NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS
February 5-6, 1998
Summary Minutes

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NTP BOARD OF SCIENTIFIC COUNSELORS MEETING
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The National Toxicology Program (NTP) Board of Scientific Counselors (the Board) met on February 5 and 6, 1998, at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. (*Attachment 1: Federal Register* meeting announcement; *Attachment 2* : Agenda and Roster of Members.) Members of the Board are Drs. John Stegeman (Chairperson), Eula Bingham, Clay Frederick, George Friedman-Jimenez, Carol Henry, Kim Hooper, Frank Mirer, John Mulvihill, Curtis Parker, Richard Peterson, and Patricia Rodier. Expert Consultant to the Board is Dr. Hiroshi Yamasaki. All were present except Drs. Bingham, Friedman-Jimenez, and Mirer. Three members of the Board's Technical Reports Review Subcommittee who were present to assist with the first day's review of the transgenics program were Dr. James Bus, Dow Chemical Company, Dr. Gary Carlson, Purdue University, and Dr. Susan Fischer, the University of Texas M.D. Anderson Cancer Center. Additionally, there were four *ad hoc* expert consultants present to assist with the transgenics program review. They were Dr. William Farland, USEPA, Dr. Michael Lieberman, Baylor College of Medicine, Dr. James MacDonald, Schering-Plough Research Institute, and Dr. Bernard Schwetz, FDA.

I. Report of the Director, NIEHS & NTP: Dr. Kenneth Olden, Director, reported that the process for the FY 1999 NIH budget was moving forward and the NIEHS was projected to receive about a 6.8% increase. He reported two major personnel losses, one being the departure of Mr. Charles Leasure, Associate Director for Management, to a similar position in the National Human Genome Research Institute, and the other being the departure of Dr. Gerald Poje, Director of NIEHS International Programs and the Environmental Justice initiative, to a presidential appointment on the Chemical Safety Commission. Dr. Olden said a national search would begin soon for a replacement for Mr. Leasure's position and he would welcome any referrals for either position. He commented that the NIEHS along with other agencies had been involved in recent hearings of the House Government Reform and Oversight Committee concerning the health threats of the *Pfiesteria* organism, and the Institute was supporting studies attempting to isolate and characterize the toxic agents including dermal necrotic and neurotoxic factors through our Marine and Freshwater Biomedical Research Centers at Duke University and the University of Miami. Human health effects were being studied at the University of Maryland and Johns Hopkins University. Other NIH institutes also were involved in studies and interpretation of data. Dr. Olden said that the Institute represented the NIH at the President's Conference on Global Climate Change and has played a leadership role in getting human health effects more prominence on the agenda. He commented on several recent major peer reviews. First, the Epidemiology Branch was reviewed by a committee of outside epidemiologists whose charge was to advise us on whether the Branch program was positioned to take advantage of opportunities in environmental health. Further, the committee was asked to assess how well the Branch interacted with the other components of the NIEHS intramural and extramural programs. Dr. Olden said that the overall external review of the Institute had been completed and we were implementing the recommendations from the report of the external review committee. The report was a blueprint for making the NIEHS stronger. He said that he and other program leaders and scientists had just returned from a two-day retreat with members of our national advisory committee and other outside scientists where discussion of the report was a major topic.

Dr. Olden concluded by discussing a collaborative study with the NCI and the EPA on the effects of agricultural chemicals on health with our interest focused primarily on non-cancer endpoints.

Dr. Olden presented certificates and acknowledged the contributions of retiring members of the Board, Dr. Henry and Dr. Stegeman.

II. Evaluation of Transgenic Models to Assess Carcinogenicity:
A. Introduction - Purpose of Meeting -- Dr. George Lucier, Director, Environmental Toxicology Program (ETP), NIEHS, listed the overall goals of the NTP: (1) to provide toxicological evaluation on substances of public health concern; (2) to develop and validate improved methods (sensitive - specific - faster); (3) to develop approaches and generate data to strengthen the science base for risk assessments; and (4) to communicate with all stakeholders. He said that each of these goals is really important to the use of transgenic animals in carcinogenesis and toxicity evaluation, and gave examples. Dr. Lucier pointed out that not all of the work at NIEHS with transgenic or knockout models will be presented although these other efforts may feed into or interact with the projects to be presented. There is also a significant effort in transgenic systems in the NIEHS extramural program, and in the summer of 1997 there was a meeting at the Institute where grantees and intramural scientists made presentations on their projects and there will be another such meeting this year. He said there is also considerable interaction and collaboration with Federal regulatory agencies including FDA, where NTP transgenic data on phenophthalein and methylphenidate have been used in safety assessment, and with EPA, where transgenic data are or will be developed on drinking water disinfection byproducts. There are interactions with the International Life Sciences Institute (ILSI), which is sponsoring studies looking at the use of various transgenic systems for assessing safety of drugs and pharmaceuticals. In the international arena, the NIEHS is collaborating with the Japanese National Institute of Health Sciences in evaluating the *ras* H2 mouse model, and Dr. Mitsumori from that institute will be presenting along with Dr. Maronpot from NIEHS on findings with that system.

Dr. Lucier listed the specific questions that the Board and expert consultants were being asked to respond to:

- Is the NTP approach to evaluation and validation of transgenic models for use in cancer bioassays sufficient and appropriate?
- Are the scientific needs of regulatory agencies being adequately addressed?
- How can existing models be best utilized? What are their limitations?
- What new models are needed, i.e., should NTP seek to develop organ-specific tumor models?, e.g., for prostate or brain tumors.

B. History and Rationale for Using Transgenic Animals to Identify Carcinogens -- Dr. Raymond Tennant, NIEHS, said he hoped to present the hypothesis for the use of transgenic models in chemical and drug safety assessment. He said development of transgenic models for carcinogenicity bioassays is part of an evolving effort over the past

two decades to develop a variety of means for being able to predict carcinogenicity, and in particularly relating to chemical structure and *in vitro* and *in vivo* genetic toxicity. This information is used in selection of chemicals, design of studies and interpretation of the results. Dr. Tennant noted that there is also an ongoing project using computer based methods to predict carcinogenicity. He said the progress in molecular biology and genetics over the past 20 years leading to cloning and transferring genes across species made transgenic systems a logical outgrowth. He elaborated on the genetic basis for the use of transgenic models. The use of inbred rodent strains has led to achievement of homozygosity in individual strains but with the consequence of high incidences of certain spontaneous tumors characteristic of a particular strain. This then leads to the problem in using these inbred animals in bioassays of trying to interpret whether tumors which arise or increase in incidence result as an effect of the chemical or are a property of the genetic substrate on which the chemical is imposed. This homozygosity or allelic enrichment has resulted in a large number of bioassays which are strain, species or sex specific in carcinogen response and often with only one tumor site. He said the corallary is that those chemicals that induce tumors in both rats and mice have a higher probability of doing so in other species and represent a much clearer target for human health effects. Dr. Tennant said that our hypothesis that expectations for transgenic models as carcinogenicity bioassays are that by providing target genes that represent highly conserved genes intrinsic to pathways of tumorigenesis both in rodents and humans that can minimize strain or species specific responses to chemicals. These models will identify chemicals with the capacity to cross species barriers. Conversely, the transgenic model may not pick up some of the strain or species specific carcinogens, and thus, there is not a uniformity of concordance between the models and 2-year bioassays. Dr. Tennant said his next point was to discuss a strategy for utilizing these transgenic models. He commented that a majority of chemicals recently nominated to the NTP are nongenotoxic, while about 25-30 % of chemicals found mutagenic either *in vitro* or because of structural alerts are not carcinogenic in 2-year bioassays. He said the best strategy is to utilize the models within the context of the subchronic (13-week) study, extending it to 26 weeks, which may provide information on dose setting, target organ toxicity, and presumptive carcinogenicity which would be useful if the decision was made to go on to a 2-year bioassay. The models under study, the $p53^{def}$ and the Tg.AC are complementary systems that will identify trans-species carcinogens, will not identify non-carcinogens, and will not identify chemicals inducing strain-specific responses. He said that not only can one count tumors but also can analyze the tumors for molecular effects indicative of specific genetic changes. Dr. Tennant summarized by stating that the goal is to identify <u>human</u> health risks from environmental carcinogens and the principal experimental tools have been the long term, two species rodent bioassays with the correlation being that a majority of all known human carcinogens are positive in rodent bioassays, whereas the concerns are whether all rodent carcinogens are human carcinogens. He stated that the alternative to the 2-year bioassay are short-term transgenic rodent bioassays, with the correlation being that all human carcinogens tested sofar have been positive, while the concerns are whether long-term bioassay positive agents that are not detected by transgenic biosssays are of concern for human health. Finally, he said there is the problem of trying to extrapolate data from genetically engineered rodents to humans, just as there has been the problem of trying to

extrapolate data from highly inbred rodents to humans. Dr. Tennant concluded that much work needs to be done.

