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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 11-12, 1996

Research Triangle Park, N.C.

The meeting began at 8:30 a.m. on December 11 and 12 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), Arnold Brown, Thomas Goldsworthy, Robert LeBoeuf, Janardan Reddy, Irma Russo, Louise Ryan, Robert Taylor, Frederick Tyson, and Jerrold Ward. All members were present. These minutes have been reviewed and approved by all members of the Subcommittee. They were written by Dr. Larry G. Hart, Executive Secretary.

When available, a limited number of final NTP Technical Reports for the studies may be obtained free of charge from the NTP Central Data Management, MD A0-01, P.O. Box 12233, Research Triangle Park, N.C., 27709. Telephone: 919/541-3419. Subsequently, they may be purchased from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, Va., 22161, 703/487-4650.

The next NTP technical reports peer review meeting will be held December 9 and 10, 1997, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, 919/541-3971.

Contents

DRAFT SUMMARY MINUTES
NTP TECHNICAL REPORTS REVIEW SUBCOMMITTEE MEETING
December 11-12, 1996

Discussion of *Helicobacter* in NTP Toxicology and Carcinogenesis Studies. Dr. J. Bucher introduced the topic by noting that about two years ago, the NTP first reported a study in which B6C3F$_1$ mice were found to have a concurrent infection that was later determined to be a strain of the bacteria *Helicobacter*. The original observation was seen in the triethanolamine study which was peer reviewed by the Subcommittee on November 29, 1994. Subsequently, *Helicobacter* was found in other NTP long-term toxicology and carcinogenesis studies. Dr. Bucher said that Dr. J. Ward, Subcommittee member and expert on *Helicobacter,* would present an overview of the *Helicobacter* problem. Dr. J. R. Hailey, NIEHS, would then present findings from the several NIEHS/NTP studies affected by *Helicobacter* and present the NTP's position on the impact of the bacteria on interpretation of neoplastic findings in affected studies. Dr. G. N. Rao, NIEHS, would then make a short presentation on disease control in NTP animal studies. Dr. Bucher concluded by acknowledging scientists at NIEHS and elsewhere who have made significant contributions to studying this problem.

Dr. Ward -- Dr. Ward said he had become involved with research on *Helicobacter* at Frederick Cancer Research Center, NCI, about four years ago, and would try to emphasize points that might be important in evaluating the NTP studies with the infection. He said there are now about 20 named *Helicobacter* species in humans and animals with about six of these identified in rats and mice. The species of concern in the NTP studies is *Helicobacter hepaticus*. Dr. Ward reported that the problem that drew his attention to *H. hepaticus* occurred when a high incidence of toxic hepatitis was found in control A/JCr mice. He said they were able to isolate the organism, identify it molecularly as a new organism, and transmit the disease with liver suspensions. *Helicobacters* are identified biochemically by certain characteristics and differences between them, but most importantly by the sequence of the 16S ribosomal RNA. Negative stain in culture works well to differentiate *hepaticus* from other mouse or rodent *Helicobacters*. This organism seems to be the most pathogenic *Helicobacter* found in mice as yet, and causes hepatitis and liver tumors which have been reproduced at least to some degree. The strains most susceptible were those at Frederick. Dr. Ward described the histogenesis of the hepatic lesions in mice. There is an acute hepatitis that occurs over a period of one to a few months, characterized by focal necrosis and focal inflammation, followed by a chronic active hepatitis. Up to 12 months, there is hepatocytomegaly, cholangitis, oval cell hyperplasia, and focal necrosis and a fibrosis, Dr. Ward termed "mouse cirrhosis". After 12 months, preneoplastic foci are seen followed by development of heptocellular adenomas and carcinomas. The *H. hepaticus* organism can be identified in tissue with silver stains; the Steiner is preferred because it is more specific, although the Warthin-Starry method works well too. Dr. Ward said that from the standpoint of the affected NTP studies, it was important to note that female mice are also infected but at a lesser degree, e.g., females generally do not have much hepatitis. Inflammatory large bowel disease, especially in immunodeficient animals, is another manifestation of *H. hepaticus* infection. Dr. Ward concluded by acknowledging the collaborative nature of their studies on the *Helicobacters,* noting especially the presence in the room of a major collaborator, Dr. James Fox, MIT.

Dr. Hailey -- Dr. Hailey thanked Dr. Ward for the continuing help that he has provided in exploring the effects of *Helicobacter hepaticus* in the NTP studies. Dr. Hailey said his

discussion would deal with the organism specifically within the context of the NTP two-year studies. He noted that the Subcommittee members had received an appendix that will be included in each of the Technical Reports for which *Helicobacter* is an issue. Specific information for a study then would be included in the body of the Report. He reported that *Helicobacter* was first identified as a problem in 1993 when a pathologist identified suspicious lesions in treated and control animals in one study. Silver stains were used to identify organisms within the liver consistent with *Helicobacter*. To determine the scope of the problem, pathology tables starting with 1984, a total of 67 studies, were reviewed. Nine studies were identified with the characteristic liver lesions, one from 1988, and eight from late 1990 to early 1991, with all animals coming from one of two major suppliers at that time.

Dr. Hailey described the *Helicobacter*-associated liver disease process in NTP studies. The disease appears to be insidious with no obvious clinical signs. Characteristic liver lesions have not been observed in any 90-day study in $B6C3F_1$ mice, consistent with Dr. Ward's findings. In the affected NTP studies, from 12-18 months, 34% of early death mice had the characteristic lesions contrasted with 51% of early death animals between 18 and 24 months. The only significant lesions were observed in the liver, primarily in males. Dr. Hailey listed the nine studies, four of which were conducted with AZT and were combined into one report, while three of the others, cobalt sulfate, chloroprene, and theophylline also were to be reviewed at this meeting. In control mice, the non-neoplastic liver lesions were characteristic of *Helicobacter.* as described earlier by Dr. Ward. Generally, a low incidence in controls was paralleled by low incidences of lesions in treated groups. The time sequence of liver lesions was consistent with Dr. Ward's studies. In identifying the presence of bacteria, the silver stains were only moderately sensitive, e.g., 26% of male mice with mild to moderate liver lesions were negative. Both Dr. Fox's and NTP laboratories have conducted polymerase chain reaction (PCR) assays for detecting the organisms and found PCR to be more sensitive than silver staining. Dr. Hailey reported there were problems with using PCR, one being that none of the PCR based assays had proven reliable for use with formalin fixed tissues and only formalin fixed tissues are available from six of the nine studies. And even in studies with frozen tissue, there was a limited amount and frozen liver was almost always limited to tissue from a neoplasm.

