NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS

Summary Minutes

Peer Review of Draft Technical Reports of
NTP Toxicology and Carcinogenesis Studies
by the Technical Reports Review Subcommittee

September 5-6, 2002

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The meeting began at 8:30 a.m. on September 5, 2002 in the Rodbell Conference Center of the David P. Rall Building, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the subcommittee are Drs. Norman R. Drinkwater (chairperson), Kim Boekelheide, Michael R. Elwell, Shuk-Mei Ho, James E. Klaunig, Walter W. Piegorsch, Stephen M. Roberts, Richard D. Storer, Mary Anna Thrall, Cheryl Lyn Walker, and Mary Vore. For further information, contact Dr. Mary S. Wolfe, Executive Secretary, at 919-541-0530 or wolfe@niehs.nih.gov.

Dipropylene Glycol Dr. Michelle Hooth, NIEHS, introduced the report on the toxicology and carcinogenesis studies of dipropylene glycol by describing the uses of the chemical, the design and dose selection for the drinking water studies, and the body weights, survival, and nonneoplastic lesions observed in rats and mice in the 3-month and 2-year studies. The proposed conclusions were:

> Under the conditions of these 2-year drinking water studies, there was *no evidence of carcinogenic activity* of dipropylene glycol in male or female F344/N rats exposed to 2,500, 10,000, or 40,000 ppm. There was *no evidence of carcinogenic activity* of dipropylene glycol in male or female B6C3F$_1$ mice exposed to 10,000, 20,000, or 40.000 ppm.
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> Exposure to dipropylene glycol in drinking water resulted in increased incidences and severities of nephropathy in male rats, increased incidences of focal histiocytic and focal granulomatous inflammation of the liver in male rats, increased incidences of bile duct hyperplasia in male and female rats, and changes in the olfactory epithelium of the nose in male and female rats.

Dr. Thrall, the first principal reviewer, agreed with the conclusions of the study. She felt the top dose for the male rats was excessive, particularly because the animals drank even larger quantities of water than the control group, and noted that all the animals in that group died of nephropathy. She also noted that dipropylene glycol was metabolized differently than ethylene glycol and that the metabolites of dipropylene glycol were generally much less toxic.

Dr. Klaunig, the second principal reviewer, agreed with the conclusions but suggested that the changes noted in the olfactory epithelium be specified. He offered the generic comment that the precision of the subclassification of hepatocellular foci in NTP studies may be misleading.

Dr. Vore, the third principal reviewer, agreed with the conclusions and inquired about the use and significance of the bile acid measures in the 3-month rat study.

Dr. Hooth noted that there were no indications from the 3-month studies that the 40,000 ppm dose would produce overt toxicity. Dr. Rick Hailey, NIEHS, described the sampling procedures for liver foci and indicated that they were used as a supplemental indicator of proliferative processes. Dr. Gregory Travlos, NIEHS, explained that bile acid measures were used as one of the biomarkers for hepatobiliary health and that the measures did contain some variability, so that some apparent decreases in rats in the 3-month study were within historical ranges.

Dr. Elwell inquired about a statement linking the adrenal gland changes observed in rats in the 3-month study to stress. He also asked about the characteristics of the nephropathy, which became lethal in the 2-year study, and about the cytoplasmic clefts in the liver and the significance of the salivary gland changes. Dr. Ronald Herbert, NIEHS, replied that while the adrenal gland changes appeared treatment related, the biological significance was unclear and stress was proposed as one possibility. He confirmed that the nephropathy was different from the normal rat kidney nephropathy, with greater incidences and severity of protein casts even after 3 months. While some histiocytic changes were seen in the livers of control animals, the clefts were observed only in dosed animals. Dr. Herbert also agreed that the salivary gland changes were worthy of mention.

Public Comment: Dr. Marcy Banton, Lyondell Chemical Company, on behalf of Dow Chemical Company, Huntsman Chemical Company and the American Chemistry Council Propylene Oxide/Propylene Glycol Panel, said that the top doses in the rodent studies were quite high and suggested that some of the effects in those groups could be attributable to substantially lower body weights rather than to chemical toxicity.

Dr. Thrall moved that the conclusions be accepted as written. Dr. Vore seconded the motion, which was accepted with 9 affirmative votes and one abstention (Dr. Boekelheide).