Discussion: Dr. Yamasaki noted that the basis for the utility of the model was that the transgene is a gene that is phylogenetically conserved in a variety of species, yet he said that Dr.Tennnant claimed it would pick up rat carcinogens, and if the test went on long enough other tumors would be detected. Dr. Tennant responded that if the exposure period is continued as in a 2-year bioassay tumors will occur that are influenced by the genetic background and independent of the transgene. Dr. Lieberman suggested that introduction of these active mutations may lower the threshold allowing one to see carcinogens that might not be seen in the parent mouse. Dr. Tennant said that threshold in the sense of creating a genetically initiated model he would agree with. Dr. MacDonald asked whether there was more to be gained from these models than just the ability to predict the outcome of rodent bioassays, i.e., concordance, such as information on carcinogenic mechanisms. Dr. Tennant said the ability to obtain mechanistic information was most important. Dr. Hooper said his concern was with the single species carcinogens, often non-mutagenic, and whether a negative finding in the transgenic model represented a true or false negative. Dr. Tennant replied that the transgene might not answer that question but rather the HCS locus in the mouse would need to be cloned and sequenced and determination made as to whether this locus was represented in humans.

C. Studies With the $p53^{def}$ Mouse Model -- Dr. John French, NIEHS, restated the hypothesis that a mouse with an inducible oncogene (such as Tg.AC) or an inactivated tumor suppressor gene (such as $p53^{def}$) will rapidly develop cancer in susceptible tissues when exposed to a transspecies carcinogen. The goal is to identify those chemicals which are the most proximate risk to human health. The focus with the p53 model is on detecting mutagenic transspecies carcinogens and it is well recognized that wild type p53 protein suppresses cancer in humans and rodents. In addition, it is critical to recognition of DNA damage and DNA repair, cell cycle control and apoptosis, serving to maintain genomic stability. Dr. French described the development and characteristics of the heterozygous $p53^{def}$ mouse by researchers at Baylor College of Medicine. Mice rendered heterozygous for the p53 tumor suppressor gene are viable and exhibit a low background incidence of tumors during their initial 12 months of life. They are at elevated risk for induced tumor development since a mutational event may result in inactivation or loss of the wild type p53 allele, thereby removing proliferative restraint. Dr. French said there were two approaches used in the animal studies, the first being retrospective using a range of expected outcomes based on findings in the 2-year bioassay in F344 rats and $B6C3F_1$ mice. Taking advantage of the window of low background incidence of tumors, 26-week replications were conducted of the 2-year bioassay under the same exposure conditions followed by gross and microscopic tissue examination. Some experiments had co-isogenic homozygous wild-type p53 mice as controls. He said the first set of studies were of both mutagenic and nonmutagenic carcinogens as well as one mutagenic noncarcinogen, *p*--anisidine, and he compared the results obtained to the 2-year bioassay results. Mutagenicity was defined as a positive result in the *in vitro Salmonella* mutagenesis assay and/or the *in vivo* micronucleus assay. Of five mutagenic mostly transspecies

carcinogens, benzene, *p*-cresidine, 4-vinyl-1-cyclohexene diepoxide, and phenolphthalein were positive in the $p53^{def}$ mouse, while, one, glycidol was negative. Four nonmutagenic carcinogens in the 2-year bioassay were negative for carcinogenicity in the $p53^{def}$ model, chloroprene, N-methyl-*o* -acrylamide, reserpine, and methylphenidate, as was the mutagenic noncarcinogen, *p*-anisidine. Dr. French then presented the results from six prospective studies with chemicals for which the 2-year bioassays were recently completed and the draft technical reports peer reviewed. The six were 1-chloro-2-propanol, coconut oil acid diethanolamide, lauric acid diethanolamide, oleic acid diethanolamide, pentachlorophenol, and pyridine. The first two were weakly mutagenic in one of the two mutagenesis assays, the others were nonmutagenic. For the six 2-year bioassays, only one, pyridine, turned out to be a transspecies carcinogen, while three of the others were single species carcinogens. All six produced negative results in the 26-week $p53^{def}$ assay. Thus, the overall outcome for predictions in the C57BL/6 heterozygous $p53^{def}$ mouse was that for mutagenic carcinogens -- 4/5 were positive, for essentially nonmutagenic carcinogens -- 10/10 were negative, and for the mutagenic noncarcinogen -- 1/1 was negative. Dr. French said, that in summary, for these assays, (1) mutagenic carcinogens demonstrated a marked decrease in tumor latency, (2) there was tissue specificity and a similar tumor phenotype between the $B6C3F_1$ mouse and the C57BL/6 mouse, and (3) this model may be useful for predicting mutagenic transspecies carcinogens. Dr. French then proceeded to look at a strategy for looking at the mechanistic understanding of tumor induction with this model. Both control and tumor tissue can be examined for loss of the wild type allele by Southern analysis. If there is still a significant signal for the p53 wild type allele, mutation analysis can be done employing a single stranded conformational polymorphism analysis. If the tumor is small, suggested mutations can be looked for through p53 immunohistochemistry and *in situ* hybridization and PCR. He exemplified this approach with three case studies -- phenolphthalein, *p*-cresidine, and benzene. With regard to phenolphthalein, increases in thymic lesions at higher doses paralleled loss of heterozygosity (allele loss). With regard to benzene, a human carcinogen, at the high dose primary tumors formed were sarcomas of the head and neck region, and in 13/16 tumors examined there was loss of heterozygosity. While for *p*-cresidine, where the primary tumors were bladder and liver, less than 25% of the bladder tumors showed loss of heterozygosity. Dr. French described further studies with transitional cell carcinomas of the bladder that failed to observe inactivating mutations in exons 5-9 of the p53 alleles. However, other studies with mice of the same strain carrying the *lac*I neutral reporter gene, they were able to show that the bladder was mutagenized. He concluded that in this model, they have observed mutagenic transspecies carcinogens that have retained both chemical and tissue specificity making it possible to do a more complete dose-response characterization, and allowing us to look for both inactivation of p53 alleles or induction of mutations in other endogenous genes or gene expression.

Discussion: Dr. MacDonald asked whether there were losses of wild type alleles in spontaneous tumors arising later in homozygous control animals. Dr. French said there were although the incidence was quite variable. Dr. Lieberman wondered what percentage of mutations were missed by looking at the polymorphism changes and not looking at all the exons. Dr. French responded that at best they are looking at 30% of the

coding regions of the genome including the flanking regions. Dr. Frederick asked whether in view of what the transgenic model misses, e.g., glycidol, would *in vivo* mutagenesis be a simpler and cheaper substitute. Dr. French replied that with current information, *in vivo* mutagenesis would not be enough, and obviously, with exceptions such as glycidol, the p53 system is good at identifying those chemicals most likely to be human carcinogens.

