar cells and replacement by adipocytes (fat cells). Dr. Chatman urged the NTP to pursue evaluation of possible pancreatic effects in view of a possible association with increased pancreatic cancer in the workplace.

Dr. Chatman moved that the Technical Report on 1-chloro-2-propanol, technical grade, be accepted with revisions discussed and the conclusions as written for male and female rats

and mice, **no evidence of carcinogenic activity**. Dr. Goldsworthy seconded the motion, which was accepted unanimously with seven votes.

Discussion of New Statistical Methodology Used by the NTP in Evaluating Tumors in NTP Toxicology and Carcinogenesis Studies.

Dr. J. Haseman, NIEHS, said that the statistical methods used in evaluating tumors in the present set of draft Technical Reports are somewhat different than used previously, so for the benefit of Subcommittee members he would briefly describe the new methods and explain why changes were needed. He went over the three sets of statistical analyses that have been used previously. First is the Fisher's exact/Cochran Armitage trend test based on the overall proportion of tumor-bearing animals. These were the methods of choice by the NCI and have been included primarily for historical continuity. The other two methods are the logistic regression test, a survival-adjusted method appropriate for incidental neoplasms, and the life table analysis, a survival-adjusted method appropriate for rapidly fatal neoplasms. With regard to potential problems, the first set of methods ignores survival differences, while the other two methods assume that all tumors of a given type are either all fatal or all incidental. In fact, it often is not known whether a tumor is fatal or incidental. The second disadvantage is that when survival differences do exist and are very large, these survival-adjusted methods may produce markedly different results, and, further, the logistic regression test, which is normally the method of choice since most tumors are assumed to be incidental, can have markedly reduced power in these situations.

Dr. Haseman reported that after considerable study, newer statistical methods have been proposed that are not linked to the fatal tumor-incidental tumor paradigm. He cautioned that these new methods may not be the final word. Currently, the most appropriate procedure is the Poly-k test derived by Bailer and Portier. This is generally known by the more familiar name of the Poly-3 test as generally a value of 3 is chosen for k. Before describing the test, Dr. Haseman illustrated with two sets of actual data from the NTP study of 1,2,3-trichloropropane the sort of problems that can be encountered using the older methods in a study with marked survival differences. Two tumors were analyzed, leukemias in high dose male rats and squamous cell carcinoma of the tongue in high dose female rats.

Dr. Haseman explained that the basic approach of the Poly-k method is to modify the denominator of the estimated prevalence of neoplasms to more closely approximate the total number of animal years at risk. This method yields a tumor prevalence rate that depends only on the choice of k, which is a shape parameter for a Weibull hazard function describing cumulative tumor incidence over time. After estimating k for a variety of tumor types using the large NTP control tumor incidence database, Bailer and Portier recommended k=3 as a reasonable choice for a general test. Key features of the Poly-3 test are that: (1) tumor-free animals contribute to the tumor rate denominator the fraction of the entire study time that they survived raised to the third power. For example, an animal dying at week 52 of a two-year study would contribute - $(52/104)^3 = 0.125$ to the denominator.; (2) tumor-bearing animals or tumor-free animals that survive to the end of

the study contribute full weight to the denominator (1.0). So, eight tumor-free animals at 52 weeks are regarded as equivalent to one that makes it to the end of the study. ; and (3) statistical comparisons are then made of these survival-adjusted neoplasm rates. A potential limitation is that the choice of k=3 may not be appropriate for all tumor types. However, Bailer and Portier showed that the Poly-3 test gave valid results under a Weibull model with k values in the range from 1 to 5. He said that it is necessary to assume some value of k because there are insufficient data in any given study, especially one without interim sacrifices, to estimate k. The NTP routinely carries out "behind the scenes" Poly-1.5 and Poly-6 tests. He said that we have found that these procedures generally give results similar to the Poly-3 test, but the flexibility is maintained of using these or other values of k on a case-by-case basis.