Elmiron® Dr. Kamal Abdo, NIEHS, introduced the report on the toxicology and carcinogenesis studies of Elmiron® by describing the therapeutic uses and mechanisms of the drug, the design and dose selection for the gavage studies, and the body weights, survival, and the nonneoplastic lesions observed in rats and mice in the 3-month and 2-year studies and the neoplastic lesions seen in mice in the 2-year studies. The proposed conclusions were:

> Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of Elmiron® in male F344/N rats administered 14, 42, or 126 mg/kg or in female F344/N rats administered 28, 84, or 252 mg/kg. There was *some evidence of carcinogenic activity* of Elmiron® in male B6C3F$_1$ mice

based on increased incidences of liver hemangiosarcoma. The increased incidences of hepatocellular neoplasms in male mice may have been related to Elmiron® administration. There was *some evidence of carcinogenic activity* of Elmiron® in female $B6C3F_1$ mice based on the increased incidences of liver hemangiosarcoma and hepatocellular neoplasms (predominately adenomas). The increased incidences of malignant lymphomas may have been related to Elmiron® administration.

Elmiron® administration caused increased incidences of nonneoplastic lesions (presence of vacuolated histiocytes) of the rectum, lung, mesenteric lymph node, and spleen (males) in rats and of the liver, rectum, mesenteric lymph node, and spleen in mice.

Dr. Klaunig, the first principal reviewer, asked if the occurrence of hemangiosarcomas in male mice might have been related to changes in hemosiderin or hemolysis or to macrophages and also if there was any relationship between the hemangiosarcomas and lysosomal storage malfunction. He also noted that the liver neoplasms in female mice were predominantly adenomas.

Dr. Ho, the second principal reviewer, asked for clarification of the interpretive conclusion for the lymphomas in female mice.

Dr. Roberts, the third principal reviewer, asked whether the strength of evidence of the hemangiosarcomas in female mice contributed to the call of *some evidence*.

Dr. Abdo replied that neither hemolysis nor hemosiderin deposition were observed. Dr. Abraham Nyska, NIEHS, said that while some inflammation was seen in the livers of mice, it was not of sufficient severity to contribute to tumor formation. He noted that other sulfated polysaccharides that induced a similar type of histiocytic infiltration induced tumors in the large intestine, an effect not seen in the present study. Explaining the rationale for the conclusions, Dr. Joseph Haseman, NIEHS, noted that hemangiosarcomas are very uncommon tumors in female mice, and their association with chemical administration was supported by a similar effect in the males. The lymphomas, where the incidences were also of borderline statistical significance with a highly variable background rate, were considered *equivocal evidence* in part because no corresponding increase was seen in the males. Dr. Hailey, NIEHS, added that these rapidly developing tumors did not spread to other organs any more in the exposed animals than in the control groups.

Dr. Elwell noted that the few hemangiomas were not included with hemangiosarcomas for analysis, though in some other studies these tumors were pooled. Dr. Nyska replied that the three hemangiosarcomas observed in the top dose group of female mice occurred in organs other than the liver, so in this case combining them for analysis may not have been appropriate. Dr. Hailey added that in studies in which vascular tumors increased, the vast majority were hemangiosarcomas.

Dr. Piegorsch suggested that the statistical significance of the lymphomas in female mice might warrant a stronger conclusion than *equivocal evidence*. Dr. Boekelheide questioned whether the contribution of the hepatocellular neoplasms to the conclusion for male mice was weakened by most of the neoplasms being adenomas. Dr. John Bucher, NIEHS, suggested that terminology consistent with previous reports would be "hepatocellular neoplasms, predominantly adenomas" and that of the effects observed in female mice, the hemangiosarcomas were the most significant biologically.

Dr. Roberts moved that the conclusions be accepted as written, including Dr. Bucher's suggestion, and Dr. Klaunig seconded the motion. Dr. Piegorsch argued for inclusion of the malignant lymphomas into the *some evidence* conclusion for female mice. Dr. Bucher observed that lymphomas historically had a highly variable incidence, and Dr. Hailey described some other NTP studies in which similar incidences of lymphomas were judged *equivocal evidence* or *no evidence*. He added that the site of origin (spleen) of the lymphomas in this study was not unusual.

Dr. Piegorsch offered an amendment that the lymphomas be included in the list of neoplasms supporting the conclusion of some evidence for female mice. Dr. Ho seconded the motion, which failed by a vote of 7 no to 3 yes. Dr. Roberts offered an amendment that the hepatocellular neoplasms be listed before the hemangiosarcomas in the female mouse conclusion. The amendment failed for lack of a second. The original motion was then approved unanimously with 10 votes.