D. Studies with the Tg.AC Mouse Model -- Dr. Jud Spalding, NIEHS, said he would describe the features of the Tg.AC transgenic mouse model as used to evaluate chemically induced carcinogenesis, protocols used, and give a discussion of the results on chemicals from both retrospective and prospective studies. The Tg.AC mouse line was created in the Leder laboratory by pronucleus injection of a construct carrying an activated v-Harvey (Ha)-*ras* gene in the FVB/N background strain. He said this was an appropriate model for these studies as mutations in the *ras* family of genes are associated with 30% of human tumors and with many spontaneous and induced tumors in rodents. All of the studies have been performed by topical application or skin painting (sp). The chief characteristic of the model is that the presence of the transgene confers the property of genetically initiated skin. The transgene is not constitutively expressed in normal skin or other tissues with the exception of the bone marrow. He said that expression of the transgene is inducible and activation of the transgene is required for tumorigenesis to occur. Induction of papillomas represents a reporter phenotype that defines chemical activity. Spontaneous tumor incidence is low to zero in dorsal skin during the period of chemical exposure. The model identifies both mutagenic and nonmutagenic carcinogens. Dr. Spalding stated that the hypothesis is that these transgenic models are most likely to identify transspecies carcinogens, including most of the major human carcinogens, and least likely to identify single sex, single species carcinogens. The protocol includes using female homozygous and hemizygous Tg.AC mice with a chemical applied either in acetone or ethanol topically to the shaved dorsal area for 20-26 weeks. A negative solvent control group and a TPA positive control group are always run concurrently with the test article. At least three doses were used with the top dose usually a maximal tolerated dose. The endpoint was tabulation of skin papillomas, which could be observed as early as 5-7 weeks. Dr. Spalding said the basis of chemical selection from 2-year bioassay studies was quite broad and included mutagenic and nonmutagenic carcinogens and noncarcinogens, and carcinogens which caused tumors in only a single sex/species, and finally, chemicals with verified findings (retrospective) and bioassays still in progress (prospective). An early bias was for nonmutagenic chemicals that were NTP skin paint studies. Dr. Spalding displayed a chart comparing 2-year bioassay findings in the $B6C3F_1$ mouse with those in the Tg.AC mouse for a variety of chemicals. He focused a more detailed discussion on two chemicals, benzene, a mutagenic transspecies human carcinogen, and mirex, a nonmutagenic carcinogen. Dr. Spalding then turned to the prospective studies of eight chemicals, six of which had been studied in the $p53^{def}$ models as related earlier by Dr. French. He chose to discuss further, results from diethanolamine and the three diethanolamine condensates, all of which had been conducted by the skin paint route of administration in the 2-year bioassay and the Tg.AC. In all cases, the range of doses in the Tg.AC well exceeded the range in either rat or mouse chronic studies. Looking at diethanolamine, which was administered at three doses five times weekly for 20 weeks, he showed there was no

response in papilloma formation other than to the positive control, TPA. With lauric acid diethanolamine, there was a papilloma response, with a slight incidence of papillomas at the mid-dose (10 mg), and a greater response in the high dose (20 mg), particularly in the homozygous compared with the hemizygous animals. Dr. Spalding turned to other chemicals in the prospective study, and noted that there was concordance between Tg.AC and the 2-year bioassay for pentachlorophenol which was positive in both, and between Tg.AC and the bioassay for 1-chloro-2-propanol which was negative in both. Dr. Spalding reported that protocol development resulted from their experiences and also collaborations with ILSI. We now recommend that animals should be singly housed, chemical administration should be for up to 26 weeks, the 14-day dose finding study could be done in FVB/N wild-type mice, and provision should be made for gross and histopathologic examination of 10-12 tissues at necropsy. He added, that other routes of chemical administration such as dosed feed, gavage or inhalation were being investigated. Dr. Spalding reiterated the advantages of transgenic mouse bioassays: (1) reduced latency period as time-to-tumor occurs within 6 months; (2) very low spontaneous tumor incidence during the exposure period; (3) reduction in number of animals per dose group; and (4) dosimetrics - dose-response and no effect levels can be determined. In summary, he said that the Tg.AC transgenic model is a short-term *in vivo* bioassay that detects both mutagenic and nonmutagenic chemical carcinogens, although it is most likely to detect transspecies carcinogens, with induction of skin papillomas acting as a reporter phenotype for indicating chemical activity. He emphasized that activation of transgene expression is required for tumor induction.

E. Molecular and Cellular Tg.AC Studies -- Dr. Ronald Cannon, NIEHS, began by describing the construct of the transgene, and then described what the papillomagenic response looked like in the skin. After applications of phorbol ester, there is a hyperplastic response which subsides, and at 8-10 days there is a follicular hyperplasia which expands into further follicle involvement, and after another week a localized hyperplasia is formed which appears much like a papilloma. By using *in situ* hybridization, the transgene was shown to be closely associated with the hair follicle. Dr. Cannon also described the full thickness wounding technique which allows more precise localization of the transgene. He speculated that there is a cell associated with the hair follicle ('cell at risk') for which after chemical or physical 'tweaking' there is a epigenetic or genetic change leading to a change in gene expression; the transgene is transcriptionally activated leading to clonal expansion resulting in a papilloma. Dr. Cannon said the next question was -- at the molecular level is the transgene constitutively expressed or is it induced? Data from full thickness wounding experiments indicated that transgene mRNA detection was restricted to wound associated skin tissues on day 16 and thereafter. The data indicate that the transgene is transcriptionally silent in normal non-treated (non-induced) skin and requires transcriptional activation to initiate the *ras* dependent papillomagenesis. Dr. Cannon discussed experiments concerned with assessing possible epigenetic modification of Tg.AC genomic DNA. Results indicated that a time dependent and site-specific hypomethylation of the transgene occurs following wounding. He spoke of experiments looking at the role of GATA transcription factors in expression of the zeta globin promoter gene. Their data indicate that GATA binding sites are required for expression of the transgene and that,

unexpectedly, GATA-3 is the sole family member expressed in mouse follicular epithelium, and implicate GATA--expressing follicle cells as a target for induced tumorigenesis in Tg.AC mice. Dr. Cannon summarized saying that: (1) one in four Tg.AC founder lines express the transgene giving credence to a positional effect; (2) all Tg.AC tumors express the transgene with a unique transcriptional complex central to tumorigenesis in mice; (3) there is a 'cell at risk' for genetic or epigenetic changes closely associated with the hair follicle; and (4) this is a stable transgene for which they have identified some transcription factors.

F. Results of NTP Transgenic Evaluations -- Dr. William Eastin, NIEHS, said he wanted to cover the driving force behind NTP's involvement in evaluation of the two transgenic models. He said that at a workshop in January 1995, Dr. Tennant presented preliminary data just completed in his laboratory that suggested Tg.AC and heterozygous p53 transgenic mice might be good models to identify chemicals with carcinogenic potential. Dr. Eastin displayed a prediction scheme incorporating NTP *Salmonella* assay and two-year rodent study results. In deciding what chemicals to study in evaluation of the models, a search of the NTP database was done to bring up representative chemicals that were (1) positive for carcinogenicity and mutagenicity, (2) positive for carcinogenicity and negative for mutagenicity, (3) negative for carcinogenicity and positive for mutagenicity, and (4) negative for carcinogenicity and mutagenicity. Dr. Eastin reviewed the study design which included using both sexes of both strains individually caged, dosing of 15 mice per group with 13-30 controls for up to 24 weeks, recording of body weights, clinical observations, complete necropsy/microscopic examination of major organs and tissues and NTP pathology working group review, and statistical evaluations. Dr. Eastin said that in their evaluations it was important to test known human carcinogens. There were four objectives in initial studies, being to determine if the models will: (1) detect human carcinogens; (2) detect carcinogens with different mechanisms of action; (3) detect a carcinogen administered by different routes of exposure; and (4) have sex differences in response. There were four human carcinogens chosen for study: diethylstilbestrol (DES), melphalan, cyclosporin A, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). He described the experimental conditions and neoplastic findings for each. For DES, a synthetic estrogen, there were increases in skin/squamous cell papillomas in both sexes of Tg.AC, while as expected since DES is nonmutagenic, there was no neoplastic response in $p53^{def}$. For melphalan, a mutagenic nitrogen mustard drug, there were increases in papillomas of the skin and forestomach in male and female Tg.AC mice, and in lung tumors in females, while there were increases in sarcomas of the skin and malignant lymphoma in male $p53^{def}$ mice. For cyclosporin, a nonmutagenic immunosuppressive fungal polypeptide, there were increases in squamous cell papillomas of the forestomach and malignant lymphoma in high dose male Tg.AC mice and in squamous cell papillomas and keratoacanthomas of the skin in females, while there were no increases in tumors in $p53^{def}$ mice. For TCDD, the nonmutagenic phenoxy herbicide contaminant, there were increases in squamous cell papillomas of the skin in male and female Tg.AC mice, and in keratoacanthomas of the skin in females, while there were no neoplastic responses in $p53^{def}$ mice. Dr. Eastin discussed results for N-methylolacrylamide, a nonmutagenic chemical that was positive in the two-year bioassay at several sites in mice. When administered by both oral and dermal

