Board of Scientific Counselors
National Toxicology Program

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

on

June 21, 1994

Research Triangle Park, N.C.

The meeting began at 8:30 a.m. on June 21 in the Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the Subcommittee are: Drs. Arnold Brown (Chairperson), Paul Bailey, Meryl Karol, Curtis Klaassen, Claudia Miller, Janardan Reddy, Irma Russo, Louise Ryan, Robert Taylor, Matthew van Zwieten, Mary Jo Vodicnik, and Jerrold Ward. 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 November 29 and 30, 1994, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, 919/541-3971.

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NTP TECHNICAL REPORTS REVIEW SUBCOMMITTEE MEETING
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Acetonitrile. Dr. J. R. Bucher, NIEHS, introduced the toxicology and carcinogenesis studies of acetonitrile by discussing the uses of the chemical and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on possible compound-related neoplastic lesions in male rats and compound-related non-neoplastic lesions in male and female mice. The conclusions for the studies were that:

> Under the conditions of these two-year inhalation studies, there was **equivocal evidence of carcinogenic activity** of acetonitrile in male F344/N rats based on an increased incidence of hepatocellular adenomas and carcinomas. There was **no evidence of carcinogenic activity** of acetonitrile in female F344/N rats exposed to 100, 200, or 400 ppm. There was **no evidence of carcinogenic activity** of acetonitrile in male or female $B6C3F_1$ mice exposed to 50, 100, or 200 ppm.
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> Exposure of male and female mice to acetonitrile by inhalation resulted in an increased incidence of squamous hyperplasia of the forestomach.

Dr. Taylor, a principal reviewer, agreed with the conclusions. He suggested that a note be added to the conclusions that in the two-year studies in male rats there might be some hepatotoxic effects based upon the findings of basophilic, eosinophilic, and mixed foci. Dr. Bucher agreed. Dr. Taylor noted the statement that tobacco smoke contains acetonitrile and wondered if there were data quantifying the levels in cigarette smoke in the literature that could be cited.

Dr. Klaassen, the second principal reviewer, agreed with the conclusions. He thought the dose of acetonitrile in the two-year study might have been higher in rats, likely 800 ppm.

Dr. Karol, the third principal reviewer, agreed with the conclusions. She agreed with Dr. Klaassen that based on survival in 13-week studies, 800 ppm would have been appropriate, and said that if gross and histopathologic changes seen in rats exposed to 800 ppm was part of the rationale for 400 ppm, that should be so stated. Dr. Bucher disagreed. He explained that when setting doses based on lethality, the aim is to not set it much higher than a quarter of the lethal dose in prechronic studies unless there is good evidence for a pharmacologic action that is the cause of death. Dr. Karol commented on the "uncertain" association between acetonitrile exposure and liver neoplasms in male rats that appears to be based on historical control data showing a 10% incidence of liver neoplasms in dosed-feed studies. She said that concurrent controls and historical data from inhalation studies would be more relevant and likely would support a causal relationship. Dr. Bucher acknowledged that an argument could be made for **some evidence** but based on a lack of a dose-response, no increase in preneoplastic lesions or atypical foci, and up to four tumors in control groups in some inhalation studies **equivocal evidence** was considered to be the best conclusion.

Dr. Taylor moved that the Technical Report on acetonitrile be accepted with the revisions discussed and with the conclusions as written for male rats, **equivocal evidence of carcinogenic activity**, and for female rats and male and female mice, **no evidence of carcinogenic activity**. Dr. Klaassen seconded the motion, which was accepted unanimously with eleven votes.

1-Amino-2,4-Dibromoanthraquinone. Dr. J. E. Huff, NIEHS, introduced the toxicology and carcinogenesis studies of 1-amino-2,4-dibromoanthraquinone (ADBAQ) by discussing the uses and rationale for study including it being part of a class study of anthraquinone derivatives, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and non-neoplastic lesions in male and female rats and mice. The conclusions for the studies were that:

> Under the conditions of these two-year feed studies, there was **clear evidence of carcinogenic activity** of 1-amino-2,4-dibromoanthraquinone (ADBAQ) in male and female F344/N rats based on increased incidences of neoplasms in the liver, large intestine, kidney, and urinary bladder. There was **clear evidence of carcinogenic activity** of ADBAQ in male and female B6C3F_1 mice based on increased incidences of neoplasms in the liver, forestomach, and lung. Exposure of male and female rats to ADBAQ for two years was associated with basophilic focus (males only), clear cell focus, eosinophilic focus, and pigmentation in the liver; renal tubule hyperplasia, renal tubule pigmentation, and transitional cell hyperplasia in the kidney; transitional cell hyperplasia, squamous metaplasia, and fat proliferation (females only) in the urinary bladder; squamous hyperplasia, hyperkeratosis, ulceration, and inflammation of the forestomach mucosa; and seminal vesicle atrophy. Exposure of male and female mice to ADBAQ for two years was associated with centrilobular hepatocellular hypertrophy (males only), basophilic focus, clear cell focus (females only), eosinophilic focus, and pigmentation in the liver; pigmentation in the kidney; and hyperplasia, basal cell hyperplasia, hyperkeratosis, and inflammation of the forestomach mucosa.