Dr. Haseman concluded with some general comments on the Poly-k test. The application of the Poly-k test to tumor data from NTP studies has been evaluated for more than a year. The resulting p-values are generally similar to those from previously used tests except when survival differences are pronounced, as he had illustrated earlier. He noted that the Poly-k test has been used in earlier NTP studies with survival problems, e.g., the second 1,3-butadiene study (NTP TR 434), and the chloroprene study (NTP TR 467), and has gained general acceptance in the scientific community. Eliminating the need to decide if a tumor is fatal or incidental is a major advantage of the Poly-k test. Finally, he said that we see no need to systematically review and re-evaluate previous NTP studies using the new methodology. Dr. Carlson asked whether the older tests would continue to be done and included in the Technical Reports. Dr. Haseman replied that the older tests will continue to be done but the analyses will not be routinely included in the Technical Report. Thus, the intention is to report only the Poly-3 test results unless special circumstances indicate otherwise.

Presentation Concerning Data Obtained on Carcinogenicity from Transgenic Mouse Models – Tg.AC and p53^{deficient}

Dr. Lucier introduced the topic by stating that over the last several years, the NIEHS and NTP has had a major program involving evaluation of transgenic mouse models for potential use in cancer bioassays. This evaluation has primarily focused on two lines, the Tg.AC and the p53^{def}, and more recently a collaborative study with the Japanese on the H-*ras* 2 model. The responses of transgenics to chemicals in the NTP database are being compared, including known human carcinogens, weak carcinogens, strong carcinogens, genotoxic carcinogens, nongenotoxic carcinogens, and noncarcinogens. Dr. Lucier reported that Dr. Raymond Tennant, who has directed many of the studies, will present an overview and Dr. Tennant, Dr. Judson Spalding, and Dr. John French from the Laboratory of Environmental Carcinogenesis and Mutagenesis, NIEHS, will discuss results from many of the chemicals for which draft Technical Reports were reviewed the past two days. Dr. Lucier concluded by announcing the NTP Board of Scientific Counselors will meet February 5 and 6 to help the NTP evaluate strategies for the use of transgenic models in assessing carcinogenic potential of chemicals. He said that Dr. Carlson, Dr. Bus and Dr. Fischer would participate in that review meeting.

Dr. Tennant said the examples reviewed today and yesterday of the long-term bioassays for which there is now transgenic data provide the conceptual framework for the question -- Why transgenics? For over two decades, scientists have been searching for plausible alternatives to long-term animal carcinogenicity studies, and although there has been some success, the information derived has not been useful enough to supplant the 2-year bioassay. Dr. Tennant said that beyond the cost and time of conducting a two to four year experiment, there is the issue of the genetics of the test organism, and trying to distinguish which effects are intrinsic to a chemical or are reflective of the genotype on which the chemical has been imposed. He said the strategy behind trying to utilize transgenics is based upon the principle that if we impose on the animal a genetic target that represents a gene that is known to be intrinsic to the pathway of carcinogenesis both in mice and in humans, then we should be able to minimize the influences of all the other genes that can influence the development of cancers. To this end, the focus has been put on the two lines. The conceptual framework within which one might utilize these models is based upon recognition of either genotoxic or non-genotoxic carcinogenesis. It is principally the Tg.AC that has shown the ability to identify non-genotoxic carcinogens.

Dr. Tennant said that a derivative effort from an initial NIEHS meeting to establish partnerships by the International Life Sciences Institute (ILSI) is coordinating the work of a consortium of 30 pharmaceutical companies and other organizations with a goal of evaluating the use of these models for drug safety assessment, and also involving the *ras* H2 line and an XPA deficient mouse. He said that the evaluations that ILSI is conducting and the NTP's previous evaluations have been primarily retrospective. The results to be presented today are prospective. The prospective method eliminates selection bias, and allows evaluation of chemicals currently being studied. Thus, from the bioassays evaluated at this meeting, six agents were evaluated in the $p53^{def}$ line and eight agents were evaluated in the Tg.AC line.