routes to Tg.AC mice, there was no tumor response by either route, an unexpected finding. Dr. Eastin next discussed responses with closely related isomers, 2,4-diaminotoluene (2,4-DAT) and 2,6-diaminotoluene (2,6-DAT), where both isomers were mutagenic while 2,4-DAT was positive in the 2-year bioassay and 2,6-DAT was negative. In the two transgenic strains, 2,4-DAT was positive for carcinogenicity in the Tg.AC but only marginally so in the $p53^{def}$ while 2,6-DAT was negative in both strains. Dr. Eastin said they also wanted to test chemicals that were negative in the 2-year bioassay. Of the four he addressed, *p*- anisidine HCl and 8-hydroxyquinoline were positive in *Salmonella* while resorcinol and rotenone were negative. *p* -Anisidine and 8-hydroxyquinoline were negative in the Tg.AC while all four were negative in $p53^{def}$. However, resorcinol induced a high incidence of squamous cell papillomas of the skin in both sexes of Tg.AC. Topical exposure of Tg.AC mice to rotenone resulted in a systemic disease known as myelodysplasia, which is a complex phenomenon combining inflammatory, hematopoietic and neoplastic features. Dr. Eastin then displayed a summary table comparing results with predictions for the chemicals he had discussed. N-methylolacrylamide predicted to be positive in Tg.AC was negative by two routes of exposure, while 2,4-DAT predicted to be positive in $p53^{def}$ was negative, and resorcinol and rotenone predicted to be negative in Tg.AC were actually positive. Dr. Eastin concluded his presentation by noting how their four initial objectives had been met: (1) detection of human carcinogens -- all four human carcinogens gave a positive response; (2) detection of carcinogens with different mechanisms of action -- human carcinogens were; (3) detection of a carcinogen administered by different routes of exposure -- the chemical chosen, N-methylolacrylamide, was negative by both routes so this objective is unresolved; and (4) whether there were sex differences in response -- there were no apparent differences in response between sexes except in Tg.AC mice receiving cyclosporin A. Finally, he said it should be noted that it was initially proposed that skin papilloma response can act as a reporter phenotype in the Tg.AC mouse to identify potential carcinogens. In the Tg.AC studies, except for rotenone, chemicals causing internal tumors also produced skin papillomas.

Discussion: In response to questions about whether the same biological mechanisms are operative in tumor response, e.g., to the carcinogen/noncarcinogen pair (2,4-DAT and 2,6-DAT), in transgenic mice compared with the animals used in the 2-year bioassay, Dr. Lucier responded that we intend to go back in cases like this and explore possible mechanisms.

G. Studies with the *ras* H2 Mouse Model -- Dr. Robert Maronpot, NIEHS, reported that the NIEHS interest in the *ras* H2 mouse model originated about two years ago when scientists from the Japanese Ministry of Health and Welfare presented data from several chemicals in this animal which contained the human *ras* protooncogene with its endogenous normal promoter. Further, the tumors derived from these animals had mutations in the human *ras* gene and not the murine endogenous gene. Dr. Maronpot said the decision was made to conduct an interlaboratory comparison with animals being shipped from Japan. He said that Dr. Mitsumori would share his experiences with their battery of results and also provide current perspective of the regulatory authorities in Japan with regard to the use of transgenic animals. The *ras* H2 is on a background of a

Balb/C mouse crossed with a C57BL mouse and if kept for 18 months develops a number of spontaneous tumors. The basic study design involves transgenic males and females as well as nontransgenic litter mates

Dr. Kunitoshi Mitsumori, National Institute of Health Sciences, Tokyo, Japan, said they have completed studies on 29 chemicals including 17 Ames test positive (mutagenic) carcinogens (as determined primarily in NTP 2-year bioassays), six nonmutagenic carcinogens, three mutagenic noncarcinogens, and three nonmutagenic noncarcinogens. He displayed data showing tumor target organs in *ras* H2 mice compared with target organs from 2-year bioassays in 12 of the mutagenic carcinogens, and there was 90% concordance. Of the 17 mutagenic carcinogens, there was a significantly increased tumor response in treated vs. control animals for 13, while incidence/multiplicity of tumors was significantly increased for 12 chemicals leading to the conclusion that the *ras* H2 model is useful for detecting carcinogenic potential of mutagenic carcinogens. Dr. Mitsumori then presented comparative tumor target organ data for the six nonmutagenic carcinogens which indicated about 70% concordance. He said that further evaluation studies are needed to determine the usefulness of *ras* H2 mice for detection of nonmutagenic carcinogens. He presented data on comparisons for the mutagenic and nonmutagenic noncarcinogens and concluded that further evaluation studies are required to confirm the absence of false positive responses. Dr. Mitsumori reported on the current test guidelines in Japan for carcinogenicity of pharmaceuticals, pesticides, food additives, and other chemicals, noting that for pharmaceuticals the requirement for long-term studies of two rodent species could be replaced by one long-term study plus one short-term study such as transgenics. He reviewed the current attitude of regulatory authorities in Japan on use of transgenic mice in safety assessment of chemicals other than pharmaceuticals, and said that for these other types of chemicals, long-term studies using two rodent species are still required.
Dr. Maronpot listed the six compounds tested simultaneously in the interlaboratory comparison. They were *p* -anisidine, *p* -cresidine, cyclosporin A, melphalan, resorcinol, and vinyl carbamate, which is the primary metabolite of urethane. He presented data for four of the chemicals. Vinyl carbamate is a potent carcinogen which after one intraperitoneal injection produced high incidences of lung adenomas and carcinomas in both male and female *ras* H2 mice in both NIEHS and Japanese studies. There was also a high incidence in the nontransgenic animals, primarily adenomas. There were high incidences of splenic hemangiosarcomas in the transgenic animals but not in the nontransgenics. Dr. Maronpot said that *p* -cresidine was selected as it was a known urinary bladder carcinogen, and urinary bladder tumors are rare in the *ras* H2 animal, so although the incidences of such tumors were low, the responses were credible. The tumor response to *p* -anisidine was expected to be negative and, indeed, it was in both NIEHS and Japanese studies. He reported the findings on resorcinol which also was expected to be negative, and in both NIEHS and Japanese studies it was negative for neoplasia. Dr. Maronpot stated that for the six chemicals there was interlaboratory agreement for the four chemicals he had reported on, and lack of agreement for melphalan and cyclosporin A for which neoplastic findings had been provided in the Board's handout. In conclusion, Dr. Maronpot said there were a number of issues that needed to be considered such as whether there should have

been greater numbers of mice per group, whether the study duration should be longer than six months, or whether higher doses should have been used. He said there was agreement on continuing to use nontransgenic littermates, refining the pathology diagnostic criteria, development of a historic control data base to help in interpretation of the findings, and defining what constitutes a positive response. He said that our data along with data on nongenotoxic carcinogens in the ILSI initiative should provide some answers within the next two years on the usefulness of the *ras* H2 in human risk assessment.

Discussion: Dr. Frederick asked whether the same routes of exposure were used as were used in the chronic bioassay. Dr. Maronpot responded that in most cases they were. In the case of vinyl carbamate there was no chronic bioassay so information from the literature aided in choosing the appropriate route.