Dr. Huff reviewed the carcinogenic responses in anthraquinone derivatives that had been studied, noting that liver seemed to be a major site and that ADBAQ was the most active as far as the number of sites. Interpretative conclusions that could be drawn on the cumulative NTP studies on this class were that anthraquinones are typically mutagenic and clastogenic, they are carcinogenic to both sexes of both rodent species, and they are predicted to represent likely carcinogenic hazards to humans exposed to these agents, especially occupationally. Dr. Huff stated that other than anthraquinone itself, no other substituted anthraquinones need to be tested for carcinogenic activity. In response to a question, Dr. Bucher said that toxicology and carcinogenesis studies on anthraquinone were in progress.

Dr. van Zwieten, a principal reviewer, agreed with the conclusions. He thought there should be more discussion in the report of the findings from the start/stop experimental groups in rats. (Stop-exposure groups were evaluated at nine and 15 months as part of an attempt to gain insight into the progression and/or regression of chemical-induced lesions.) Dr. Huff said the start/stop studies would be explained in more depth. Dr. van Zwieten noted the high impurity levels in the first lot of chemical used for the 13-week and first two months of the two-year studies and said that a statement indicating this did not affect the integrity of the studies might be helpful. Dr. Huff responded that the impurities had been characterized and more mention would be given.

Dr. Ward, the second principal reviewer, agreed with the conclusions. He commented that no hyaline droplets were reported in kidneys of rats after nine months, and since this might be a chemical causing accumulation of alpha-2μ-globulin, the report should indicate that droplets were searched for but not found or found but not reported. Dr.

Huff said he would address this issue in the Discussion. Dr. Ward objected to characterizing cholangiofibrosis found in rat liver in a 90-day study as premalignant. He stated that this lesion is almost only induced by liver carcinogens but itself usually does not progress to bile duct tumors. Dr. M. Elwell, NIEHS, said this interpretation was from the literature and we would revise our wording on neoplastic potential to also reflect Dr. Ward's experience.

Dr. Reddy, the third principal reviewer, also agreed with the conclusions. He said it would have been useful to characterize the chemical nature of the pigment that accumulated in liver, kidney, and other organs as well as in the fur and tail. Dr. Huff responded that logically the pigment was either the chemical or one of its metabolites but we would have to determine the feasibility of going back and attempting to define it better.

Dr. Russo had observed evidence of chronic inflammation in one of the micrographs and wondered whether the liver lesions were associated with hepatitis. Dr. Karol asked whether there was inflammation of the eosinophilic foci which would suggest a hypersensitivity type reaction. Dr. Elwell said there was some inflammation with the cholangiofibrosis but this was really limited to focal lesions where there is fibrosis and to cystic bile ducts, and there was not an eosinophilic inflammation; the term "eosinophilic foci" referred to focal cellular alteration of hepatocytes. Dr. Bailey cited a statement from the use, production and human exposure sections that "no individualized information was located regarding amounts produced or specific uses of 1-amino-2,4-dibromoanthraquinone," leading him to wonder if this was a chemical no one is using. Dr. Huff said this was a valid question and not just for ADBAQ but also the other derivatives. He said proprietary information was hard to obtain although he was hopeful that a request to the American Pharmaceutical Association concerning anthraquinone dyes in either over-the-counter or prescription items might yield some data on human exposure. There was some discussion that primary exposure to these dyes would be from topical application or exposure. Dr. Bailey offered to provide information that his company had on percutaneous absorption of some of these dyes.

Dr. van Zwieten moved that the Technical Report on 1-amino-2,4-dibromoanthraquinone be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, **clear evidence of carcinogenic activity**. Dr. Reddy seconded the motion, which was accepted unanimously with eleven votes.

Benzethonium Chloride. Dr. J. R. Bucher, NIEHS, introduced the toxicology and carcinogenesis studies of benzethonium chloride by discussing the uses of the chemical, 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 studies were that:

> Under the conditions of these two-year dermal studies, there was **no evidence of carcinogenic activity** of benzethonium chloride in male or female F344/N rats receiving 0.15, 0.5, or 1.5 mg/kg. There was **no evidence of carcinogenic activity** in male or female $B6C3F_1$ mice receiving 0.15, 0,5, or 1.5 mg/kg.
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> Exposure of rats and mice to benzethonium chloride by dermal application in ethanol for two years resulted in epithelial hyperplasia in male and female rats and mice and sebaceous gland hyperplasia and ulcers in female rats at the site of application.