Dr. Hailey commented that a PCR-RFLP assay for use with formalin fixed tissues has been developed and with the aid of a laboratory at the University of Missouri has been used to assay 32 two-year studies, including the nine, and three 90-day studies with the majority selected because they were started during the same time frame as the affected studies. Of the 32 studies, frozen tissue was available from 22 and of these all three of the tumor positive studies were also positive for *H. hepaticus*, while of the other 19 with no lesions, three were also positive. Of the 10 studies with formalin fixed livers, only one of the six affected studies was positive for *H. hepaticus*, while of the four studies with no lesions all were negative with PCR. Dr. Hailey reported that another study was designed to determine whether *Helicobacter* had an impact on other tumors where there high enough spontaneous rates to allow comparison between affected and unaffected studies with frozen tissue available. Both male and female mice were evaluated for the nine affected and 13 unaffected studies. Statistically significant increases in malignant and all tumors in male mice could be accounted for by the increases in liver tumors and hemangiosarcomas of the liver. A similar although less dramatic pattern was observed in females. He noted that a confounding factor is that there has been an increase in the

incidence of liver neoplasms in control $B6C3F_1$ mice over the last few years, and much of this increase can be attributed to increased body weights that have also occurred. Dr. Hailey reported that study start dates for most of the affected studies (8/9) were later in the time frame while dates for most of the unaffected studies (11/13) were earlier. Then, he said that a reasonable argument could be made for considering studies in which there are no liver lesions as unaffected. Using this criterion, an additional 13 studies were added to the group of 13 unaffected studies. Even with 26 studies, the unaffected studies are still clustered toward the early time points and the increase in liver tumors in the affected male mice is not as dramatic but still present, while in female mice the slight increase is not statistically significant

Since the tumor effect in male mice appears real as an increase associated with *Helicobacter,* a mutational spectrum was examined for a mutation for which the NTP has a large database, H-*ras* codon 61 AAA mutations, with a historical control incidence of 32%. Two affected studies were examined, with only 4-5% incidence of these mutations, while two unaffected studies combined for a 32% incidence, right at the historical control rate, providing further evidence that the increased liver neoplasms in the affected studies were related to *Helicobacter*. As additional evidence, animals from the nine affected studies were categorized into three groups: (a) animals with mild to moderate inflammatory lesions considered related to *H. hepaticus* infection; (b) animals with minimal to mild lesions that may have been associated with *H. hepaticus*; and (c) animals with no liver lesions considered associated. Within affected studies, the incidence of liver neoplasms was significantly increased ($p<0.05$) in males with mild to moderate *Helicobacter*-associated liver lesions when compared to animals without such liver lesions. In other studies, cell kinetics were evaluated in exploring the link between animals with lesions and development of tumor. Proliferating nuclear antigen (PCNA), an endogenous marker of cell proliferation was used in comparing animals in affected studies with lesions, animals in affected studies without lesions, and animals in unaffected studies. Using PCNA, the S-phase labeling index (LI) and proliferating index (PI), which represents the percentage of cells cycling, were determined. Male mice with lesions from affected studies had significantly ($p<0.001$) higher PCNA values for LI and PI than did male mice without lesions, both from affected and unaffected studies. Increased apoptosis (programmed cell death) usually accompanies increased cell proliferation. For males, the apoptotic index in one affected study was significantly greater than that observed in two unaffected studies. Finally, labeling indices in livers of male mice from two 90-day studies started during the time of the nine affected studies were compared with values cited in the literature and there were no differences.

Dr. Hailey said that the most important aspect of our investigation is how we interpret our studies in the face of this infection (*H. hepaticus*) or this disease process (hepatocellular neoplasms and hemangiosarcomas). He said that clearly the increase in liver neoplasms seen in the male mouse make this particular endpoint in the male mouse confounded. However, tumors at other sites in affected studies can be interpreted in the usual manner. For the female mouse, the liver response likely can be interpreted in the usual manner, and, sites other than the liver in females can be interpreted in the usual manner. Dr. Hailey mentioned two projects proposed to strengthen interpretation of neoplastic effects in the livers of female mice. One would be to determine incidence of H*ras* codon 61 AAA mutations in liver neoplasms from control females in affected studies in which liver neoplasms are increased in treated females. The other project would be to confirm that

studies in which only formalin fixed tissue is available are indeed "unaffected". One approach is to use PCR to confirm them negative for *H. hepaticus*. Another is to confirm that studies positive for *H. hepaticus* without liver disease are not adversely affected.

Discussion: Dr. Fox reported that they had just characterized *Helicobacter* in rats and wondered if the NTP had made any effort to identify the organism in rats. Dr. Hailey responded that evidence had not been seen of any problem in NTP studies although there had not been an opportunity to explore the possibility at this point. Dr. Goldsworthy asked for an assessment of the strength of evidence in the affected and unaffected categories. Dr. Hailey said the category of affected was solid in that the organism was definitively identified in the three studies where frozen tissue was available and for five of the six studies where only formalin fixed tissues were available, the liver lesions characteristic of *Helicobacter* were present, and as well the organism was identified with the Warthin-Starry stain. He said the findings for unaffected are a bit more tenuous although the PCR studies with frozen tissues along with cell proliferation assays tend to support the conclusion that there is no effect. In response to a question by Dr. Ward as to whether organisms had been looked for in the cecum, Dr. Hailey said that frozen intestinal sections were negative for *Helicobacter*. Dr. Fox commented that with *H. pylori* there are virulent and non-virulent strains while with *H. hepaticus* this characterization has not been made and can't be made on retrospective data so he might disagree with lesions being interpretable in the "usual manner". Dr. Goldsworthy asked whether chemical treatment might not exacerbate the incidence of *H. hepaticus*-associated nonneoplastic liver lesions. Dr. Hailey said the incidence was similar in control and treated groups in the nine affected studies.