Decalin Dr. Po-Chuen Chan, NIEHS, introduced the report on the toxicology and carcinogenesis studies of decalin by describing the uses of the chemical, the design and dose selection for the inhalation studies, and the nonneoplastic and neoplastic lesions in the 3-month and 2-year studies. The proposed conclusions were:

> Under the conditions of these studies, there was *clear evidence of carcinogenic activity* of decalin in male F344/N rats based on increased incidences of renal tubule neoplasms. The increased incidences of benign or malignant pheochromocytoma (combined) of the adrenal medulla in male rats were also considered to be exposure related. There was *no evidence of carcinogenic activity* of decalin in female F344/N rats exposed to 25, 100, or 400 ppm. There was *no evidence of carcinogenic activity* of decalin in male $B6C3F_1$ mice exposed to 25, 100, or 400 ppm. There was *equivocal evidence of carcinogenic activity* of decalin in female $B6C3F_1$ mice based on marginally increased incidences of hepatocellular and uterine neoplasms.
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> Exposure of male rats to decalin resulted in nonneoplastic lesions of the kidney characteristic of α2u-globulin accumulation. Nonneoplastic lesions of the liver were observed in male mice exposed to decalin.

Dr. Walker, the first principal reviewer, queried whether the adrenal gland pheochromocytomas in male rats merited a call of *some evidence*. She also asked about the relevance to humans of the mechanism involving α2u-globulin.

Dr. Thrall, the second principal reviewer, agreed with the conclusions and observed a correlation between pheochromocytoma occurrence and nephropathy severity.

Dr. Boekelheide, the third principal reviewer, inquired about the biologic significance of the benign uterine stromal polyps and the hepatic lesions in female mice as indicators of carcinogenic activity. He also asked about the significance of the effects on the reproductive system in the 3-month male mouse study.

Dr. Chan explained that, while pheochromocytoma occurs commonly as a spontaneous neoplasm in male Fischer rats, the increased incidences in this study were significantly increased and exceeded the historical control range and thus were considered to be related to chemical exposure. Dr. Haseman, NIEHS, added that the pheochromocytomas were not included in the strongest conclusion statement, *clear evidence of carcinogenic activity*, which was applied to the kidney neoplasms, but the language adopted did indicate that the pheochromocytomas were exposure related. Dr. Chan said that the liver neoplasms in female mice were considered *equivocal evidence* because while increased incidences were seen in two of the three exposed groups, the pattern was not significant by the trend test. Conversely, the uterine stromal polyps were also considered *equivocal evidence*, because the incidences in the top dose group were not very significantly increased relative to the controls, despite an overall positive trend. Dr. Hailey confirmed that these polyps were indeed neoplasms and not simply an inflammatory response.

Dr. Elwell observed that decalin has been reported to be an inhaled irritant, but in the present studies no effects on the laryngeal or nasal mucosa were seen. He also asked if the infiltration and fibrosis in the pleura of female rats merited inclusion in the summary table.

Dr. Drinkwater raised the question of whether the conclusion based on kidney tumors should carry a qualifying comment that the tumors were a secondary effect of 2u-globulin accumulation. Dr. Bucher, NIEHS, replied that the standard practice had been simply to mention the occurrence of both lesions and permit future readers to draw their own inferences about causality based on the evolving hypotheses and knowledge.

Dr. Boekelheide asked whether the uterine stromal polyps should be considered neoplasms or a nonneoplastic response. Dr. Walker indicated that while sarcomas are irreversible, there was less certainty about stromal polyps. Dr. Hailey said that stromal polyps occasionally progress to sarcomas and generally the two are grouped for purposes of analysis.

Dr. Walker moved that the conclusions be approved as written and Dr. Roberts seconded the motion. Dr. Piegorsch offered an amendment that the conclusion in male mice be changed to *equivocal evidence* based on a marginal increase in liver neoplasms. The amendment failed for lack of a second. The original motion was then accepted unanimously with 10 votes.