Dr. Bailey, a principal reviewer, agreed with the conclusions. He thought the dose levels selected were adequate to evaluate the carcinogenic potential of this chemical in rats and mice.

Dr. Vodicnik, the second principal reviewer, agreed with the conclusions. She questioned one of the justifications for chemical selection for study, i.e., that there was 'a suspicion of carcinogenicity.' She said this statement was based on results of a dated, isolated study in which commercial grade benzethonium chloride was given subcutaneously to rats. The localized sarcomas described were typical of those resulting from repeated irritation. Dr. Vodicnik said sufficient rationale for study was the widespread human exposure and the fact that it has not been adequately tested. Dr. Bucher noted that there are human carcinogens, e.g., the nickels, that are very difficult to show as being carcinogenic in animal studies by other than an injection route. He thought it an appropriate response by the NTP to do this study by the dermal route to clarify whether there was, in fact, any suspicion of carcinogenicity. Dr. Vodicnik added that part of her concern had to do with the lack of characterization of the test material and impurities in the earlier study.

Dr. Reddy, the third principal reviewer, also agreed with the conclusions. He thought the highest dose used for the two-year study in male animals could have been higher. Dr. Bucher agreed. Dr. Reddy asked whether it would be appropriate to modify dermal study protocols in the future to examine the potential promoting effect of a compound such as this one. Dr. Bucher responded that whether studies of promotion were worthwhile efforts was a question we wanted the Subcommittee to advise us on.

Dr. Ward stated that he agreed with the study rationale, commenting that this study provides more evidence that chronic irritation alone does not cause tumors. Dr. Ryan asked for clarification of a statement that sebaceous gland carcinomas in treated male rats were consistent with the spectrum of neoplasms found in adjacent control skin of treated and untreated animals. Dr. Bucher said that the lesions seen in treated animals were similar to this type of lesion seen in control animals suggesting less likelihood that these neoplasms were associated with chemical exposure. Dr. Klaassen inquired as to why there was a wider dosing interval between the top and low doses, i.e., ten-fold compared with the often used four-fold. Dr. D. Marsman, NIEHS, said that there was a suggestion that between the 16-day and 13-week studies there was a progression of lesions into

lower doses, thus the wider dose range might be more likely to pick this up in two-year studies. Dr. Miller commented that in view of the wide human exposure in skin products it would be useful to relate the doses applied to typical concentrations in consumer products. Dr. Bucher agreed.

Dr. Bailey moved that the Technical Report on benzethonium chloride be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, **no evidence of carcinogenic activity**. Dr. Vodicnik seconded the motion, which was accepted unanimously with eleven votes.

t-Butyl Alcohol. Mr. J. D. Cirvello, NIEHS, introduced the toxicology and carcinogenesis studies of *t*-butyl alcohol by discussing the uses and routes of human exposure for the chemical, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and non-neoplastic lesions in rats and mice. Because of increased incidences of rare proliferative lesions of the renal tubule in dosed male rats, additional step sections from kidneys of all control and exposed male rats were prepared and evaluated. The conclusions for the studies were that:

> Under the conditions of these two-year drinking water studies, there was **some evidence of carcinogenic activity** in male F344/N rats exposed to 1.25, 2.5, or 5 mg/ml of *t*-butyl alcohol based on increased incidences of renal tubule adenoma or carcinoma (combined). There was **no evidence of carcinogenic activity** in female F344/N rats exposed to 2.5, 5, or 10 mg/ml. There was **equivocal evidence of carcinogenic activity** in male $B6C3F_1$ mice exposed to 5, 10, or 20 mg/ml of *t*-butyl alcohol based on the marginally increased incidences of follicular cell adenoma or carcinoma (combined) of the thyroid gland. There was **some evidence of carcinogenic activity** in female $B6C3F_1$ mice exposed to 5, 10, or 20 mg/ml based on increased incidences of follicular cell adenoma of the thyroid gland.
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> Exposure to *t*-butyl alcohol was associated with mineralization and increased incidences of renal tubule hyperplasia of the kidney in male rats, increased incidences of transitional epithelial hyperplasia and increased severity of nephropathy of the kidney in male and female rats, follicular cell hyperplasia of the thyroid gland in male and female mice, and chronic inflammation and hyperplasia of the urinary bladder in male mice and to a lesser extent in female mice.