Dr. Rao -- Dr. Rao addressed the control and prevention of infectious diseases in the production colonies and the testing facilities conducting studies for the NTP, giving a brief overview of the procedures in place. With regard to production colonies, he said the foundation colony is derived with known microflora and feeds into an expansion colony to provide adequate breeder animals for production, and both colonies are one way. He described the comprehensive health evaluation program used, stating that the NTP production colonies have not supplied any animals with known infectious agents to NTP studies since April 1983; however, the existence of *Helicobacter* was not known until about three years ago. With regard to the testing facilities, Dr. Rao described the sentinel animal program which was begun by the NCI carcinogenesis program in 1978 and has been in use ever since. From 1978 to 1984, almost all NTP studies had one or more viral infections; from 1984 to 1988, studies with viral infections decreased; and since 1988, most of the studies were free of viral infection with the last 25 studies reviewed by the Subcommittee completely free, with one recent exception. The AZT studies in the current set of reports to be reviewed had a viral infection. The infection was limited to the AZT studies by not allowing the facility to start any new studies. Dr. Rao confirmed the time frame within which the studies having *Helicobacter* were initiated, noting that mice for all of the studies came from one contractor although not just one production colony. He said that the production colony from whence came the first study with *Helicobacter* was terminated, not because of *Helicobacter* but because of the detection of opportunistic pathogens. Dr. Rao said that currently in 22-month old mice there is no indication of the organism. Rats have been included in serology assays to determine whether *Helicobacter* could be a problem.

Discussion: Dr. Fox cautioned against depending on ELISA assays or serological surveillance, and particularly with rats where there are no data in terms of seroconversion in terms of absence or presence of the organism. Rather, he said the organism should be cultured, identified by biochemical and molecular techniques, and then a sensitive and specific PCR should be developed. Dr. Rao said that the Program is depending on the culture but is validating serology assays.

Public Comments and Discussion: Dr. Fox urged that future work proceed toward development of molecular markers such as mentioned by Dr. Hailey in terms of looking at H-*ras*. He would also highlight research being done by Dr. Lucy Anderson at Frederick Cancer Research Center with modified DNA adducts. Dr. Fox urged reevaluation of the interpretation of the impact of the infection in livers of female mice. Dr. Hon Wing Leung, Union Carbide Corporation, representing the CMA Alkanolamines Panel, expressed concern that the *Federal Register* announcement of the meeting did not indicate there would be a discussion of the *Helicobacter* problem. He noted also that the Appendix on *Helicobacter* was made publicly available only recently, and thus, there was insufficient time for the Panel to provide written comments. Dr. Bucher expressed regrets that the Appendix was not available sooner. He said that the NTP would certainly accept comments subsequent to the meeting. He said that he didn't think there any studies coming up where the infection was present. The study on triethanolamine that was peer reviewed and accepted by the Subcommittee in 1994 was being reevaluated in terms of *Helicobacter*. Dr. LeBoeuf asked for clarification as to why Dr. Fox thought *Helicobacter* to be a confounder in female mice. Dr. Fox said he had not time to evaluate the present studies but based his statements on review of the triethanolamine data. He stated that in the triethanolamine study, there is almost identical prevalence in both male and female mice of culturable *H. hepaticus* identified by sensitive molecular techniques after culture and also by PCR. Dr. Jerry Rice, IARC, commented that there seems to be a dramatic nonuniformity in different lobes of the liver with regard to severity of the *H. hepaticus* induced lesions, even in susceptible strains of mice.

NTP Draft Technical Report Reviews

3'-Azido-3'-deoxythymidine (AZT) & Interferon AD + AZT. Dr. R. D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of 3'-azido-3'-deoxythymidine (AZT) & interferon AD + AZT by discussing the uses and rationale for study, describing the experimental design including a recovery group of mice to assess reversibility of bone marrow changes, reporting on the lack of survival and body weight effects, and commenting on treatment-related neoplastic and non-neoplastic lesions in male and female mice. The conclusions for the two-year studies in mice were that:

> Under the conditions of these 2-year gavage studies there was **equivocal evidence of carcinogenic activity** of AZT in male mice based on increased incidences of renal tubule and Harderian gland neoplasms in groups receiving AZT alone. There was **clear evidence of carcinogenic activity** of AZT in female mice based on increased incidences of squamous cell neoplasms of the vagina in groups that received AZT alone or in combination with α-interferon A/D.

Hematotoxicity occurred in all groups that received AZT.

Treatment with AZT alone and AZT in combination with α-interferon A/D resulted in increased incidences of epithelial hyperplasia of the vagina in all dosed groups of females.

Dr. LeBoeuf, a principal reviewer, agreed with the conclusions. He noted the presence of *H. hepaticus* as a confounding factor for interpretation of liver lesions but in this case would not have an impact on the level of evidence for males or females.

Dr. Reddy, the second principal reviewer, agreed with the conclusions. He asked for a rationale for why the studies were not also done in rats, and asked whether there were any case reports indicating an increase in vaginal tumors in human females. Dr. Irwin responded that Burroughs Wellcome had conducted a satisfactory study in rats so there would be nothing gained by repeating it. Dr. Ken Ayers, Glaxo-Wellcome, reported that the findings have been cited in the *Physicians Desk Reference* (PDR) since about 1990. Dr. Irwin said there are no reports in the literature of genital tumors in human females associated with use of AZT. Dr. Reddy said that, nonetheless, the findings could raise concerns because of the genital papillomas and warts reported in human papilloma virus infected HIV-positive males and females.

Dr. Tyson, the third principal reviewer, agreed with the conclusions. He asked about the rationale for the strain of mice used and thought it would have been of interest to have used a strain of immuno-compromised mice. Dr. Tyson commented that useful insights might have been gained from looking for molecular markers found in human vaginal tumors, such as activated oncogenes or certain papilloma viruses. Dr. Irwin said that with the complexity of the study, it was preferred to use the $B6C3F_1$ model for which there was a large historical database.

Dr. Goldsworthy asked whether a higher incidence of hepatoblastomas in mice might be associated with *Helicobacter*. Dr. Hailey said that we had seen hepatoblastomas more frequently in recent studies as part of what seems to be a progression of that lesion.

Dr. LeBoeuf moved that the Technical Report on 3'-azido-3'-deoxythymidine (AZT) & interferon AD + AZT be accepted with revisions discussed and the conclusions as written for male mice, **equivocal evidence of carcinogenic activity**, and for female mice, **clear evidence of carcinogenic activity**. Dr. Reddy seconded the motion, which was accepted unanimously with eight votes.