Urethane/Ethanol Dr. Fred Beland, NCTR, introduced the report on the toxicology and carcinogenesis studies of urethane and ethanol by noting that the purpose of the studies was to develop dose response data for use in risk assessment evaluations for the known carcinogen, urethane. Urethane is a by-product of fermentation and occurs in alcoholic beverages. Dr. Beland described two competing hypotheses for possible metabolic interactions between urethane and ethanol, one which could increase the carcinogenic response, the other which could diminish it, and described the drinking water studies that incorporated combinations of the two chemicals. He described the water consumption, body weights, survival, patterns of neoplasm development, DNA adducts, and enzyme induction observed in the studies. The proposed conclusions were:

> Under the conditions of this 2-year drinking water study, there was *clear evidence of carcinogenic activity* of urethane in male B6C3F_1 mice based on increased incidences of liver, lung, harderian gland, and forestomach neoplasms and of hemangiosarcoma, primarily of the liver and heart. There was *clear evidence of carcinogenic activit*y of urethane in female B6C3F_1 mice based on increased incidences of liver, lung, harderian gland, mammary gland, and ovarian neoplasms and of hemangiosarcoma, primarily of the liver, heart, and spleen. The occurrences of hemangiosarcoma of the spleen in males and of the uterus and skin in females were also considered to be exposure related.
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> Exposure to urethane resulted in increased incidences of nonneoplastic lesions of the liver and heart in males and females and of the uterus in females.
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> Under the conditions of this 2-year drinking water study, there was *equivocal evidence of carcinogenic activity* of ethanol in male B6C3F_1 mice based on increased incidences of hepatocellular neoplasms. There was *no evidence of carcinogenic activity* of ethanol in female B6C3F_1 mice exposed to 2.5% or 5%,
>
> The coadministration of urethane and ethanol resulted in marginal changes in the incidences of some neoplasms that were attributed to urethane alone. In males, increasing the ethanol concentration may have decreased the alveolar/bronchiolar and harderian gland adenoma or carcinoma responses to urethane. In females, increasing the ethanol concentration may have increased the hemangiosarcoma of the heart and alveolar/bronchiolar adenoma or carcinoma responses to urethane. Overall, the findings were insufficient to establish a definitive effect of ethanol on the carcinogenicity of urethane in B6C3F_1 mice.

Dr. Piegorsch, the first principal reviewer, questioned whether it would be appropriate to draw conclusions on the potential carcinogenicity of ethanol from this study. Dr. Roberts, the second principal reviewer, shared those concerns, but felt the other conclusions about the interaction of urethane and ethanol were reasonable.

Dr. Elwell, the third principal reviewer, questioned the inclusion of certain sites, such as spleen, skin, and uterus, in the list of hemangiosarcomas considered related to urethane administration.

He noted the similarity in carcinogenic effects of urethane, butadiene, and isoprene. Regarding the conclusions, Dr. Elwell suggested that squamous cell neoplasms be added as an effect in male mice and that hemangiosarcomas in the spleen of males and uterus and skin of females be considered "may have been related" to chemical exposure. He agreed with the conclusion that the data were insufficient to establish an effect of ethanol on the carcinogenicity of urethane.

Dr. Drinkwater observed that underlying several of the reviewers' comments was the question of whether the conclusions should be framed in the same format as standard NTP studies. Dr. Beland replied that the primary intent of these studies is to provide data for risk assessment rather than to serve as a traditional carcinogenicity screen, and thus other forms of conclusion statements could be considered. Dr. Christopher Portier, NIEHS, added that the single-chemical groups of this study might be consistent with the standard carcinogenicity bioassay, and the subcommittee could determine the adequacy of the study design.

Dr. Beland agreed that the incidences of hemangiosarcomas in the spleen, skin, and uterus were not significant, unlike those in the heart and liver. Dr. Paul Mellick, Pathology Associates, Inc., observed that the overall incidences of hemangiosarcomas for all sites combined were markedly increased in the high dose urethane groups. Dr. Elwell said that the usual approach has been to treat vascular tumors as site specific.

After group discussion on the phrasing for hemangiosarcomas, Dr. Piegorsch proposed that the first portion of the conclusion statement be *clear evidence of carcinogenic activity* in male mice based on increased incidences of liver, lung, harderian gland, skin, and forestomach neoplasms and hemagiosarcomas primarily of the liver and heart. For female mice he proposed *clear evidence of carcinogenic activity* based on increased incidences of lung, liver, harderian gland mammary gland, and ovarian neoplasms and hemangiosarcomas primarily of the liver and spleen. Hemangiosarcomas of the spleen in males and of the uterus and skin in females may have been exposure related.