Dr. Ward, a principal reviewer, disagreed with the conclusions for male rats. He commented that with the standard pathology protocol, there were no significantly increased incidences of renal tumors or renal tubular hyperplasia, so **no evidence of carcinogenic activity** would be appropriate. With the step section technique, the incidence of tumors increased significantly only in the mid-dose group. Thus, he considered the correct conclusion for male rats based on the extended evaluation to be **equivocal evidence of carcinogenic activity**. Mr. Cirvello responded that **equivocal evidence** based on the standard evaluation would be our choice because there was only one adenoma in controls and none in control animals in previous drinking water studies while there were increased adenomas in all dose groups as well as a carcinoma in the high dose group. Mr. Cirvello asked for Subcommittee discussion on the level of evidence based on the extended evaluation. Dr. J. Haseman, NIEHS, recommended that the NTP include a formal statistical analysis of tumor data from the extended evaluations which might reduce some of the confusion. Dr. Ward said the details of the pathology protocol should be more precisely defined, e.g., numbers of sections in the step sectioning of the kidney, an explanation of the grading system for hyperplasias, and numbers of lesions per rat or section. Dr. A. Radovsky, NIEHS, said the severity of hyperplasia is graded in terms of how closely the lesion approaches an adenoma, i.e., the size of the lesion and extent of cellular atypia. Step sectioning was more extended than usual, being 16-17 sections per animal instead of eight, which was standard in previous studies. She said this information would be added to the report.

Dr. Ryan, the second principal reviewer, questioned the conclusions for male rats and male and female mice. With regard to male rats, she supported **equivocal evidence of carcinogenic activity** based on none of the pairwise tests being strongly significant and only the lifetable trend test being significant when the logistic regression test is probably more relevant. For mice, she proposed **no evidence** for males and **equivocal evidence** for females. Although the high dose in females was significantly different from controls for thyroid adenomas, there was not a clear dose-related trend. Dr. Haseman stated that for male rats, looking at the combined evaluation, the incidence in the mid-dose group was statistically significant by any test and the high-dose incidence is significant with the addition of the one tumor from the interim sacrifice. Other factors arguing for **some evidence** were tumor multiplicity at the mid dose, increased hyperplasia at the high dose, and the occurrence of the uncommon carcinomas. With regard to **some evidence** for the thyroid tumors in female mice, Dr. Haseman said this is a fairly uncommon tumor and the incidence of 15% at the top dose was three times the highest incidence seen in water controls and almost double the highest incidence in feeding study controls, and there were supporting increases in hyperplasia at the mid and top doses. For male mice, he said that increased hyperplasias and similar but less impressive tumor findings to those in females suggested **equivocal evidence**. Dr. Ryan asked why the results from 18-day and 13-week inhalation studies were not included. Mr. Cirvello said inclusion of the inhalation studies would have made the report too cumbersome and they will be reviewed and published separately in a toxicity series report. Dr. Ryan asked for explanation of why there were 11 male rat controls with renal tubule hyperplasia in the extended evaluation, yet the lesion is considered rare. Dr. Radovsky explained that these lesions are uncommon in the routine single section of kidney from control male rats but by increasing the sample size to 16 sections per animal the incidence of this lesion is increased.

Dr. Russo, the third principal reviewer, agreed with the conclusions but had reservations about the conclusion in male rats. She speculated that in this strain of rats the chemical might be acting more as a promoter. Dr. Bucher responded that the study was not designed as a promotion study and further, the numbers of tumors observed in the extended evaluation would argue against a promotion type effect. Dr. Russo said the report could benefit from some discussion of the lower susceptibility of female rats to the chemical, and of the species-related organ specificity, i.e., kidney in rats vs. thyroid in mice. Mr. Cirvello agreed to expand on this in the discussion.

There was more discussion on the merits of extended pathological evaluations and when they should be done. Dr. Haseman likened it to the differences between a partial evaluation and a definitive evaluation and said he would tend to give higher weight to the more definitive evaluation, which should be closer to the true tumor incidence. Dr. Russo urged more uniformity in the number of sections and the way they are taken in extended evaluations. Dr. Bucher commented that we are careful to keep the control data from standard histopathological evaluations separate from data collected under step-section techniques. Dr. van Zwieten said he saw step-sectioning as most useful where one has an equivocal finding and the extended evaluation might help resolve whether or not there is an association with chemical exposure. Dr. Haseman concurred.

Dr. Ward moved that the Technical Report on *t*-butyl alcohol be accepted with the revisions discussed and with the conclusions as written for male rats and female mice, **some evidence of carcinogenic activity**, for male mice, **equivocal evidence of carcinogenic activity,** and for female rats, **no evidence of carcinogenic activity.** Dr. van Zwieten seconded the motion, which was accepted unanimously with eleven votes.