Dr. French said he would describe the p53 model, review the summary results collected to date and then talk about the prospective analyses. Why p53? The p53 tumor suppressor gene suppresses cancer in humans and laboratory animals. In humans, the majority of cancers usually present with a mutation in one allele of p53 and loss of heterozygosity in the other allele. The model was developed by researchers at Baylor College of Medicine. Dr. French said they have used a 26-week exposure regimen with both mutagenic and non-mutagenic carcinogens. This time frame is important in that the $p53^{def}$ consistent with its genetic background is free of tumors up to and even beyond 10 months of age, thus avoiding some of the properties of long-term studies where tumors spontaneously arising late in life can confound the interpretation. This led to the hypothesis that this mouse will rapidly develop cancer in the target tissues when exposed to a trans-species carcinogen in the absence of sporadically arising cancer during the exposure period. He said the model may respond to non-mutagenic carcinogens given sufficient exposures but within the 26-week exposure period it was expected that only the trans-species carcinogens could be identified. Dr. French reported that several years ago, a series of mutagenic carcinogens, non-mutagenic carcinogens, and at least one mutagenic non-carcinogen were chosen for study in the $p53^{def}$. For the mutagenic carcinogens, *p-*

cresidine, benzene, 4-vinyl-1-cyclohexene diepoxide, and phenolphthalein, tumors were induced within a six month period at the same sites, at the same dose levels and by the same route of exposure as used in the 2-year studies. The non-mutagenic carcinogens, N-methyl-*o*-acrylamide, reserpine, and methylphenidate, were negative in these studies. Thus, Dr. French said that it was no surprise that for six of the chemicals for which the long-term bioassay reports were just reviewed, all were nonresponsive as carcinogens during the six-month exposure period in the $p53^{def}$ mouse. These were coconut oil acid diethanolamine condensate, lauric acid diethanolamine condensate, oleic acid diethanolamine condensate, 1-chloro-2-propanol, pyridine, and pentachlorophenol.

Dr. Spalding said -- why the activated *ras* oncogene? It has been known for a long time in both human tumors as well as spontaneous and chemically induced tumors in rodents that one of the three or four different *ras* family genes have mutations, so the Tg.AC is a very appropriate model to evaluate. He said the Tg.AC mouse was created in the Leder laboratory at Harvard by pronuclear injection of a v-Harvey-*ras* gene. The transgene has point mutations in codons 12 and 59, and the integration of the transgene confers on these mice the characteristic of genetically initiated skin as a target for tumorigenesis. With the exception of bone marrow, the transgene is nonconstitutive in the tissues. The route of administration is dermal (skin paint) and the response of skin papillomas are looked upon as a reporter phenotype that identifies the chemical activity. Another important feature is that the spontaneous incidence is zero to very low, less than one papilloma per mouse, in the area of application and the experiments are completed before the profile of spontaneous tumors begin to occur for the most part. Dr. Spalding described the basic protocol with topical application of three doses for each chemical five days a week for 20 weeks. Papillomas are usually seen within the first 10 weeks when there is a response. He then reviewed the responses seen with the chemicals for which long-term bioassays had just been reviewed. The eight chemicals were diethanolamine, coconut oil acid diethanolamine, lauric acid diethanolamine, oleic acid diethanolamine, 1-chloro-2-propanol, furfuryl alcohol, pyridine, and pentachlorophenol. Of these there was correspondence for four of the chemicals with 1-chloro-2-propanol and oleic acid diethanolamine negative in both the bioassay and Tg.AC, and positive responses in Tg.AC for lauric acid diethanolamine and pentachlorophenol corresponding with positve responses in the bioassay. In most cases, the doses used were higher than in the bioassay such that one could be more confident that a negative response really was a negative response.