For the second portion of the conclusion, Dr. Piegorsch proposed "the design of the 2-year drinking water study was inadequate to determine the carcinogenic activity of ethanol in male and female $B6C3F_1$ mice."

For the third portion of the conclusions, the subcommittee had an extensive discussion on whether the term "marginal" best described the changes caused by ethanol in the urethane-induced tumor incidences. Dr. Piegorsch then proposed "The coadministration of urethane and ethanol resulted in alterations in the incidences of some neoplasms that were attributed to urethane alone. In males, increases in ethanol concentration may have decreased the alveolar/bronchiolar and harderian gland adenoma or carcinoma responses to urethane. In females, increasing the ethanol concentration may have increased the incidence of hemangiosarcoma of the heart and alveolar/bronchiolar adenoma or carcinoma response to urethane. Overall, the findings were insufficient to establish a definitive effect of ethanol on the carcinogenicity of urethane in $B6C3F_1$ mice."

Dr. Boekelheide queried the implications of the word "definitive" in the final sentence. Dr. Piegorsch suggested replacing it with "interactive." Dr. Piegorsch then moved that these three conclusions be adopted. Dr. Boekelheide felt there was an inconsistency between the first and last sentences of the third conclusion. Dr. Piegorsch suggested changing "resulted in" to "may have affected" in the first sentence. Dr. Klaunig asked if the final sentence could be deleted, and Dr. Roberts argued that without it there would be no conclusion. Dr. Storer proposed adding the word "consistent" before "interactive."

At this point there was no second to the proposed rewording of the third conclusion, so the subcommittee acted on the first two portions separately. Dr. Klaunig seconded Dr. Piegorsch's motion to adopt the first two portions of the conclusions, on the urethane study and the inadequacy of the ethanol alone study. After further discussion the motion was approved unanimously with 10 votes.

Returning to the conclusion for the interaction, Dr. Roberts suggested saying mice cotreated with urethane and ethanol had altered incidences of some neoplasms. Dr. Boekelheide asked how the language in this report might set precedents for future studies of chemical interactions. Dr. Storer felt using the same levels of evidence categories as for carcinogenicity would be too complex.

Dr. Ho proposed for the final sentence "Overall there was some evidence of interactive effects of ethanol on the carcinogenicity of urethane in $B6C3F_1$ mice." Dr. Drinkwater suggested the word "equivocal" could replace "some." Dr. Portier stated that the NTP would prefer not to use the same terms as occur in the levels of evidence of carcinogenic activity categories and suggested instead "strong" and "weak" as two levels of interaction. Dr. Piegorsch moved "weak evidence of an interaction" as the conclusion statement. Drs. Storer and Ho thought the terminology was an improvement, and Dr. Klaunig seconded the motion. Dr. Roberts proposed an amendment that that sentence be the first in the paragraph and Dr. Klaunig concurred. The final phrasing was "Overall there was weak evidence of an interaction of ethanol on the carcinogenicity of urethane in $B6C3F_1$ mice. In males, increasing the ethanol concentration may have decreased the alveolar/bronchiolar and harderian gland adenoma or carcinoma responses to urethane. In females, increasing the ethanol concentration may have increased the incidence of hemangiosarcoma of the heart and alveolar/bronchiolar adenoma or carcinoma responses to urethane." The motion was approved unanimously with 10 votes.

***trans*-Cinnamaldehyde** Dr. Hooth, NIEHS, introduced the report on the toxicology and carcinogenesis studies of *trans*-cinnamaldehyde by describing its use as a flavoring agent, the process of including the chemical in starch microcapsules, the design of the 3-month and 2-year studies where the microcapsules were mixed with the feed of rodents, and the survival, body weights, and nonneoplastic lesions observed in the studies. The proposed conclusions were:

> Under the conditions of this 2-year feed study, there was *no evidence of carcinogenic activity* of *trans*-cinnamaldehyde in male or female F344/N rats exposed to 1,000, 2,100, or 4,100 ppm. There was *no evidence of carcinogenic*

activity of *trans*-cinnamaldehyde in male or female B6C3F$_1$ mice exposed to 1,000, 2100, or 4,100 ppm.

The three principal reviewers, Dr. Vore, Dr. Boekelheide, and Dr. Ho all agreed with the conclusions. Dr. Elwell noted the decreases in liver tumor incidences in male and female mice and asked if these could be related to decreased body weights. Dr. Hooth replied that, extrapolating from known body weight relationships, the decrement in body weight in this study would account for some, but not all of the liver tumor decrease. She also mentioned reports in the literature of antimutagenic and anticarcinogenic effects of *trans*-cinnamaldehyde. She confirmed the one weakly positive mutagenic response in the *Salmonella* TA100 strain.