Comparative Initiation/Promotion Skin Paint Studies of B6C3F$_1$, Swiss (CD-1®), and SENCAR Mice. Dr. W. C. Eastin, NIEHS, introduced the report by stating that in 1983, an Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation that was commissioned by the NTP Board had reviewed the basic biology and chemistry of chemical carcinogenesis and recommended methods that NTP should use for the detection and evaluation of chemical carcinogens. One recommendation was that there should be an increased emphasis on short-term tests to detect agents that do not exert genetic effects such as some promoting agents. Mouse skin initiation/promotion is one model routinely used to study this process. However, for the B6C3F$_1$ mouse, the strain commonly used for NTP carcinogenesis studies and for which a large database exists, the skin tumor response using the initiation protocol was not known. Therefore, the objectives of this research project were to compare the tumor response sensitivity of B6C3F$_1$ mouse skin to that of two often used responsive strains, Swiss (CD-1®) and SENCAR mice, using known chemical initiators and promoters and also complete carcinogens.

Dr. Eastin described the study design and techniques used for in-life data collection for these 52-week studies. There were basically three overall designs. For Protocol Design A, the combination of 7,12-dimethylbenz(a)anthracene (DMBA) initiation and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) promotion was selected because this pair is routinely used to study tumorigenesis. However, DMBA requires metabolic activation to achieve initiation and it was possible that the B6C3F$_1$ mouse might not make this metabolic conversion. Therefore, a second study was conducted using *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG), a direct acting carcinogen, as the initiator (Protocol Design B). In addition to the promoter TPA, benzoyl peroxide (BPO), a non-phorbol ester and known promoter following DMBA initiation, was also used in both designs. Finally, the complete carcinogen studies used repetitive applications of low concentrations of the carcinogens, DMBA and MNNG.

Dr. Eastin gave a detailed reporting of the results. The conclusions that could be drawn for the initiation/promotion studies were that all three strains of mice demonstrated sensitivity by developing skin tumors after topical application of the chemicals under study (DMBA, MNNG, TPA, and BPO). At the concentrations of the chemicals tested, the most sensitive of the three strains appeared to be SENCAR mice, in the sense that lower doses of test chemical were generally required to produce effects equivalent to the other two strains. Skin tumors also tended to develop earlier and to exhibit increased multiplicity in SENCAR mice relative to the other two strains. By these criteria, the overall sensitivity of Swiss (CD-1®) mice was intermediate, and B6C3F$_1$ mice showed the least overall sensitivity to dermal carcinogenicity. In the complete carcinogen studies, the skin tumor response in all three strains was more similar than in the initiation/promotion studies, and there was a high incidence of skin tumors in all three strains with both carcinogens. More B6C3F$_1$ and SENCAR mice developed skin tumors and averaged more tumors per mouse than did Swiss (CD-1®) mice. Skin tumors developed earlier in SENCAR mice than in B6C3F$_1$ and Swiss (CD-1®) mice.

Dr. Ryan, a principal reviewer, suggested that there should be some discussion regarding the increased sensitivity of the SENCAR strain in terms of survival under the TPA/TPA promoter reference group and whether this was a detriment to use of this strain, at least in the sense of complicating the statistical analyses. Dr. Eastin explained that many of these animals were not really dying but were being removed from the study after lesions had developed and he would explain this better in the report. Dr. Ryan asked as to the

implication of tumors appearing in the groups receiving TPA without DMBA or MNNG initiation. Dr. Eastin said there should not have been tumors in any groups except those that received initiation with promotion and those receiving repetitive application of carcinogens (DMBA or MNNG). Dr. Ryan also asked why a standard survival analysis on time to tumor was not done. Dr. J. Haseman, NIEHS, responded that the analysis was based on the time of appearance of the first tumor, an in-life observation.

Dr. Bailey, the second principal reviewer, noted that, as stated in the report, these studies were designed to provide mechanistic tumorigenesis data and to determine if this model would be a useful adjunct to the NTP toxicity/carcinogenesis studies. He said he would like to see this addressed in the discussion. Dr. Eastin agreed to expand the discussion to talk about the objectives and how they were met. He said whether this model was a useful adjunct was a question that we wanted the Subcommittee to advise us on. Dr. Bailey said there should be a statement in the front of the report that the most sensitive strains of mice to tumor promotion were also those that were significantly more sensitive to the irritant effects of the chemicals as evidenced by a marked inflammatory reaction.

Dr. Miller, the third principal reviewer, said that possible effects of dose errors in Study Design A, promoter reference group, upon the study results need explanation. Dr. Eastin noted that the correct dose was given for 50 of the 52 weeks so doubted that the error would have affected the outcomes. Dr. Miller thought that the effect on the findings of the much lower dose of TPA promoter in SENCAR as compared with the other two strains should be discussed. Dr. Miller stated that her primary concern was that there should be in the abstract a better explanation of why this study was conducted and what conclusions can be drawn about performing such studies in $B6C3F_1$ mice, and in terms that are accessible to non-toxicologists.