Chloroprene. Dr. R. L. Melnick, NIEHS, introduced the toxicology and carcinogenesis studies of chloroprene by discussing the use and rationale for study, describing the experimental design, and commenting on the numerous compound-related neoplastic and non-neoplastic lesions in male and female rats and mice. He emphasized that chloroprene is the 2-chloro analogue of 1,3-butadiene. The 2-year studies were done by inhalation exposure at concentrations of 0, 12.8, 32, and 80 ppm. Dr. Melnick noted that reduced survival in male and female mice at the top two doses was associated with tumor responses as in the NTP studies on 1,3-butadiene. To give a better sense of what the actual tumor effects may have been, supplemental statistical analyses were performed by the survival-adjusted "Poly-3" quantal response test currently being developed by the NTP which modifies the Cochran-Armitage/Fisher exact test by adjusting the denominators of the neoplasm rates to take into account survival differences. Dr. Melnick reported that in a further comparison with the 1,3-butadiene studies, the survival adjusted tumor data were fit to a Weibull model to get a characterization of the shape of the dose-response curves. As with 1,3-butadiene, the dose response was generally linear, and when deviating from linear, it was supralinear. He said that from that data, it could be concluded that chloroprene's potency as a carcinogen in mice was equal to or greater than that of 1,3-butadiene. Dr. Melnick concluded by noting that unlike 1,3-butadiene, chloroprene was not genotoxic in the usual NTP assays. Dr. Sills described studies evaluating lung neoplasms and Harderian gland neoplasms in mice for mutations in the K-*ras* gene. Looking at the mutational frequency and spectra for chloroprene-induced tumors, the mutational spectra were shown to be different from that of controls in that there were increased frequencies of mutations in lung and Harderian gland neoplasms, and in lung neoplasms there was a unique CTA mutation at codon 61 which had never been detected before in terms of the NTP historical database. The conclusions for the two-year studies in rats and mice were that:

> Under the conditions of these 2-year inhalation studies, there was **clear evidence of carcinogenic activity** of chloroprene in male F344/N rats based on increased incidences of neoplasms of the oral cavity; increased incidences of neoplasms of the thyroid gland, lung, and kidney were also attributed to chloroprene exposure. There was **clear evidence of carcinogenic activity** of chloroprene in female F344/N rats based on increased incidences of neoplasms of the oral cavity; increased incidences of neoplasms of the thyroid gland, mammary gland, and kidney were also attributed to exposure to chloroprene. Low incidences of urinary bladder neoplasms in male and female rats and lung neoplasms in female rats may also have been related to exposure to chloroprene.
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> There was **clear evidence of carcinogenic activity** in male B6C3F$_1$ mice based on increased incidences of neoplasms of the lung, circulatory system (hemangiomas and hemangiosarcomas), and Harderian gland; increased incidences of neoplasms of the forestomach and kidney were also attributed to exposure to chloroprene. There was **clear evidence of carcinogenic activity** of chloroprene in female B6C3F$_1$ mice based on increased incidences of neoplasms of the lund, circulatory system (hemangiomas and hemangiosarcomas), Harderian gland, mammary gland, liver, skin, and mesentery; increased incidences of neoplasms of the forestomach and Zymbal's gland were also attributed to exposure to chloroprene.

Exposure of male and female rats to chloroprene was associated with increased incidences of alveolar epithelial hyperplasia in the lung; nephropathy; and several nonneoplastic effects in the nose including olfactory epithelium atrophy, fibrosis, adenomatous hyperplasia, basal cell hyperplasia, chronic inflammation, respiratory metaplasia, and necrosis. Exposure of male and female mice to chloroprene was associated with increased incidences of bronchiole hyperplasia and histiocytic cell infiltration in the lung; epithelial hyperplasia in the forestomach; renal tubule hyperplasia (males only); several effects in the nose including olfactory epithelium atrophy, respiratory metaplasia, and adenomatous hyperplasia; and hematopoietic cell proliferation of the spleen.

Dr. Ward, a principal reviewer, agreed with the conclusions. He commented that there were many nonneoplastic lesions in the nasal cavity of rats and mice but no nasal tumors. In light of present theory/hypotheses that chronic lesions may lead to cancer, he said the Discussion should make note that the many toxic and reparative nasal lesions did not lead to tumors. Dr. Ward said it was important to know whether the hyperplasias in many organs were focal or diffuse. Dr. Melnick said most of the hyperplasias were focal and this would be emphasized in the text. Because so many tissues were involved, Dr. Ward suggested an additional summary table for comparison to 1,3-butadiene which might list target organs of toxicity and carcinogenesis. Dr. Melnick agreed.

Dr. Goldsworthy, the second principal reviewer, agreed with the conclusions. He said the conclusions should note the significant changes in survival and body weights that occurred in the study. Dr. Goldsworthy thought the differing decreases in body weights between the different doses might call into question the numbers derived from the dose-response curves. Additionally, he stated that the conclusions should note the presence of *Helicobacter* infection, and the fact that this resulted in confounding the interpretation of liver hepatocellular tumors and hemangiomas in male mice.

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

Dr. Goldsworthy moved that the Technical Report on chloroprene be accepted with revisions discussed and with the conclusions as written for male and female rats and mice, **clear evidence of carcinogenic activity.** Dr. Russo seconded the motion, which was accepted unanimously with nine votes.

Cobalt Sulfate Heptahydrate. Dr. J. R. Bucher, NIEHS, introduced the toxicology and carcinogenesis studies of cobalt sulfate heptahydrate by discussing the uses and describing the experimental design which derived from NTP prechronic inhalation studies. He reported there were no survival or body weight effects in rats or mice, and commented on the extensive compound-related neoplastic and non-neoplastic lesions 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 cobalt sulfate heptahydrate in male F344/N rats based on increased incidences of alveolar/bronchiolar neoplasms. A marginal increase in incidences of pheochromocytomas of the adrenal medulla may have been related to exposure to cobalt sulfate heptahydrate. There was **clear evidence of carcinogenic activity** in female F344/N rats based on increased incidences of alveolar/bronchiolar neoplasms and pheochromocytomas of the adrenal medulla in groups exposed to cobalt sulfate heptahydrate. There was **clear evidence of carcinogenic activity** of cobalt sulfate heptahydrate in male and female $B6C3F_1$ mice based on increased incidences of alveolar/bronchiolar neoplasms. Exposure to cobalt sulfate heptahydrate caused a spectrum of inflammatory, fibrotic, and proliferative lesions in the respiratory tract of male and female rats and mice.

Dr. Tyson, a principal reviewer, agreed with the conclusions. Concerning the genetic mechanisms involved in murine lung tumorigenesis, he said that although a comprehensive study of K-*ras* activation was done in lung tumors, other molecular markers could have been assessed as well. The study of LOH or homozygous deletions on regions of chromosome 4, which are syntenic to regions of human chromosome 9p21 where frequent deletions are observed in human lung cancer, could have been studied to determine if similar mechanisms are at work in both murine and human lung tumorigenesis via exposure to this chemical. Dr. R. Sills, NIEHS, reported that further studies were planned with the next step being to look at loss of heterozygosity not only on chromosome 4, but also to look at chromosomes 6 and 11, where the p53 genes are located.