Dr. Vore moved that the conclusions be accepted as written, and Dr. Roberts seconded the motion, which was approved unanimously with 10 votes.

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Dr. Portier, NIEHS, introduced the review by explaining that, as a result of consultation with the Subcommittee prior to the meeting, the original plan to evaluate the conclusions concerning possible carcinogenic hazard of the two multifunctional acrylates had changed. The revised purpose of the review would be to evaluate the design and appropriate methods of analysis for these transgenic mouse studies. Further questions to be addressed were whether the Levels of Evidence of Carcinogenic Activity categories currently used for standard 2-year cancer bioassays would be applicable and what reporting format would be appropriate.

Dr. Bucher, NIEHS, outlined the agenda of presentations and described a variety of design and interpretation issues related to the use of genetically modified mouse models for cancer assessment. These included choice of animal model, group size, study duration, route of exposure, dose selection, use of positive or negative controls, extent of pathology evaluation, and appropriate statistical analysis method. Other fundamental questions were whether these should be considered carcinogenicity studies or promotion studies and what sort of interpretive conclusions could be drawn.

Dr. John French, NIEHS, described the Tg.AC transgenic mouse model, the construction of the v-Ha-*ras* transgene, and the use of the model as a squamous epithelium reporter phenotype. Dr. David Dunson, NIEHS, described a generalized Poisson system to analyze the incidence, multiplicity, and onset times for the skin papillomas that are the primary endpoint of the Tg.AC model.

Dr. Rajendra Chhabra, NIEHS, described the study nomination and uses of the two chemicals, trimethylolpropane triacrylate (TMPTA) and pentaerythritol triacrylate (PETA), the results of the traditional 2-week and 3-month toxicity studies, and the protocol and results for the 6-month transgenic mouse study of TMPTA. Effects observed were hyperplasia, hyperkeratosis, inflammation, and squamous cell papillomas of the skin at the site of chemical application in males and females, plus skin carcinomas in females, and myelodysplasia (a hematopoietic disorder) in males and females. In the standard mouse bioassay system the tumor response

would have been judged "clear evidence of carcinogenic activity." Dr. Hailey, NIEHS, described the histologic characterization of the skin lesions observed and contrasted the more severe inflammatory responses to chemical exposure in the skin of B6C3F1 mice to the milder inflammatory response in the Tg.AC mice.

Dr. Elwell, the first principal reviewer, agreed that the squamous cell neoplasms observed in the TMPTA study could be considered a positive response. He asked for more information on the response to the positive control (TPA) and expressed concern that the papilloma response was observed only at doses that also caused skin inflammation.

Dr. Storer, the second principal reviewer, suggested that a different form of conclusion other than "evidence of carcinogenic activity" would be more appropriate to describe results in the Tg.AC model. He also argued that it was unclear that the model would give equivalent responses as the classic skin promotion model. He inquired if a time sequence of histologic observations might help distinguish between two mechanisms for tumor formation, particularly in the forestomach: systemic exposure to inflammatory cytokines as a consequence of skin irritation, or direct oral exposure to the chemical from grooming.

Dr. Piegorsch, the third principal reviewer, felt the Dunson statistical model was reasonable, noting it may be specific for the Tg.AC system.

Dr. Chhabra noted that the systemic effect was seen only in one animal group, the high dose female group in the TMPTA study. Dr. French added that the dose regimen was determined operationally based on TPA doses that provided a robust response without being overly toxic. In response to Dr. Storer's question about time progression of tumorigenesis, Dr. Chhabra noted that the papillomas formed quickly, in a matter of a few weeks, and Dr. French added that the papillomas kept developing with chemical administration, so there was no acclimation or adaptation to exposure. Dr. Hailey said that the hematopoietic proliferation was thought to be associated with the myeloid rather than the erythroid component and thus more likely attributable to the inflammatory response rather than systemic exposure. Dr. Storer asked if one could infer that the ζ-globin promoter construct of the transgene was responsive to inflammatory cytokines. Dr. French answered that while that was a possibility, the proliferation more likely was a generalized response of the hematopoietic system.

Dr. Thrall said that use of complete blood count (CBC) would have helped discern whether myelodysplasia, a preleukemic condition, or just an inflammatory response, occurred. Dr. Hailey agreed.