Dr. Tennant noted that the presentation of the transgenic data from the prospective evaluation was timely, while the results of the two-year bioassays, just reviewed, were clear in the minds of the review panel. An important conclusion that can be drawn at this point is that the long feared super sensitivity of these transgenic models does not appear to be evidenced by the data presented. Dr. Tennant said that an important issue to be resolved is concerned with those chemicals where the transgenic bioassay results were discordant with the 2-year bioassay results. This leads to the question, how important for human health risk are the responses that are missed by the transgenic models? This is a fundamental issue that is going to have to be resolved in figuring out how these or other transgenic lines are going to be useful.

Discussion: Dr. Bailer commented that this exercise around the predictivity of the transgenic models was reminescent of the discussions a decade or so ago about the predictivity of the short-term genotoxicity assays such as *Salmonella* for predicting

carcinogenicity. He said that based on the studies presented he would be concerned about the poor predictivity, and cautioned against completely moving away from long-term studies. Dr. Bailer said that in all fairness, the chemicals prospectively evaluated may not be the best for doing any kind of risk projection. Dr. Tennant responded that these are the chemicals of today. Dr. Bus agreed saying that increasingly we are going to be dealing with compounds with less and less biologic activity because it is not in anybody's best interest to manufacture more toxic chemicals. Dr. Tennant remarked that by the time the ILSI initiative is completed there will be well over 80 agents available in a database. He believed that a matrix of results is being established against which any future assay model also can be evaluated. Perhaps a modest goal would be to calibrate the models to discriminate the substances that most would agree have the most unambiguous carcinogenic potential. Dr. Tennant said that within a six month exposure period, these models would identify the vast proportion of agents that are trans-species carcinogens in 2-year bioassays, and conversely would detect very few of those that are negative in mice and rats in 2-year assays. Dr. Bus commented that through use of pharmacokinetics and other technologies, the way we are setting doses is also evolving. Dr. Lucier concluded that there are a number of issues to be wrestled with including the mechanistic issues, the dosimetry issues, the predictivity issues, as well as the policy issues in determining regulatory needs pertaining to regulatory acceptance of these kinds of models.

By immunodampening the dominant epitope the immune system then reacts to other more conserved epitopes.

The prospective exclusive license will be royalty-bearing and will comply with the terms and conditions of 35 U.S.C. 209 and 37 CFR 404.7. This prospective exclusive license may be granted unless within 90 days from the date of this published notice, NIH receives written evidence and argument that established that the grant of the license would not be consistent with the requirements of 35 U.S.C. 209 and 37 CFR 404.7.

Applications for a license filed in response to this notice will be treated as objections to the grant of the contempleted license. Comments and objections submitted to this notice will not be made available for public inspection and, to the extent permitted by law, will not be released under the Freedom of Information Act, 5 U.S.C. § 552.

Dated: November 5, 1997.

Barbara M. McGarey,

Deputy Director, Office of Technology Transfer.

[FR Doc. 97-30202 Filed 11-17-97; 8:45 am]

BILLING CODE 4140-01-M

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

National Toxicology Program; National Toxicology Program (NTP), Board of Scientific Counselors' Meeting; Review of Draft NTP Technical Reports

Pursuant to Public Law 92-463, notice is hereby given of the next meeting of the NTP Board of Scientific Counselors' Technical Reports Review Subcommittee on December 9 and 10, 1997, in the Conference Center, Building 101, South Campus, National Institute of Environmental Health Sciences (NIEHS), 111 Alexander Drive, Research Triangle Park, North Carolina. The meeting will begin at 1:30 p.m. on December 9, and at 8:30 a.m. on December 10, and is open to the public. The agenda topic is the peer review of draft Technical Reports of long-term toxicology and carcinogenesis studies from the National Toxicology Program. Additionally, there will be a presentation made concerning data obtained from transgenic mouse models on several of the chemicals being reviewed.