Dr. Ward, the second principal reviewer, agreed with the conclusions. He agreed with the rationale for the doses chosen for the two-year study but because there was no dose-related body weight gain depression he thought that both rats and mice could have tolerated higher doses. With regard to the extensive lesions in the nasal cavity and larynx, he stated that this was a classic case showing the association between toxic and regenerative/reparative lesions resulting in no tumors.

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

Dr. Tyson moved that the Technical Report on cobalt sulfate heptahydrate 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, **clear evidence of carcinogenic activity**. Dr. Russo seconded the motion, which was accepted unanimously with eight votes.

Ethylbenzene. Dr. P. C. Chan, NIEHS, introduced the toxicology and carcinogenesis studies of ethylbenzene by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weights, and commenting on compound-related neoplastic 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 **clear evidence of carcinogenic activity** of ethylbenzene in male F344/N rats based on increased incidences of renal tubule neoplasms. The incidences of testicular adenomas were also increased. There was **some evidence of carcinogenic activity** of ethylbenzene in female F344/N rats based on renal tubule adenomas. There was **some evidence of carcinogenic activity** of ethylbenzene in male B6C3F_1 mice based on increased incidences of alveolar/bronchiolar neoplasms. There was **some evidence of carcinogenic activity** of ethylbenzene in female B6C3F_1 mice based on increased incidences of hepatocellular neoplasms.
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> Exposure of male and female rats to ethylbenzene resulted in increased incidences of renal tubule hyperplasia and an increased severity of nephropathy. Exposure of male mice to ethylbenzene resulted in increased incidences of alveolar epithelial metaplasia, syncytial alteration of hepatocytes, hepatocellular hypertrophy, hepatocyte necrosis, and thyroid gland follicular hyperplasia. In female mice, ethylbenzene exposure caused increased incidences of eosinophilic foci of the liver, pituitary gland pars distalis hyperplasia, and thyroid gland follicular cell hyperplasia.

Dr. Reddy, a principal reviewer, agreed with the conclusions. He said that for purposes of contrasting findings with those of Maltoni, published in 1985, there should be information cited on types, sites and incidences of tumors from that study. Dr. Chan said the total number of tumors were given but not differentiated by target organ in that paper. Dr. Reddy noted that the methods used, e.g., immunohistochemistry, to rule out α-2μ-globulin nephropathy in male rats should be described. Dr. J. Mahler, NIEHS, responded that H and E stain is a good screen for hyaline droplet accumulation and was used.

Dr. Goldsworthy, the second principal reviewer, agreed with the conclusions. He agreed that the inhalation route was appropriate although it was noted that ethylbenzene has been detected in surface water and ground water. Dr. Goldsworthy thought the further information obtained from renal step sections was helpful but asked for clarification about the decision to do step sections and why not with other organs such as thyroid or pituitary. Dr. Hailey said the major reason to conduct a step section was to help in interpreting an equivocal or uncertain effect. Endocrine organs such as thyroid or pituitary are too small to step section. Dr.. Goldsworthy suggested **clear evidence** might have been a better call in male mice based on a positive dose-response trend and presence of metaplasia in the target tissue. Dr. Mahler said that metaplasia is a rather unusual lesion and is not generally recognized as being a precursor to neoplasia.

Dr. Ryan, the third principal reviewer, agreed with the conclusions. She said that one of the reasons for studying the chemical was its structural similarity to benzene and toluene, and wondered why there wasn't more discussion comparing toxic effects of the three chemicals. Dr. Ryan expressed concern that the high dose in female rats and male and

female mice may have been too low as there were really no survival or body weight effects in these groups. Dr. Bucher commented that prechronic studies were performed on ethylbenzene and an NTP study report published in 1992. There essentially were no histopathologic findings in the prechronic studies so the dose selection for the chronic study was based on body weight deficit in male rats. Dr. Ryan said you could argue for there being **clear evidence** in male mice based on a dose-related increase of combined benign and malignant lung neoplasms and in female mice based on a dose-related increase of combined benign and malignant hepatic neoplasms. Dr. J. Haseman, NIEHS, said there were three reasons for the level of evidence chosen. Most importantly, the tumor increases fell within the historical control range; secondly, they were primarily benign tumors; and thirdly, the tumor increases were seen in only one sex: lung tumors in males and liver tumors in females.

Dr. LeBoeuf commented that survival at the high dose in male rats was only 4% and the level of evidence was driven by increased incidences of renal tubule neoplasms in the high dose group. He said he was uncomfortable on basing the level of evidence on findings where there was such poor survival. Dr. Bucher responded that the fact we see the effect in both sexes and accompanied by severe nephropathy, the latter effect rarely if ever seen in females, suggests an intrinsic carcinogenic activity of ethylbenzene.

Dr. Ryan moved that the Technical Report on ethylbenzene be accepted with revisions discussed and the conclusions as written for male rats, **clear evidence of carcinogenic activity,** and for female rats and male and female mice, **some evidence of carcinogenic activity**. Dr. Reddy seconded the motion, which was accepted unanimously with nine votes.

Later in the meeting, Dr. LeBoeuf made a motion to reopen the discussion primarily on the tumor response in male rats. Dr. Taylor opined that the maker and seconder of the original motion on ethylbenzene should have to agree. Drs. Ryan and Reddy agreed to reopening the discussion. Dr. Goldsworthy seconded the motion, which was accepted by six yes votes to two no votes (Brown, Reddy). Dr. Ward was not present.

Dr. LeBoeuf stated that his concern was primarily the mortality in high dose male rats and the implication in terms of the interpretation of the data at that dose. He said in the original NCI guidelines for the two-year bioassay, one had to do with particular treatments not causing a significant change in survival unless reduced survival was a result of neoplasia and as a general guideline not to exceed a minimum of 10% decrement in body weight gain. In the ethylbenzene report, he said it was clearly stated that the majority of the neoplasms in male rats were considered to be incidental to the cause of death. This led him to propose reducing the conclusion in male rats to **some evidence of carcinogenic activity**. Dr. Haseman pointed out that at week 84, the survival in high dose male rats was still 70%. Dr. Goldsworthy said an issue here has to do with when the first tumors arose. Dr. Bucher commented that nephropathy likely was the primary contributor to mortality. Dr. Haseman suggested that as with the oxazepam study, there be wording in the conclusions to the effect that there was **clear evidence only** at doses resulting in enhanced nephropathy. Dr. Bucher noted that in many studies over the years the conclusion for carcinogenic activity was affirmed even though the MTD was exceeded. In most studies where there are renal tubular neoplasms associated with nephropathy in male rats, carcinomas are generally not seen, a like response in females is generally not

seen, and 21 tumors are certainly not seen. Dr. Goldsworthy reminded the reviewers of the caveat - "Under the conditions of these studies...". Dr. Ryan pointed out that in the low dose group without the extended evaluation, the tumor incidence was outside the historical control range.