Dr. Brown commented that the Program wanted the Subcommittee's advice on the usefulness of the initiation/promotion model for providing mechanistic data as an adjunct to the NTP toxicology and carcinogenesis studies. He thought the approval of the report should be dispensed with first. Dr. Ryan moved that the Technical Report on comparative initiation/promotion skin paint studies of $B6C3F_1$ mice, Swiss (CD-1®) mice, and SENCAR mice be accepted. Dr. Bailey seconded the motion, which was accepted unanimously with eleven votes.

Discussion: Dr. Reddy said that with limited resources the Program should not be doing initiation/promotion studies on most test chemicals. Dr. Bailey thought that there had been a forum or review several years ago by EPA. Dr. R. Griesemer, NIEHS, said that was correct and there was also a review by the International Agency for Research on Cancer (IARC) that dealt with initiation/promotion in all organs where data existed, not just skin. The newer approaches to understanding cell cycle stage specificity might diminish priority for standard initiation/promotion studies. Dr. Klaassen said use would need to be selective and based on some scientific rationale. As an example, he opined that many chemicals associated with thyroid tumors were acting through a promotional mechanism so such studies might inform us about the biology. Dr. Klaassen cautioned that a major goal of toxicology was not to find the most sensitive test or species but rather the species or test most predictive for humans. Dr. Reddy commented that most promoters were organ specific. The question is what does one do with the data obtained. Dr. G. Lucier, NIEHS, stated that the Program would like to be in a position to select from a variety of possibilities including initiation/promotion but also use of transgenic mice,

mechanistic studies looking at chemical interactions with receptors or target genes, or use of alternative methods, with the thrust being to develop information that will be more predictive of what might happen in humans. He thanked the Subcommittee for their input.

1-Trans-Delta^9-Tetrahydrocannabinol. Dr. P. C. Chan, NIEHS, introduced the toxicology and carcinogenesis studies of 1-trans-delta^9-tetrahydrocannabinol (THC) by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and non-neoplastic lesions in mice. He also presented toxicokinetic data for male rats. The conclusions for the studies were that:

> Under the conditions of these two-year gavage studies, there was **no evidence of carcinogenic activity** of 1-trans-delta^9-tetrahydrocannabinol (THC) in male or female F344/N rats administered 12.5, 25, or 50 mg/kg. There was **equivocal evidence of carcinogenic activity** of THC in male and female B6C3F$_1$ mice administered 125, 250, or 500 mg/kg based on the increased incidences of thyroid gland follicular cell adenomas in males and females at 125 mg/kg. Increased incidences of thyroid gland follicular cell hyperplasia occurred in male and female mice, and increased incidences of hyperplasia and ulcers of the forestomach were observed in male mice.

Dr. Klaassen, a principal reviewer, agreed overall with the conclusions. He asked whether the decreased incidences in some tumors should be indicated in the conclusions and thought a summary table in the Discussion section would be helpful, including comment on any correlation with decreased body weight. Dr. Chan said such a table would be added. Dr. Klaassen asked for an explanation as to why there were 13-week study groups with 9-week recovery periods in both species since this is not a usual design. Dr. Chan commented that the effects of THC are known to linger so a recovery period was added especially regarding effects on the reproductive system to aid in possible extrapolation to humans. Dr. Klaassen was pleased with the presentation of plasma level data and urged that it be included along with similar data for the other three experimental groups in the final Report.

Dr. Taylor, the second principal reviewer, agreed with the conclusions. He noted the statement that "THC also appears to modify arachadonic acid metabolism..." and said it would be helpful to expand on this by indicating the extent and direction and the possible implications therapeutically or physiologically. Dr. Chan agreed. Dr. Taylor said a comment should be added on selection of gavage as the dose route since this differs from usual routes of human exposure. Dr. Chan responded that there was insufficient compound available to do an inhalation study while intraperitoneal injection was less akin to human exposure and also, there is a very small historical database for the injection route.

Dr. van Zwieten, the third principal reviewer, also agreed with the conclusions. He asked for comment on the apparent inverse dose-response relationship for the thyroid gland tumor incidences in mice. Dr. R. Sills, NIEHS, said this would be expanded.