Tentatively scheduled to be peer reviewed on December 9-10 are draft Technical Reports of 10 two-year studies, listed alphabetically, along with supporting information in the attached table. All studies were done using Fischer 344 rats and B6C3F$_1$ mice. The order of review is given in the far right column of the table. Copies of the draft Reports may be obtained, as available, from: Central Data Management, MD E1-02, P.O. Box 12233, Research Triangle Park, NC 27709 (919/541-3419), FAX (919/541-3687), email: CDM@niehs.nih.gov.

Public comment on any of the Technical Reports is welcome. Persons wanting to make a formal presentation regarding a particular Technical Report must notify the Executive Secretary by telephone, by FAX, by mail, or by email no later than December 4, 1997, and provide a written copy in advance of the meeting so copies can be made and distributed to all Subcommittee members, *ad hoc* expert consultants, and staff, and made available at the meeting for attendees. Written statements should supplement and may expand on the oral presentation. *Oral presentations should be limited to no more than five minutes.*

The program would welcome receiving toxicology and carcinogenesis information from completed, ongoing, or planned studies by others, as well as current production data, human exposure information, and use patterns for any of the chemicals listed in this announcement. Please contact Central Data Management at the address given above, and they will relay the information to the appropriate staff scientist.

The Executive Secretary, Dr. Larry G. Hart, P.O. Box 12233, Research Triangle Park, North Carolina 27709 (telephone 919/541-3971; FAX 919/541-0295; email hart@niehs.nih.gov) will furnish agenda and a roster of Subcommittee members and expert consultants prior to the meeting. Summary minutes subsequent to the meeting will be available upon request to Central Data Management.

Attachment.

Charles E. Leasure, Jr.,

Acting Director, National Toxicology Program.

SUMMARY DATA FOR TECHNICAL REPORTS TENTATIVELY SCHEDULED FOR REVIEW AT THE MEETING OF THE NTP BOARD OF SCIENTIFIC COUNSELOR'S TECHNICAL REPORTS REVIEW SUBCOMMITTEE

[December 9-10, 1997]

Chemical CAS No.	Technical route/ report number	Primary uses	Exposure levels	Review order
1-Chloro-2-Propanol, Technical, 127-00-4.	TR-477	Chemical intermediate for propylene oxide & other organic compounds.	Dosed-Water (deionized water): Rats: 0, 150, 325, or 650 ppm; Mice: 0, 250, 500, or 1000 ppm (50/sex/group).	10
Coconut Oil Acid, Diethanolamine Condensate, 68603-42-9.	TR-479	Foam stabilizer in shampoos and dishwashing liquids; dyeing assistant in textile processing.	Topical (95% ethanol): Rats: 0, 50, or 100 mg/kg; Mice: 0, 100, or 200 mg/kg (50 sex/species/group).	2
Diethanolamine, 111-42-2	TR-478	Production of textile lubricants. Rubber chemicals intermediate; emulsifier in agricultural chemicals, cosmetics, and pharmaceuticals; gas conditioning agent.	Topical (ethanol): Male Rats: 0, 16, 32, or 64 mg/kg; Female Rats: 0, 8, 16, or 32 mg/kg; Mice: 0, 40, 80, or 160 mg/kg (50/sex/species/group).	1

SUMMARY DATA FOR TECHNICAL REPORTS TENTATIVELY SCHEDULED FOR REVIEW AT THE MEETING OF THE NTP BOARD OF SCIENTIFIC COUNSELOR'S TECHNICAL REPORTS REVIEW SUBCOMMITTEE—Continued

[December 9–10, 1997]