Dr. LeBoeuf moved that the conclusions for male rats be changed to **some evidence of carcinogenic activity**. Dr. Ryan seconded the motion, which was defeated by six no votes to two yes votes (LeBoeuf, Russo). Dr. Ward was not present.

Isobutyraldehyde. Dr. K. M. Abdo, NIEHS, introduced the toxicology and carcinogenesis studies of isobutyraldehyde by discussing the uses and rationale for study, describing the experimental design, 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 the two-year studies in rats and mice were that:

> Under the conditions of these 2-year inhalation studies, there was **no evidence of carcinogenic activity** of isobutyraldehyde in male or female F344/N rats or male or female B6C3F$_1$ mice exposed to 500, 1,000 or 2,000 ppm isobutyraldehyde.
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> In male and female rats, exposure to isobutyraldehyde induced squamous metaplasia and suppurative inflammation of the nasal respiratory epithelium, and degeneration of the nasal olfactory epithelium. In male and female mice, exposure to isobutyraldehyde vapor caused degeneration of the nasal olfactory epithelium.

Dr. Tyson, a principal reviewer, agreed with the conclusions. He said a discussion of the possible reasons for the discrepancy between genotoxicity reported in previous studies cited and what was observed in this study would be helpful.

Dr. Brown, the second principal reviewer, agreed with the conclusions. He suggested that some portion of the discussion in the Results regarding the insignificance of the nasal tumors found in rats be included in the Conclusions/Discussion in view of the rarity of nasal tumors of any kind. Dr. Brown acknowledged the appropriateness of the inhalation route but noted that significant human exposure can occur from food or water and said a comment on the natural availability of the compound would be helpful. Dr. Abdo replied that when put in water or food, the chemical is conjugated or combines with other chemicals and some degradation of the isobutyraldehyde is observed. Further, the chemical was nominated because of concerns about worker exposure.

Dr. Tyson moved that the Technical Report on isobutyraldehyde be accepted with revisions discussed and the conclusions as written for male and female rats and mice, **no evidence of carcinogenic activity**. Dr. Brown seconded the motion, which was accepted unanimously with eight votes. Dr. Ward was not present.

Oxazepam. Dr. J. R. Bucher, NIEHS, introduced the toxicology and carcinogenesis studies of oxazepam in rats by discussing the use and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic lesions in male rats, and non-neoplastic lesions in female rats. Dr. Bucher reported that the Subcommittee had reviewed a bioassay of oxazepam in Swiss-Webster and B6C3F$_1$ mice in 1992. The findings were that there was **clear evidence of carcinogenic activity** in both strains based on hepatocellular neoplasms, and there were also hepatoblastomas in B6C3F$_1$ mice. For the current study, there were no prechronic (13-week) studies performed. Dr. Bucher said that plasma oxazepam concentrations were taken at the end of the study in surviving animals. For comparison, terminal plasma levels were generally higher in mice as expected. In the current rat study, plasma levels in the low and mid-dose groups were in the range of human therapeutic plasma levels. The conclusions for the two-year studies in rats were that:

> Under the conditions of these 2-year dosed feed studies, there was **some evidence of carcinogenic activity** in male F344/N rats based on increased incidences of renal tubule adenomas in exposed groups also exhibiting significantly enhanced nephropathy. There was **no evidence of carcinogenic activity** of oxazepam in female F344/N rats exposed to feed containing 625, 2,500, or 5,000 ppm for 2 years or 10,000 ppm for 6 months.
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> Administration of oxazepam to rats resulted in nonneoplastic lesions in the forestomach, glandular stomach, and small intestine as well as centrilobular hypertrophy of hepatocytes in the liver. In addition, nephropathy was increased in incidence in female rats and was markedly increased in severity in male rats resulting in early mortality at the higher exposure concentrations.

Dr. Taylor, a principal reviewer, agreed in principle with the conclusions. He stated that because there was substantial reduction in body weight gain and survival in high and mid-dose male rats, along with dose response that was not very dramatic, he would argue for changing the conclusion in male rats to **equivocal evidence of carcinogenic activity**. Noting the figure illustrating the metabolism of oxazepam in F344 rats, Dr. Taylor opined that some discussion of rat versus mouse metabolism would be useful as an aid in trying to explain the difference between mouse and rat in sites of toxicity and neoplasia.

Dr. Brown, the second principal reviewer, agreed with the conclusions. He said that it would be helpful if additional information could be provided in the Abstract regarding the background against which this bioassay was conducted, i.e., the unpublished study by industry in Sprague-Dawley rats. Dr. Bucher observed that the current *Physicians Desk Reference* provides description of the rat study performed by Wyeth, although no doses are listed. The citation indicated there were increases in prostatic adenomas, interstitial cell adenomas of the testes, and thyroid follicular cell adenomas, none of which were replicated in the current study in F344 rats. Dr. W. Allaben, NCTR/FDA, pointed out that the data are considered proprietary information and by law cannot be released publicly. Dr. Bucher mentioned that this was one of four benzodiazepines nominated and selected for study. Three were products of Hoffman-LaRoche, who agreed to carry out the studies with our assistance in study design. The studies were completed and data submitted to the FDA.

There ensued a lengthy discussion about the appropriateness of step sectioning of kidneys in male rats and on the issue of the doses used. Dr. Bucher said that in retrospect the 1,250 ppm dose group that was terminated after 26 weeks with the thought it would be uninformative would have been the best top dose. Since the nephropathy was enhanced at the top two doses used (2,500 and 5,000 ppm), it was thought to have impacted on formation of the renal adenomas. Dr. LeBoeuf argued that if the top dose group were excluded from analysis because of very poor survival and one looks at the results for renal adenomas in the mid and low dose groups compared with controls, he would conclude there was **equivocal evidence**. Dr. Haseman noted that the increase in renal adenomas in the mid dose group was significant at P= .018. Dr. Goldsworthy asked under what circumstances the NTP would consider a study to be inadequate for evaluation. Dr. Bucher said generally, the NTP might consider a study as being inadequate if there is poor survival and there is no tumor response such that the ability of the study to detect a response may have been compromised. Dr. Goldsworthy asked whether not including a 13-week study is going to be done more in future bioassays. Dr. G. Boorman, NIEHS, pointed out that in the case of oxazepam a 26-week study did not predict very well; however, the decision whether to employ a 13-week study would have to be decided on a case-by-case basis drawing on other toxicity information that is available.