Dr. Ward inquired whether step sectioning of the thyroid glands in mice had been considered in view of the equivocal findings. Dr. M. Elwell, NIEHS, said that because of the small size of the mouse thyroid, one cross-section is fairly representative for the whole organ. Dr. Ward wondered if the body weights being lower than controls in all dose groups suggested exceeding of the maximal tolerated doses. Dr. Bucher said that interpretation about possible exceeding of an MTD is difficult when one of the pharmacologic effects of a chemical is on weight gain. Dr. Chan commented that a

complicating factor was that because THC is taken up and stored in adipose tissue levels, buildup on chronic administration might exceed an MTD. Dr. Russo asked for comment on the lower levels of FSH and LH in female animals. Dr. Bucher said that although the reproductive effects of THC were well studied we could not explain why these hormone levels differed between males and females in this study. Dr. van Zwieten observed that for many of the neoplasms with decreased incidences in dosed groups the incidences were still within the historical control range. Dr. Miller suggested including data on human plasma levels so as to contrast animal levels and typically achieved human levels so that people don't get the idea that THC is something they could take as a medication to ward off cancer. Dr. Bucher commented that one of our concerns about this report was that it might be misinterpreted as showing large beneficial therapeutic effects. This is why the NTP has tried to make very clear that most of these changes are believed to be related to weight gain.

Dr. Klaassen cited a report in the text that the amount of THC taken in by habitual smokers was estimated to range from 0.3 to 12.0 mg/kg which would be in the same range as the doses given to rats. Dr. Taylor pointed out that plasma levels for a dose given by inhalation would be much higher than for the same dose given orally.

Dr. Klaassen moved that the Technical Report on 1-trans-delta^9-tetrahydrocannabinol be accepted with the revisions discussed and with the conclusions as written for male and female rats, **no evidence of carcinogenic activity**, and for male and female mice, **equivocal evidence of carcinogenic activity**. Dr. Bailey seconded the motion, which was accepted unanimously with ten votes.

Short-Term Study

1-Nitropyrene. Dr. J. R. Bucher, NIEHS, introduced the discussion by noting that the NTP has been evaluating ways to better set priorities for selection of chemicals for two-year studies, and specifically mentioned a priority setting plan presented to the NTP Board and others by Dr. B. A. Schwetz while still at NIEHS. Under this scheme, chemicals strongly predicted to be carcinogenic would not be chosen for two-year studies, nor would chemicals believed not to be carcinogenic. He said the toxicology report series will be the vehicle for these decisions, and he hoped eventually these well documented predictions would be accepted by the regulatory agencies, and also would be included in the deliberations on the *Biennial Report on Carcinogens*. Thus, the report on 1-nitropyrene is the first where we have concluded that a chemical is a likely carcinogen in the absence of neoplasms in an NTP study. Following our agreed on procedure for peer review of short-term toxicity study reports, the report received thorough mail review by two scientists, one a Board member and one outside chosen for expertise with the chemical. Dr. Bucher said that insofar as the Subcommittee has jurisdiction over approving the appropriateness of the interpretative conclusions that the Program draws, it seemed that the Subcommittee might want to have jurisdiction over the predictions made by the Program as well.

Dr. Bucher proposed that there might be a number of options for the Subcommittee to discuss. First, would be to continue with mail review of the short-term toxicity study reports, only bringing those reports to the full Subcommittee when we had a chemical on which we were making a prediction. Here the Subcommittee would be asked if they agree or disagree with conclusions drawn and the conclusions that the reviewers have drawn. A second option would be to bring the report directly to the Subcommittee as is done with the two-year study reports, and have a full hearing in the open session. A third option would be to continue strictly with mail reviews. Dr. Bucher concluded by noting that the Subcommittee had received copies of the two mail reviews prior to the meeting and a written review by Dr. Ward as well was enclosed in their meeting folders.

Dr. Brown said there could be other options but asked for discussion on the three presented. Dr. Reddy commented that there seemed to somewhat of a divergence of opinion between the two reviewers although the IARC assessment is that 1-nitropyrene is a carcinogen so the first option would be suitable. However, for other compounds where an in-depth study by the Subcommittee is needed, the second option should also be available. Dr. Vodicnik thought a hybrid of the first two options would work best and she would like to see more information presented on the 1-nitropyrene study. Dr. Taylor suggested a mail review to all the members of the Subcommittee as a fourth option. Dr. Griesemer commented that the mail review was intended in part to provide expertise on specific organ toxicities while providing advice on the scientific adequacy of the report. Now, we are asking a different question, which is, should a substance be or not be tested for carcinogenicity based on information in the report. Dr. Vodicnik suggested a redefinition of the fourth option such that there would be an initial limited mail review as is now done followed by Subcommittee review of the recommendations. If there was unanimous agreement that would end it, if not, the Subcommittee would proceed to a full review. Dr. W. Allaben, NCTR/FDA, called attention to the fact that the regulatory agencies cannot regulate without carcinogenicity data as the law stands now. Dr. Brown pointed out that the prediction was not made just on the basis of the 90-day study. Dr. Allaben acknowledged that the IARC classification in this case would preclude the need

for testing. Dr. Lucier reported that a workshop was planned for later in the year to deal with two issues, one being the strength of evidence needed to make a prediction and the second to try and determine the kinds of information that the regulatory agencies need to do their work.