Chemical CAS No.	Technical route/ report number	Primary uses	Exposure levels	Review order
Furfuryl Alcohol, 098–00–0	TR–482	Solvent for ethers and esters; in manufacture of phenolic and furan resins. chemical intermediate. wetting agent; liquid propellant; flavoring agent, obtained from processing corncobs, coffee beans.	Inhalation (air): Rats & Mice: 0, 2, 8, or 32 ppm (50/sex/species/group).	8
Isobutene, 115–11–7	TR–487	Production of diisobutylene, trimers, butyl rubber, antioxidants for foods, packaging, food supplements & plastics, isooctane, high-octane aviation gasoline, polyisobutene resins. (NTP Executive Summary).	Inhalation (air): Rats & Mice: 0, 500, 2000, or 8000 ppm 50/sex/species/group).	6
Isoprene 78–79–5	TR–486	Monomer and comonomer for elastomers, prepared from turpentine, petroleum products (Merck 1989).	Inhalation (air): Rats: 0, 220, 700, or 7000 ppm; 50/sex/group).	7
Lauric Acid, Diethanolamine Condensate, 120–40–1.	TR–480	Foam stabilizer for liquid household detergents and shampoos.	Topical (95% ethanol): Rats: 0, 50, or 100 mg/kg; Mice: 0, 100, or 200 mg/kg (50/sex/species/group.	3
Oleic Acid, Diethanolamine Condensate, 93–83–4.	TR–481	Surfactant in wax emulsion; to increae shampoo viscosity.	Topical (95% ethanol): Rats: 0, 50, or 100 mg/kg; 50/sex/group; Mice: 0, 15, or 30 mg/kg; 55/sex/group.	4
Pentachlorophenol, Purified, 87–86–5	TR–483	Wood preservative, fungicide, soil fumigant for termites, preharvest defoliant, seed treatment for beans, preservative for paint, leather, textiles, inks, antibacterial agent in disinfectants & cleaners (HSDB 1990).	Dosed-Feed (NIH–07); Rats: 0, 200, 400, or 600 ppm; 50/sex/group—1000 ppm Stop Study (60/sex).	5
Pyridine 110–86–1	TR–470	Solvent, organic synthesis, flavoring ingredient, manufacture of fungicides, pharmaceuticals, dyestuff, explosives, mfr. of vitamins, sulfa drugs, intermediate in manufacture of diquat & paraquat, waterproofing textiles (HSDB 1990).	Dosed-Water (deionized water): Rats: 0, 100, 200, or 400 ppm; Male Mice: 0, 250, 500, or 1000 ppm; Female Mice: 125, 250 or 500 ppm; Male Wistar Rats: 0, 100, 200, or 400 ppm (50/sex/group).	9

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DEPARTMENT OF THE INTERIOR

Fish and Wildlife Service

Notice of Receipt of Application for Endangered Species Permit

The following applicants have applied for permits to conduct certain activities with endangered species. This notice is provided pursuant to Section 10(c) of the Endangered Species Act of 1973, as amended (16 U.S.C. 1531 *et seq.*):

Applicant: Mark Hartman, Nashville, Tennessee PRT–836121.

The applicant requests authorization to take (salvage dead shells, and harass during surveys) the dwarf wedge mussel, *Alasmidonta heterodon,* throughout the species range in North Carolina, for the purpose of enhancement of survival of the species.

Applicant: David L. Leonard, Sebring, Florida PRT–836133.

The applicant requests authorization to take (harass during installation of cavity inserts) the red-cockaded woodpecker, *Picoides borealis,* at St. Sebastian River State Buffer Preserve, Brevard County, Florida, for the purpose of enhancement of survival of the species.

Written data or comments on these applications should be submitted to: Regional Permit Biologist, U.S. Fish and Wildlife Service, 1875 Century Boulevard, Suite 200, Atlanta, Georgia 30345. All data and comments must be received by December 18, 1997.

Documents and other information submitted with this application are available for review, subject to the requirements of the Privacy Act and Freedom of Information Act, by any party who submits a written request for a copy of such documents to the following office within 30 days of the date of publication of this notice: U.S. Fish and Wildlife Service, 1875 Century Boulevard, Suite 200, Atlanta, Georgia 30345 (Attn: David Dell, Permit Biologist). Telephone: 404/679–7313; Fax: 404/679–7081.

Dated: November 3, 1997.

Sam D. Hamilton,

Regional Director.

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