Dr. Taylor moved that the Technical Report on oxazepam be accepted with revisions discussed and the conclusion as written for female rats, **no evidence of carcinogenic activity**, and changed for male rats from **some evidence of carcinogenic activity** to **equivocal evidence of carcinogenic activity**. Dr. Brown seconded the motion. In discussion, Dr. Ward opined that having toxicity in an organ such as the kidney where there are also tumors strengthens the evidence for the tumors being chemically induced because the organ is a target site for the chemical. Dr. Haseman said that if one considers the increase in tumors to be chemically-related, **some evidence** would be appropriate, if one feels uncertainty as to chemical-relatedness then **equivocal evidence** would be an appropriate interpretation. Dr. Taylor's motion was accepted with five yes votes to three no votes (Goldsworthy, Reddy, Ward).

Polyvinyl Alcohol. Dr. A. Radovsky, NIEHS, introduced the toxicology and carcinogenesis studies of polyvinyl alcohol by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on the lack of compound-related neoplastic or non-neoplastic lesions in female mice. The conclusions for the two-year studies in female mice were that:

> Under the conditions of this 2-year study there was **no evidence of carcinogenic activity** in female B6C3F$_1$ mice of polyvinyl alcohol administered 25% in deionized water by the intravaginal route. No neoplasms or nonneoplastic lesions were considered related to treatment with polyvinyl alcohol.

Dr. Russo, a principal reviewer, agreed with the conclusions. She had concerns about the lack of testing in the rat and not having more than one dose. This was in view of studies reporting development of sarcomas at the site of subcutaneous implants of polyvinyl sponges in rats that led the International Agency for Research on Cancer (IARC) to recommend further studies in animals. Dr. Russo wondered if the chemical could be administered in a sponge or tampon. Dr. Radovsky said the AZT studies had shown the mouse to be susceptible to developing vaginal tumors. She said the possibility of using a pessary or tampon could be considered in a future study.

Dr. Taylor, the second principal reviewer, agreed with the conclusions. He said it would have been of merit to have developed innovative ways to administer higher doses. Dr. Radovsky said the problem with handling of the 25% weight/weight solution of polyvinyl alcohol was not so much solubility as viscosity. The dose used was the maximum concentration that could be consistently administered with the available dosing equipment.

Dr. Allaben said that the FDA was involved in the study design and the agency thought the information needed was obtained in spite of the technical difficulties. Dr. Goldsworthy asked whether Glaxo-Wellcome in their studies in rats with AZT had similar responses with systemic versus intravaginal administration. Dr. Radovsky replied that vaginal tumors were induced with AZT by both oral gavage and vaginal administration. Dr. Tyson asked whether there was any leakage after intravaginal administration. Dr. Radovsky said there was some but it was not quantifiable. Dr. Matthews reported that his group studied disposition of radiolabeled polyvinyl alcohol in the rat using measures to avoid ingestion through grooming. He said there was very slight absorption without bioaccumulation after either single or multiple doses.

Dr. Russo moved that the Technical Report on polyvinyl alcohol be accepted with revisions discussed and the conclusions as written for female mice, **no evidence of carcinogenic activity**. Dr. Taylor seconded the motion, which was accepted unanimously with eight votes. Dr. Ward was not present.

Primidone. Dr. J. K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of primidone by discussing the use and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic lesions in male and female mice, and non-neoplastic lesions in male and female rats and mice. The conclusions for the two-year studies in mice and rats were that:

> Under the conditions of these 2-year feed studies there was **equivocal evidence of carcinogenic activity** of primidone in male F344/N rats based on a marginal increase in thyroid gland follicular cell neoplasms, primarily adenomas, and a marginal increase in renal tubule neoplasms. There was **no evidence of carcinogenic activity** of primidone in female F344/N rats exposed to 600, 1,300, or 2,500 ppm. There was **clear evidence of carcinogenic activity** of primidone in male B6C3F1 mice based on the increased incidences of hepatocellular neoplasms, and the increased incidence of thyroid gland follicular cell adenomas was also considered to be chemical related. There was **clear evidence of carcinogenic activity** of primidone in female mice exposed to 300, 600, or 1,300 ppm based on the increased incidences of hepatocellular neoplasms.
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> Exposure of rats to primidone resulted in increased incidences of hepatocyte cytoplasmic vacuolization and centrilobular hypertrophy in males and females and eosinophilic foci in females. The increased severity of nephropathy and increased incidence of renal tubule hyperplasia in male rats were related to primidone exposure. Exposure of male mice to primidone resulted in hepatocyte centrilobular hypertrophy and thyroid gland follicular cell hyperplasia. Exposure of female mice to primidone resulted in hepatocyte centrilobular hypertrophy and cytoplasmic vacuolization, eosinophilic foci, and thyroid gland follicular cell hyperplasia.

Dr. Goldsworthy, a principal reviewer, agreed in principle with the conclusions. He said the poor survival in mid and high dose male rats as well as weight gain depression made the decision between **equivocal evidence** and **some evidence** unclear in male rats even though incidences of thyroid follicular cell and renal tubular neoplasms were above the historical range. Dr. Goldsworthy wondered whether it might have been appropriate with the male rat data to use the survival-adjusted "Poly-3" quantal response employed in the chloroprene study. Dr. Haseman reported that the new "Poly-K" methods will begin to be used routinely with the reports for the next review meeting. This and other newer methods have an advantage over current methods in that they do not require an assumption regarding whether a tumor is fatal or incidental. Dr. Goldsworthy thought there was an overemphasis in the Introduction and Discussion on relating all of the tumor responses to a primary metabolite, phenobarbital, and some discussion should be given to possible carcinogenic activity of primidone and the other primary metabolite, phenylethylmalanomide (PEMA).

Dr. Ryan, the second principal reviewer, agreed with the conclusions. She liked the section looking at plasma concentrations of primidone and phenobarbital and questioned whether markedly different plasma level patterns between rats and mice might explain differences in response between the species. Dr. Ryan noted the widespread human usage as an anticonvulsant and asked why some of these toxicology studies would not have been done as part of the FDA approval process. Dr. Dunnick said that primidone

was developed in the 1950s and nominated because there were no long term toxicology and carcinogenicity studies reported in the literature.

Dr. LeBoeuf, the third principal reviewer, agreed with the conclusions. He commented that the pharmacokinetics and toxicokinetics, although limited in scope, were extremely useful for cross comparisons to studies with phenobarbital, and further, this type of data should be collected routinely to aid in interpretation of other bioassays. Dr. LeBoeuf said the confirmation of an absence of *Helicobacter* in this study was comforting regarding interpretation of the tumor results in mice.