Dr. Walker inquired if there would be a qualitative difference in interpretation of response if some gene other than *ras* (for example, green fluorescent protein) were joined to and activated by the ζ-globin promoter. That is, was cancer or some other gene expression the endpoint of the model? Dr. French replied that there were two contexts for the expression of the gene in the Tg.AC model, that it was correctly turned on at day 12 of embryogenesis, and that other regulatory control regions were also being brought into play.

Dr. Ho also questioned whether Tg.AC could be termed a cancer model and inquired if functional genomics or chromosomal characterizations had been done for the observed neoplasms. Dr. French replied that the primary focus had been on the downstream events for *ras* expression: p53 mutation or inactivation. About 30% of the metaphase cells showed trisomy at chromosome 15, but changes in chromosome number did not seem a prerequisite for expression.

Dr. Vore asked about the relationship between inflammation or wounding and papilloma formation. Dr. French cited examples of studies where both effects were observed and other studies where either inflammation or papilloma formation occurred without the other. Dr. Chhabra added that one of the dose groups in the companion PETA study was another such example.

Dr. Boekelheide asked if any difference in responses had been observed between sexes, and if the available surface changed once papillomas began forming. Dr. French noted that in a study of benzene the magnitude of response was greater in males. Whether that was due to a hormonal difference or because of different animal housing conditions was speculative. He also felt that any additional dosing after papillomas had begun forming was superfluous because the process was irreversible.

Public Comments Dr. John Van Miller, Toxicology/Regulatory Services, representing the American Chemistry Council (ACC), discussed the general class of chemicals known as specialty acrylates and methacrylates (SAM), the use of monomeric forms of SAM in producing cross-linked polymers, and a series of industry studies on two representative chemicals of this group (triethylene glycol diacrylate and the corresponding methacrylate). He noted that skin irritation is characteristic of SAM, but they were not carcinogenic in the bioassays conducted by the ACC. He suggested that the skin tumors observed in the present Tg.AC studies may have been driven by irritation and urged that conclusions about carcinogenicity be withdrawn until the mechanism of papilloma formation was clarified. Dr. Jane S. Allen, Jane Allen Consulting, Inc., representing the American Chemistry Council, also addressed the severe dermal toxicity in the TMPTA and PETA studies and suggested that the observed tumors resulted from nonspecific skin toxicity.

Because of the general similarity of findings between the two acrylate studies, the Subcommittee agreed to forego a formal presentation on the companion PETA study. Dr. Walker inquired about the seeming low purities of PETA cited in that report. Dr. Chhabra explained that these were highly reactive materials and those measures were just of the monomer, whereas the technical grade material consisted of a mixture including oligomers and other monofunctional acrylates.

Dr. Drinkwater then turned the discussion to the general question of the use of transgenic models by the NTP, noting suggestions have ranged from use as a preliminary screen to complete replacement of the conventional bioassay. Dr. Ho expressed optimism that the shorter time involved in the transgenic assays would enable rapid decisions about which tests would be most appropriate for a given chemical. Dr. Storer differentiated between reporter models, such as the Tg.AC model, and oncogene or tumor suppressor gene models such as *p53* or *ras*-H2. He felt

the latter might merit conclusions about carcinogenicity, whereas systems such as the Tg.AC model would more appropriately be used as part of larger summaries of a collection of studies. Dr. Piegorsch noted that in an NTP evaluation of various strategies for identifying carcinogens, the strongest concordance came from a combination of data from *p53* and traditional rat bioassays and genotoxicity data. Responding to Dr. Drinkwater's suggestion, Dr. Storer agreed the Tg.AC studies might fit better in the Toxicity Report rather than Technical Report series.

Drs. Walker and Elwell inquired about how positive and negative results from transgenic studies would be used in decisions about whether to perform additional testing. Dr. Klaunig concurred with the notion of using the transgenic models in a triage approach for testing and also emphasized the need to understand the mechanism for tumor formation in such models. Regarding the type of interpretive conclusion that can be drawn from transgenic models, Dr. Roberts noted that many of the transgenic models might not be predictive for carcinogenicity per se. Dr. Storer felt that while the *p53* or *ras*-H2 models more closely approximated the normal tumorigenic processes, the Tg.AC model was more questionable in that regard. Dr. Walker concurred.

No vote was taken on the conclusion statements in the draft reports.