Dr. Vodicnik moved that for short-term reports where there are predictions, like 1-nitropyrene, there be an initial mail review with three reviewers, two being *ad hoc*. Copies of the three reviews along with the report would be sent to the Subcommittee prior to their next meeting and at the meeting there would be a brief discussion on whether or not to accept the recommendations in the reviews. Dr. Reddy seconded the motion. As clarification, Dr. Brown said short-term reports where there was not a prediction of carcinogenicity would be reviewed by mail as they are now. The motion was accepted unanimously with eleven votes.

Dr. Brown asked that the Subcommittee move to the specific question of how to proceed with the 1-nitropyrene report, i.e., whether to approve the conclusions or to defer action until the next meeting of the Subcommittee. Dr. Klaassen moved to defer for two reasons, one being that the public had not been informed of this discussion and thus had no opportunity to comment, and secondly, this will allow time for a third review to be obtained and for the staff to prepare a presentation. Dr. Reddy seconded the motion. Dr. Lucier commented that it is our public health responsibility to make a prediction if the mechanistic data and other information support such an action. The motion was accepted unanimously with eleven votes. Dr. Bailey said it was important that the public be apprised of this new charge for the Subcommittee. Dr. Hart said it would be announced in the *Federal Register* in advance of the next meeting of the Subcommittee, which is scheduled for November 29 and 30, 1994.

Information Report

Diet for Rats and Mice in NTP Studies -- Recommendations. Dr. G. N. Rao, NIEHS, began his report by noting that what is the right diet for rodents in long-term studies has been a constant question. He said that a workshop on diet had been held one year ago (June 23, 1993) in conjunction with the Technical Reports Review Subcommittee meeting to review the effects of changes in protein, fat, and fiber concentrations of diet on chronic disease, tumor incidences, and survival of rats, especially F344 rats, in various NTP two-year studies, and to discuss modification of diet composition and ingredients. Dr. Rao stated that resolution had been made as to the best diet and he wanted to present our recommendations to the Subcommittee.

Dr. Rao reported that the NIH-07 open formula nonpurified diet has been the selected diet for NTP studies since 1980 and was designed for reproduction, lactation, and growth of rodents in production colonies. This diet, which contains approximately 24% protein, 5% fat, and 3.5% fiber, may not be an optimal diet for rats in long-term studies, and some components such as protein may be increasing the incidence and severity of chronic diseases, e.g., nephropathy. He presented data from NIEHS studies comparing rats on NIH-07 vs. a 15% protein diet that showed a 15% protein nonpurified diet is adequate for growth and maintenance, ~ 30% decrease in protein consumption markedly decreased severity of nephropathy, while caloric restriction and lowering of body weight may not be necessary. With regard to the effects of fat, Dr. Rao presented data on the effects of corn oil, used as a gavage vehicle, which showed that corn oil type of fat appears to decrease the incidence or delay the onset of leukemia in male rats, results in increased incidences of mammary and anterior pituitary tumors in rats correlated with increased body weight, and incidence of pancreatic acinar cell tumors appears to be influenced by a combination of body weight and type of fat. Taking advantage of these observations, studies were done with diets containing higher fat and higher fiber to maintain caloric density along with decreased protein (NTP-90, NTP-91, NTP-92). These studies showed that: (1) when the caloric content of higher fat diets were adjusted with crude fiber, the body weight gain was decreased; (2) increasing fat content appeared to decrease incidence/severity of leukemia or leukemia associated mortality; (3) increasing fiber content delayed mammary tumor development and associated mortality in females; (4) higher fat or fiber, or a combination, decreased incidence of adrenal pheochromocytomas in males; (5) lower protein, higher fat and higher fiber (than in NIH-07), decreased spontaneous tumor burden in two-year studies; and (6) diets for rats in 2-year studies could be modified to decrease/delay spontaneous tumor development and to decrease severity of chronic diseases. Dr. Rao also briefly discussed recently revised dietary recommendations for rodents by the American Institute of Nutrition (AIN) and, in draft form, by the National Research Council (NRC), and compared them with the NTP diets.

Dr. Rao informed the Subcommittee that based on extensive studies of various diets developed by the Program along with input from many other sources, the NTP plans to change the laboratory rodent diet beginning in September 1994 in studies designed to investigate the biological effects of electromagnetic fields. He compared the nutrient and ingredient composition of the new open formula nonpurified diet with the NIH-07 diet used by the Program since 1980, noting that there are still some final refinements to be made. The new diet will have less protein, 15% vs. 23% in NIH-07, and higher fat (9% vs. 5%) and fiber (11% vs. 3.5%).