There was some discussion about the tumor promoting activity of primidone/phenobarbital. Dr. J. Rice, IARC and formerly NCI, noted the markedly increased incidences of hepatoblastomas in treated mice, and said that in his experience at the NCI in mice of certain strains and especially in male mice, agents capable of promoting hepatocarcinogenic effects invariably generate a significant fraction of hepatoblastomas. This was consistently seen with phenobarbital. These tumors are highly malignant, metastize readily, and are often lethal.

Dr. Goldsworthy moved that the Technical Report on primidone be accepted with revisions discussed and the conclusions as written for male rats, **equivocal evidence of carcinogenic activity**, for female rats, **no evidence of carcinogenic activity**, and for male and female mice, **clear evidence of carcinogenic activity**. Dr. LeBoeuf seconded the motion, which was accepted unanimously with eight votes. Dr. Ward was not present.

Tetrahydrofuran. Dr. R. S. Chhabra, NIEHS, introduced the toxicology and carcinogenesis studies of tetrahydrofuran by discussing 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 and female 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 tetrahydrofuran in male F344/N rats due to increased incidences of adenomas or carcinomas (combined) of the kidney. There was **no evidence of carcinogenic activity** of tetrahydrofuran in female F344/N rats exposed to 200, 600, or 1,800 ppm. There was **no evidence of carcinogenic activity** of tetrahydrofuran in male $B6C3F_1$ mice There was **clear evidence of carcinogenic activity** of tetrahydrofuran in female $B6C3F_1$ mice due to increased incidences of hepatocellular neoplasms.

Dr. Brown, a principal reviewer, agreed with the conclusions. In view of the CNS symptoms present in both species, he suggested that a comment be added to the discussion and conclusion sections regarding histologic studies of the CNS in both mice and rats in the two-year study. Dr. Chhabra agreed noting that as with many solvents these are nonspecific types of effect. He said that the EPA has asked industry to submit data on acute neurobehavioral toxicity studies.

Dr. Ryan, the second principal reviewer, agreed with the conclusions. She had initial reservations about the strength of the renal tumor data in male rats but on reviewing historical control incidences of these tumors in inhalation studies was persuaded that the level of evidence was appropriate.

Dr. LeBoeuf, the third principal reviewer, did not agree with the conclusions in male rats and female mice. He stated that there was a marginal treatment related effect in male rats and further, a treatment related effect on renal tubule hyperplasias was not observed leading to a conclusion of **equivocal evidence**. He said a detailed step sectioning of kidneys would be appropriate. Dr. Chhabra said that step sections were not called for as the staff was confident of the conclusion of **some evidence** based on the numbers of tumors in treated animals contrasted with the historical rate. Dr. Hailey noted that in almost all previous studies where step sections were done, ethylbenzene being the exception, if the level of evidence was **some evidence**, the additional sectioning did not support a change to **clear evidence.** Dr. LeBoeuf said he would defer his comments on the conclusions in female mice until after clarification of possible confounding effects of *Helicobacter* present in mice livers.

Dr. LeBoeuf asked for further discussion around the relevance or interpretation of neoplasia induction when survival is so poor, and perhaps not attributable to tumor induction. Dr. Haseman pointed out that in this study male rat survival was low in all dose groups and controls as well. In response to a comment from Dr. Allaben, Dr. Haseman agreed that high body weight could be a factor contributing to the overall poor survival in male rats. Dr. Hart read written comments into the record from Dr. Frank Mirer, NTP Board member. Dr. Mirer said that a structural analogy of tetrahyrofuran to furan is misplaced, rather it should be to diethyl ether. He commented that, because there were no differences between treated and control animals in weight gain or

mortality, a higher dose might have been tolerated increasing sensitivity for detecting carcinogenic effects. Dr. Chhabra said narcosis was induced at 1800 ppm precluding giving higher doses.

Dr. Brown moved that the Technical Report on tetrahydrofuran be accepted with revisions discussed and the conclusions as written for male rats, **some evidence of carcinogenic activity**, for female rats and male mice, **no evidence of carcinogenic activity**, and for female mice, **clear evidence of carcinogenic activity**. Dr. Ryan seconded the motion, which was accepted unanimously with seven votes. Drs. Goldsworthy and Ward were not present.

Theophylline. Dr. P. C. Chan, NIEHS, introduced the toxicology and carcinogenesis studies of theophylline by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related non-neoplastic lesions in male rats. The conclusions for the two-year studies in rats and mice were that:

> Under the conditions of these 2-year gavage studies, there was **no evidence of carcinogenic activity** of theophylline in male or female F344/N rats administered 7.5, 25, or 75 mg/kg. There was **no evidence of carcinogenic activity** of theophylline in male $B6C3F_1$ mice administered, 15, 50 or 150 mg/kg or female $B6C3F_1$ mice administered 7.5, 25, or 75 mg/kg.
>
> The incidence of chronic inflammation of the mesenteric arteries was increased in dosed male rats.

Dr. Reddy, a principal reviewer, agreed with the conclusions. He said it would be useful to include information on the theophylline concentration per cup of tea and average daily consumption in tea drinkers. Dr. Chan said it was a very small amount but wide ranging due to different kinds of tea and preparations. Dr. Reddy asked why the decision was made not to do histopathologic examination of tissues from 16-day fed mice. Dr. Chan said these animals were used only for dose setting and there was no mortality. Dr. Reddy wondered whether the vasculitis was due to the drug or to the *Helicobacter* infection. Dr. Hailey observed that *Helicobacter* is not reported to have effects on vasculature outside of the liver.

Dr. Taylor, the second principal reviewer, agreed with the conclusions. He thought a more extensive discussion of the periarteritis should be included, noting that in human medicine this can represent a fairly serious and life threatening condition which can occur after the administration of certain drugs. Dr. A. Nyska, NIEHS, commented that this lesion is characteristic for vasodilator drugs, only in rats, and only in the mesenteric arteries.

Dr. W. Allaben, NCTR/FDA, recommended that comment be made in the conclusions regarding the decreases in liver cancer in treated mice and mammary cancer in rats. Dr. Bucher said this would be done. Dr. Goldsworthy said there also should be comment on significant decreases in body weight gain in the conclusions.

Dr. Reddy moved that the Technical Report on theophylline be accepted with the revisions discussed and the conclusions as written in male and female rats and mice, **no evidence of carcinogenic activity**. Dr. Taylor seconded the motion, which was accepted unanimously with nine votes.