H. Summary of Model Studies Evaluation -- Dr. John Bucher, NIEHS, said he would summarize the data presented in such a way as to help the Board in responding to the questions posed to them. He would review the data presented for the three transgenic models, comparing that with the rodent bioassays and drawing on our knowledge and experience along with our mechanistic expectations on how the models work in trying to answer the question of whether we can reliably use these models in carcinogen identification and assessment -- and if so, how? Dr. Bucher spoke of the evaluations with the model developmental chemicals for the Tg.AC. These were acetone and ethanol - solvents for dermal studies, TPA - a strong skin tumor promoter, *o* -benzyl-*p* -chlorophenol - a weak skin tumor promoter, and benzethonium chloride - a nonmutagenic noncarcinogen. For all of these, there was concordance between Tg.AC and the bioassay with the exception of ethanol which has not been adequately evaluated in a bioassay. Dr. Bucher then reviewed how well one or more of the models had a positive neoplastic response with five human carcinogens. Benzene was positive in all three as was melphalan, while cyclosporin A was positive in Tg.AC and *ras* H2, and DES and TCDD were positive in the Tg.AC so there was concordance for all with at least one transgenic model. Dr. Bucher noted that the concordances were not so good with an amine/amide chemical class. Diethanolamine and its amides with oleic acid, coconut oil and lauric acid had been evaluated prospectively in Tg.AC, and the three amides also in $p53^{def}$, while the bioassay reports were peer reviewed in December 1997. There were positive tumor responses in the bioassay (mouse liver, kidney) for diethanolamine and the coconut oil and lauric acid amides. Interpretation was complicated because neoplasia in the amides could be attributed to diethanolamine as a contaminant. There was also lack of concordance between triethanolamine, a mouse liver carcinogen in the bioassay, and Tg.AC. Dr. Bucher noted a lack of concordance in any of the transgenic models with four other nongenotoxic carcinogens in the bioassay (primarily liver or kidney), being methylphenidate, furfuryl alcohol, pyridine, and 1,1,2-trichloroethane. Turning to two carcinogen-noncarcinogen pairs, Dr. Bucher noted concordance between the bioassay and at least one of the transgenic models for 2,4- and 2,6-diaminotoluene, and for *p* -cresidine and *p* -anisidine. With regard to *p* -cresidine, he said it was not clear whether the bladder tumors were a normal response to a potent carcinogen rather than activation of the transgene so further study was needed. Looking at a diverse group of seven

noncarcinogens, there was concordance for five, 8-hydroxyquinoline, 1-chloro-2-propanol, 2-chloroethanol, phenol, and mixed xylenes. In the Tg.AC only, there was a skin tumor response for resorcinol and for rotenone, myelodysplasia was induced with some uncertainty as to whether or not this represented a neoplastic response. Dr. Bucher next turned to some robust rodent carcinogens where there was not concordance with any of the transgenic systems -- glycidol, chloroprene, N-methylolacrylamide, and reserpine. All were multisite and three were transspecies in the bioassay. To contrast this lack of success, Dr. Bucher discussed other rodent carcinogens for which there was concordance with at least one of the transgenic models --dimethylvinyl chloride, 4-vinyl-1-cyclohexene diepoxide, pentachlorophenol, mirex, phenolphthalein, ethyl acrylate, and ethylene thiourea. Dr. Bucher commented on a number of strong, mostly mutagenic, carcinogens previously reported as being positive in the *ras* H2 by Dr. Mitsumori, including three known human carcinogens, cyclophosphamide, phenacetin and thiotepa.

Dr. Bucher began to summarize the data. Looking at concordance between outcome in the Tg.AC and $p53^{def}$ and the bioassay or human experience, 24 studies gave a concordant response and 13 were non-concordant, 65%. If the results of the 18 Japanese studies of mostly strong carcinogens in *ras* H2, the concordance increases to 76%. Further, among the three transgenic lines, all eight human carcinogens were detected. He said that of the 13 chemicals that were non-concordant -- 2 identified a positive response for a noncarcinogen; -- 2 were mouse liver tumor only; -- 1 gave Clear Evidence (CE) in mouse liver and adrenal (CE is the strongest level of carcinogenic activity in the NTP bioassay while Some Evidence (SE) is a lesser level of carcinogenic activity); -- 1 gave CE in mouse liver and SE in rat kidney; -- 2 gave CE in mouse liver and SE in mouse kidney; -- 1 gave SE in rat nasal cavity and mouse kidney. He said that to this point, the major tumor response missed was mouse liver. However, when looking at reserpine and N-methylolacrylamide, these non-concordant chemicals are multisite, strong carcinogens. Further, glycidol and chloroprene are mutisite, multisex, multispecies carcinogens, and glycidol was the only *Salmonella* positive chemical not identified by one of the transgenics. Just to give perspective to those not familar with the NCI/NTP bioassay, the overall concordance between rats and mice in 379 chemical carcinogenicity studies is 74.4%. Based on the information presented, Dr. Bucher posed the question -- What kinds of chemicals would we likely miss based on these results? Answer: Nongenotoxic mouse liver, kidney, and/or adrenal carcinogens, which would account for about 10% of the chemicals in the NTP database, and include small halogenated alkanes and alkenes, a number of drugs, industrial chemicals and pesticides. Dr. Bucher displayed several schemes that have been published for potential strategies on how one might use transgenic animals in a bioassay program. In these, the transgenic result tends to become the final word. He said that he would propose that a negative in Tg.AC or p53 might lead to consideration of a chemical for a 2-year study while a positive in any of the three transgenic models would indicate to him that it was likely to be positive in a rodent bioassay. Dr. Bucher stated that one of the questions posed to the Board was -- should the NTP begin to develop site-specific transgenic models? He said that as a scientist who has been involved in the design, conduct, and evaluation of a number of rat and mouse bioassays, there is nothing he would like better than to have a battery of site-specific transgenic models sitting on the shelf that

he could use to mimic individual mechanisms involved in human mammary, brain, prostate, and other types of cancers. He acknowledged that there is the view that a few general screens are better because unappreciated mechanisms are most certainly involved in carcinogenesis, and genetic alterations thought to be involved in human cancers may not be important in rodents. If the view is that we should, which tumor sites should be targeted? Dr. Bucher suggested that models for brain and prostate tumors, cancers that are increasing in the human population, would be helpful. He noted that in the NTP rodent bioassay database, only three chemicals have been positive for brain tumors, and none have been positive for prostate cancer. Dr. Bucher reported that there are some site-specific models at a preliminary stage of study at NIEHS, including a model for intestinal tumors developed in The Netherlands, an APC mutation which will be used with some water disinfection byproducts. A few years ago, models for mammary cancer were evaluated and found not useful, while models for BRCA1 and BRCA2 are being looked at currently. A scientist at NCI has suggested evaluation of a p16 deficient model for brain tumors. Preliminary work has been done in looking for site-specific models for prostate tumors.

Discussion: Dr. Bus suggested that to gain equivalent information to that obtained from the traditional two sex/two species bioassay, at least three transgenic models would be needed and wondered whether the additional cost would be justified. Dr. Bucher said there are certain chemicals for which we could confirm their carcinogenicity with a transgenic assay, obviating a bioassay, if the regulatory community would accept these findings, while other chemicals/classes could be predicted not to be detected by a transgenic and we would proceed directly to a bioassay. Dr. Carlson asked whether there other chemicals under study in the transgenics, and any nonorganic agents. Dr. Bucher said there were and plans were to go back and look in more depth including mechanistic studies on some chemicals already evaluated, e.g., TCDD and DES. Also, several chemicals under the ILSI initiative are being evaluated, e.g., peroxisome proliferators. Dr. MacDonald said there were 21 chemicals under the ILSI initiative, including some genotoxic carcinogens, some negative in bioassays, and of most interest and concern, pharmaceuticals that have produced tumors in rodents. Dr. Farland commented that these were correlative approaches and his concern was that there may be different modes of action in tumor responses in transgenics vs. those in the bioassay. He thought the value may be in following up on some of the mechanistic issues. Dr. Carl Barrett, NIEHS, commented that there may be many reasons why the tumor results in the transgenic model may be different from those in the bioassay. Using a value added approach, he said a firm positive in the transgenic assay may support a questionable or equivocal finding in the bioassay. Another issue requiring more study is with the potent multisite/species carcinogens in the bioassay, e.g., glycidol, chloroprene, where the transgenic assay is negative. The explanation here might lie in 26 weeks not being long enough for tumor development. Dr. Lucier said one of the value added features is the ability to get good dose response information in the transgenic compared with the bioassay. Dr.Thomas Goldsworthy, Integrated Laboratory Systems, noted that down the road it should be clearer which transgenic model to evaluate a particular chemical in thus making it more cost effective than at present where more than one model is needed.

I. Transgenic Studies and Risk Assessment -- Dr. Christopher Portier, NIEHS, said that when there is an adequate bioassay available, added information from transgenics gives mechanistic understanding. There is also the possibility of using the transgenic assay alone. In terms of risk assessment and use of transgenics, he said there were three areas he would touch on, these being hazard identification, dose-response assessment, and species extrapolation. Also there is the possible direct use in quantitative risk assessment based on the mechanistic understanding of the multistage process. First, with regard to transgenics and hazard identification, Dr. Portier said the low background of tumors in transgenic models gives greater statistical power meaning that fewer animals and more doses can be used. With the Tg.AC, one has an observable tumor enabling provision of more data on tumor size and growth. With the earlier tumor response, more compounds can be evaluated and in a shorter timeframe, a real plus for risk assessment. Dr. Portier discussed transgenics and concordance and why there were differences between NTP data only and all data. This can reflect such things as different methods of making tumor calls, e.g., with Tg.AC measuring internal tumors as well as papillomas, and differences in the chemicals selected. He said that discordance actually may lead to better understanding of mechanisms. Further, we may need different and/or additional assays than the current ones. Finally, we are working with a moving target and the data base will change as ILSI data become available and as more NIEHS studies are completed. Second, with regard to transgenics and dose-response, Dr. Portier commented that although the transgenics are cheaper and faster allowing more dose levels, this leads to design questions such as more dose levels may mean fewer animals per group and less sensitivity for detecting weaker carcinogens. Also, keeping in mind that bioassays and human exposure may be the major part of lifetime exposure should length and timing of transgenic exposures be changed in view of regression/progression of tumors such as papillomas. He said that potency evaluation is going to be limited as doses for the transgenics were set to be the same as in the bioassay with the top dose presumably being the maximal tolerated dose (MTD). Third, with regard to transgenics and species extrapolation, Dr. Portier reported that toxicokinetics evaluations are currently underway to determine how transgenic animals respond kinetically to chemicals compared with standard bioassay animals, and the likely weaker historical control data base may pose problems in doing modeling of chemicals. Then there is the issue of genomic knowledge and how this can be used to strengthen estimates of population risk. He said he was not sure how to do this and would require considerable discussion among modelers and the regulatory community, i.e., do we look for sensitive populations or turn to molecular epidemiology. He said there are other possibilities to consider such as age equivalence across species, i.e., what are the implications for appropriate human linkage between the "sped-up assay" vs. the "lifetime" rodent bioassay? Dr. Portier turned to transgenics and mechanism based risk assessment. In speaking of the initiation-promotion paradigm, he noted that with the transgenics there is genomic initiation which may not be the same as chemical initiation, and there are some real questions raised as to whether the shape of the dose-response curve would be similar in a transgenic vs. a nontransgenic animal. He said there is the same question regarding late stage mutational effects and application to human populations. Dr. Portier mentioned other mechanistic modeling considerations. He said that complete carcinogens pose problems in that they have multi-effects, e.g., TCDD has both mutational effects as well as

proliferative effects. How does the shape of the dose-response curve look in transgenic animals as contrasted with nontransgenics? Another consideration is biomarkers of effect. Because they develop faster in transgenics, we may be able to follow biomarkers in animals that get tumors in a more efficient manner than in animals on long-term bioassays. This may strengthen their use in low dose, multispecies extrapolation.

Discussion: Dr. Frederick said it seemed early to be able to draw conclusions about dose-response particularly in the Tg.AC where there appears to be a second messenger involved in tumor initiation. Dr. Portier agreed but thought the Tg.AC to be useful now even if we don't understand the signaling pathway as we have a very quantifiable response in the skin papillomas. Dr. Tennant asked how much value do you place on identifying strain or species responses in the bioassay? The key question is which effects should be extrapolated to humans from either transgenics or the bioassay. What are we learning about human risk, and from which model? Dr. Tennant mentioned a problem with Tg.AC animals from one supplier not responding to TPA and recommended steps to monitor for and avert such problems in the future.

J. FDA Perspective on Transgenic Models -- Dr. Schwetz spoke in place of Dr. Joseph Contrera, FDA, who could not be present. He began by mentioning the FDA mission statement which is to ensure safety and efficacy of drugs, foods, cosmetics, devices and veterinary agents, while noting that two additional features have been added recently by Congress, these being to carry out faster reviews of submissions and to begin to achieve less regulatory burden on industry. With that in mind, he commented on the scheme whereby a transgenic assay would be run, and then a decision made to also conduct a two-year study which could have the effect of more regulatory burden. Thus, if we cannot find a way to scientifically justify alternative *in vivo* models they will be bypassed. Dr. Schwetz said he hoped this would not be the case such that the few two-year studies would be high priority and not just screens. He said that his subsequent remarks would pertain to drugs and not to other products regulated by the FDA. Drugs are regulated on the basis of the weight of evidence. Many of these drugs are listed in the Physicians Desk Reference (PDR) and some may be animal carcinogens but are approved because the benefit easily outweighs the risk or the animal data is not considered relevant to humans. Dr. Schwetz spoke of the International Committee for Harmonization (ICH) that has been considering guidelines on how the *in vivo* transgenic models can be applied in the evaluation of pharmaceuticals. The ICH guidance is being applied by the Center for Drug Evaluation and Research (Center for Drugs) and allows for the optional application of an appropriate *in vivo* alternative to the second two-year rodent carcinogenicity study as long as there is a scientific justification. He said that considerations for choice of a transgenic or other *in vivo* model could be route of administration, comparative systemic exposure and metabolism relative to humans, toxicity/pharmacodynamics of the drug, extent of experience and scientific acceptance of the model, and potential contribution of additional useful information related to carcinogenic mechanisms not available from a second rodent bioassay. Dr. Schwetz discussed under what circumstances the transgenic models might be used. One would be as an alternative to a second two-year bioassay; however, at this point there is no intent to use to replace the first bioassay. Another use would be as a

complementary confirmatory study for drugs with equivocal findings in a two-year study where additional data could help with risk-benefit decisions. Another use would be to set priorities for 'old' drugs with no bioassay data as to whether a two-year study is needed. Another use would be as an option to repeating an equivocal or inadequate two-year study. Another could be to provide information to enable proceeding with clinical trials in the absence of a completed two-year bioassay. And finally, a use could be to assess the carcinogenic potential of contaminants or degredants not present in a drug as tested in two-year studies.

Discussion: Dr. Henry asked whether FDA has actually used transgenic data. Dr. Schwetz responded that data from the NTP on methylphenidate and phenolphthalein have been used. Dr. Raymond Stoll, Boehringer Ingelheim, commented that a mouse two-year bioassay offers little beyond what can be gained from a rat bioassay and some transgenic assay. He opined that if having a negative transgenic assay means having to go on to do a conventional bioassay, then industry will stick with the two rodent bioassays. Dr. Henry asked whether transgenic research was going on at FDA. Dr. Daniel Casciano, NCTR/FDA, said there were efforts at NCTR and the Center for Drugs. Dr. Schwetz said that most of their efforts were on the newborn mouse assay. He also urged that we not back off from development and evaluation of mechanism-based tests simply because we don't yet know how to use them or the data generated.

K. EPA Perspective on Transgenic Models -- Dr. Vicki Dellarco, EPA, said the agency operates under 12 different laws with about 30 different provisions to set national standards for clean air and water, to regulate products by registration, define criteria for identifying risk in hazardous substances, and set cleanup standards for site remediation. She illustrated the magnitude of EPA regulatory needs by noting that under the 1990 Clean Air Act Amendments there are 189 air pollutants to be evaluated, while under the 1996 Safe Drinking Water Act Amendments there are ~400 drinking water contaminants and ~600 disinfection byproducts. Different levels of information are needed to implement statutes as in cases where quantification is required to implement a cost-benefit analysis very robust data are needed. With regard to resource considerations, Dr. Dellarco commented that if traditional bioassays were employed to test just 30 drinking water disinfection byproducts, the cost would be about $120 million. There are also time constraints, and although some laws allow considerable time before promulgation of regulatory standards, e.g., 8-10 years under the Clean Air Act, others allow much less time. Also, under the new laws there is more emphasis on obtaining mechanistic data, e.g., the Food Quality Protection Act and the Safe Drinking Water Act. Thus, Dr. Dellarco stated that new assays such as transgenics will help because approaches are needed that are cost and time effective, that can be used to prioritize and rank chemicals as to their magnitude of concern, and can be directed at questions about modes of action. In addition to transgenic assays, she noted their interest in the Medaka fish assay and the newborn mouse assay. In terms of the alternative tests, Dr. Dellarco said there are some points to consider: (1) new technologies are important from the standpoint of time and resource constraints if promising; (2) we want to promote understanding and appropriate use of these assays in cancer hazard identification; (3) we need to understand the strengths and

limitations of these new assays; and (4) we need better models for nonmutagenic agents. So should we use data from these alternative assays now? She said that we should as part of the weight of evidence, in helping to understand mode of action, and even, in some cases, in lieu of the bioassay, although with programs such as FIFRA there are statutory requirements for bioassay data. However, Dr. Dellarco said there are situations within the agency where transgenic assays can be useful right now, and she specifically cited water disinfection byproducts where relative risk or hazard information can help guide safer disinfection treatments approaches. The EPA currently has a pilot study ongoing with the NIEHS for performing bioassays on some of the most prevalent of these byproducts, and other studies will be done as well including toxicokinetics on some. In November 1998, a rule will be issued to regulate some of these byproducts with consideration of additional regulation projected for 2001. She said we hope to use assays such as the transgenics to aid in rank ordering relative potencies. In conclusion, Dr. Dellarco stated that alternative tests including transgenics provide information to be used in a weight of the evidence approach, are valuable for sorting out hypotheses about mode of action, and are needed for cost and time effective approaches.

Discussion: Dr. Peterson noted the ability of the Tg.AC to detect carcinogenicity of TCDD and DES, and suggested that this system might be useful in looking for toxic equivalence factors or estrogen equivalents. He referred to Dr. Bucher's prediction scheme that among chemicals not likely to be detected with the transgenics were halogenated alkenes and alkanes and pointed out that some disinfection byproduets fell in these classes. Dr. Dellarco responded that the short chain aliphatics may be of less concern toxicologically while the brominated derivatives may be of more concern. Dr. Bucher agreed and said that the brominated derivatives might be picked up better than the chlorinated ones. Also he reported that we were looking at an intestinal tumor model as a detection system. Dr. Frederick said the metabolism of some of these compounds is complex and suggested running an *in vivo* mutagenesis assay, such as *lac*I or micronucleus, in parallel. Dr. Dellarco thought that a good suggestion. Dr. Lucier added that we were also evaluating some of these compounds in the Medaka assay.

L. Public Comments -- Dr. Thomas O'Brien, Lankenau Medical Research Center, said he had been an NIH grantee for many years whose research interests were in the mechanisms of skin carcinogenesis and tumor promotion. Recently he and colleagues had developed a transgenic mouse model that was qualitatively different from those being evaluated by the NTP and ILSI. He said the model overexpresses the enzyme ornithine decarboxylase (ODC) in skin and epithelial tissues and was thought to be a possible system for carcinogenicity testing. The model is described as the keratin 6 gene promoter driving the ODC transgene (K6/ODC Mouse). Dr. O'Brien said the advantages of this system were that (1)it is sensitive to very low single doses of initiating genotoxic carcinogens producing multiple skin tumors because (2) the tissues are constitutively promoted, (3) genotyping is not required, and (4) the mouse model has reasonably good breeding performance. Thus, he believes this to be a good surrogate model for sensitive humans in the population. Dr. O'Brien stated that there needed to be a mechanism for providing financial support for

new transgenic and knockout model development and validation and comparison with existing models.

Discussion: Dr. Stegeman said a good question had been raised as to how the NTP goes about investigating promising new models. Dr. Lucier said the question of whether new models are needed was a major discussion point for this meeting and the NTP was certainly not locked into certain models. Dr. Rodier inquired as to the connection between ODC and a sensitive human population. Dr. O'Brien responded that many human tumors and preneoplastic tissues such as intestinal polyps express ODC activity.

Dr. David Hattan, Center for Food Safety and Applied Nutrition (CFSAN)/FDA, said that if there are major quantitative differences in the tumor data obtained between the transgenic assay and the bioassay how do you use this information in risk assessment. How do you use the data if there are qualitative differences? He said another concern was whether one might miss late onset tumors with these models

M. Further Discussion on the Transgenics -- In response to questions and concerns, Dr. Tennant said he would talk about the patent issue. DuPont holds the license to the original oncomouse patent which covers any transgenic animals created by pronuclear injection in which tumor is a phenotype. They also hold the license to the Utah patent which covers knockout technology. He said DuPont has licensed Taconic Farms to produce $p53^{def}$, Tg.AC, and perhaps other models. The models are available commercially without any restrictions. However, other models developed may be subject to patent infringement unless a license is negotiated through DuPont. The $p53^{def}$ is also available through Jackson Laboratories. Dr. Tennant said that through the early association with the Leder laboratory the NIEHS has a royalty free license for the Tg.AC. Dr. Mitsumori said his institute had no problem with the licensing arrangement and there was no charge by DuPont but regarding future commercial use as a screen he couldn't say. Dr. Lucier said we've had no problems as it should be in DuPont's best interests to have the models widely developed and used. Dr. Tennant agreed that as long as the models were in research and development there was no problem but if a model were to be syndicated for screening or for research purposes, a licensing agreement might be required by DuPont.

In other discussion, Dr. Yamasaki said that it was more and more apparent that NTP was not only perceived as a national leader but also as an international leader in development and evaluation of transgenic technology for carcinogenic hazard identification so it was important to know the direction that NIEHS was going with this initiative, including the emphasis being given to it through the extramural program. Dr. Lucier said he took those comments as a response to questions 3 and 4 posed to the Board, i.e., are existing models best utilized and are they adequate, and what new models are needed. He said the NTP position is to work in parallel developing new models, evaluating them for use in chronic bioassays or carcinogen evaluation, while making brief forays into the risk assessment area to test their utility in the regulatory and carcinogen evaluation arenas. He said we are not locked into just these three models particularly since they do not encompass all mechanisms of carcinogenesis. Dr. Lucier reported that the NIEHS issued a Request for

Applications (RFA) a couple of years ago, followed by awarding of grants to primarily academic scientists, and there recently has been a meeting where intramural and extramural scientists working in this area could share their research. Dr. Henry pointed out that these discussions have indicated that the scientific community wants to be informed on the directions and strategies for the NTP in this area. Dr. Frederick observed that the discussions about the drinking water disinfection byproducts program suggested there might be a gap in the ability of the current transgenic models to identify carcinogenic hazard with some of these chemicals.

END OF THE TRANSGENICS REVIEW

III. Report of the Director, ETP:

- Dr. Lucier said he wanted to update the Board on the state of the toxicology program which he thought was very good in attempting to utilize advances in technology and in continuing to build partnerships, as exemplified by the transgenics program. We are trying to better link science and policy.
- Dr. Lucier commented that one of the centerpieces of the Program is mechanism based toxicology, again exemplified by evaluation of the transgenic models. Another example is exploration of microarray chip technology which may make it possible to look at expression of thousands of genes simultaneously. A molecular toxicologist is being recruited and a group in that discipline will be formed.
- Dr. Lucier said that the NTP has been viewed as a rodent testing program, and we think that is not appropriate and there should be a human studies component in that our findings are ultimately used to help assess human risk from exposure to chemicals. Among studies over the past few years in molecular epidemiology at NIEHS are those of Dr. Doug Bell on genetic susceptibility markers in particular with polymorphisms in drug metabolizing enzymes, of his own laboratory on developing methods to identify dioxin sensitive genes in human samples. He reported that a staff epidemiologist was being recruited who will serve to foster interactions between toxicologists and epidemiologists which should help in setting priorities for study.
- Dr. Lucier stated that the weak link in risk assessment is often exposure assessment. He said we are in the process of developing a white paper with the Centers for Disease Control and Prevention (CDC), EPA, and possibly NIOSH in developing an overall government initiative on exposure assessment. This is important to the NTP in terms of the *Report on Carcinogens* whereby every two years an assessment has to be made of known or reasonably anticipated to be human carcinogens and we need to bolster the human exposure information for that report. This kind of information will also be useful for comparing effects doses in long and short-term toxicology and carcinogenesis studies with human levels of chemicals and/or metabolites. Dr. Lucier said that this effort was stimulated by our need to obtain body burden levels on 60 to 70 chemicals that are endocrine disruptors to give a picture of human exposures from day to day living and help set priorities for which chemicals should be studied. We now have analytical methods for most of the agents and relevant metabolites. A workshop is planned tentatively for July to help further develop this initiative.

- Dr. Lucier said that a new Office of Nomination and Selection had been formed headed by Dr. H.B. Matthews, and the person being recruited to work in exposure assessment will work out of that office.
- Dr. Lucier reported that risk assessment methodology has been a major initiative during the 90s under Dr. Cristopher Portier with the emphasis being on bringing the best biological information available to developing biologically based models for risk assessment.
- Dr. Lucier listed and discussed a number of high priority agents being studied: (1) electric and magnetic fields (EMF) - several workshops have been held or will be held soon to deal with clinical, epidemiological, *in vitro*, and *in vivo* animal studies culminating in a June workshop to prepare a comprehensive report which will form the basis for a report to Congress by Dr. Olden; (2) phenolphthalein - transgenic mouse data was provided to FDA to aid in their assessment of risk; (3) drinking water disinfection byproducts, dioxins and estrogens - we are working closely with EPA to help provide data to meet their mandates; (4) natural products - we are planning a workshop on dietary supplements for September; and (5) malformed frogs and *Pfiesteria* - we are involved with these highly visible ecological issues which impinge on human health, especially the *Pfiesteria* outbreaks. In response to a query by Dr. Peterson, Dr. Lucier gave an update on the malformed frogs investigation, noting that such malformations have been reported now in 31 states. The NTP is still trying to identify a possible causative agent in the pond and well water samples that have been analyzed. Dr. Peterson asked whether there had been an increase in birth defects reported. Dr. Lucier responded that Minnesota does not have a birth defects registry so we had not been able to determine this.
- Dr. Lucier spoke of the Center for Evaluating Reproductive Risks that will provide objective narrative evaluations of human health risk and will be headed by Dr. Michael Shelby. We are still in the process of establishing the Center.
- Dr. Lucier commented on the NTP Newsletter, asked whether Board members were receiving it, and requested their feedback on it and the information included.

IV. Reports of Committee Activities:

A. Report on Carcinogens Subcommittee -- Dr. C.W. Jameson, NIEHS, stated that the basis for an annual report on carcinogens was established in 1978 by Section 301 (b) (4) of the Public Health Service Act which stipulated that the Secretary, DHHS, shall publish an annual report which contains: A list of all substances (i) which either are known to be human carcinogens or may reasonably be anticipated to be human carcinogens; and (ii) to which a significant number of persons residing in the United States are exposed. The act was amended in 1993 to provide for a biennial report which is now called the *Report on Carcinogens*. Dr. Jameson reviewed the criteria for listing agents, substances or mixtures in the *Report* as revised in 1996. Among changes in the criteria at that time was to provide for the consideration of mechanistic information for listing or delisting a substance, agent or mixture. He said the revised criteria were used in the peer review of agents, substances and mixtures to be listed in the Eighth *Report* which was recently submitted to the Secretary and should be approved and published in the spring of 1998. Dr. Jameson said he wanted to brief the Board on the status of nominations for the Ninth *Report*

which is scheduled for publication in 1999. He described the nomination review process which includes an initial scientific peer review of nominations by an NIEHS review group (RG1), followed by peer review by the NTP Executive Committee's Working Group (RG2), and then external peer review by the Board's Report on Carcinogens Subcommittee. He described the 14 agents, substances, mixtures and classes reviewed by the three groups in 1997. Dyes metabolized to benzidine, smokeless tobacco, strong inorganic acid mists containing sulfuric acid, tamoxifen, and tobacco smoking were recommended by all three groups for listing as **known to be a human carcinogen**. UV radiation was recommended by RG1 and the Board Subcommittee for listing as **known to be a human carcinogen**, and RG2 recommended deferral of action to address the full spectrum of UV radiation. Chloroprene, phenophthalein, tetrafluoroethylene, and trichloroethylene were recommended by all three groups for listing as **reasonably anticipated to be a human carcinogen**. 1,3-Butadiene, cadmium and cadmium compounds, and 2,3,7,8-tetrachlorodibenzo-*p*--dioxin (TCDD) are already listed in the *Report* as **reasonably anticipated,** and have been recommended for upgrading to **known** by all three groups. Saccharin had been petitioned for delisting from the *Report* which was supported by RG1 and RG2, while the Board Subcommittee recommended that saccharin remain listed in the *Report* as **reasonably anticipated to be a human carcinogen**. The nominations with the recommendations of the three review groups will be announced in the *Federal Register* with a request for final public comment. The recommendations along with public comments will be presented to the NTP Executive Committee. The recommendations of the three scientific peer review committees and the NTP Executive Committee will be given to Dr. Olden for his decision. Dr. Jameson described the 11 nominations to date to be reviewed in 1998, also for inclusion in the Ninth *Report*. They are crystalline silica, methyl *tert* -butyl ether, diesel particulates, environmental tobacco smoke, alcoholic beverages, ethylene oxide, isoprene, boot and shoe manufacturing (worker exposure circumstance), nickel refining (worker exposure circumstance), and nickel and nickel compounds. Ethyl acrylate was petitioned for delisting from the *Report* .

B. Technical Reports Review Subcommittee -- Dr. Rick Hailey, NIEHS, reviewed the findings from the December 9-10 meeting of the Subcommittee at which the draft Technical Reports for long-term toxicology and carcinogenesis studies on 10 chemicals were peer reviewed. As described in the previous days review of the NTP transgenics program, prospective transgenic studies, Tg.AC and/or $p53^{def}$, had been performed on most of the chemicals. Dr. Hailey reported on the findings with diethanolamine and the three fatty acid condensates noting that the degree of tumor response in the condensates correlated directly with the unreacted free diethanolamine content. Thus, the tumor responses in the coconut oil and lauric acid condensates were primarily associated with the free diethanolamine. The other reports reviewed were on 1-chloro-2-propanol, furfuryl alcohol, isobutene, isoprene, pentachlorophenol, and pyridine. Dr. Hailey announced studies to be reported and peer reviewed in 1998. On March 11, toxicology and carcinogenesis studies of 60 Hz electric and magnetic fields (EMF) in $B6C3F_1$ mice and F344/N rats will be reviewed, and as well DMBA initiation/magnetic field (50 and 60 HZ) studies in female Sprague Dawley rats. Dr. Hailey said that four toxicology and

carcinogenesis studies were scheduled for the fall of 1998 (October 30). The chemicals are ethylene glycol monobutyl ether, glutaraldehyde, methyleugenol, and oxymetholone.

C. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the NTP Interagency Center for the Evaluation of Alternative Toxicology Methods -- Dr. William Stokes, NIEHS, said the activities and the processes that these two initiatives would operate under were reviewed at a public meeting on November 7, 1997, at NIEHS, and he would summarize some of the information that was presented then and comments made. Dr. Stokes stated that there were opportunities for improved predictions of toxicity for risk assessment purposes by incorporating new science and technology, for improved efficiency in terms of cost and time, and for improved animal welfare through refinement of procedures to reduce pain and distress, through reduction of the number of animals used, and through replacement with non-animal methods or lower phylogenetic species. Dr. Stokes said ongoing NTP efforts in this area were strengthened by the mandate provided by Public Law 103-43 in 1993. He said his talk would focus on two of the directives from this law: (1) to establish criteria for validation and regulatory acceptance of alternative methods; and (2) to develop a process for regulatory acceptance of alternative methods. He briefly reviewed the process leading to a final ICCVAM report and establishment of a permanent ICCVAM, beginning with the formation of the *ad hoc* ICCVAM in 1994. Dr. Stokes said the final report has three chapters: one on validation criteria, a second on regulatory acceptance, including criteria and 29 recommendations relating to a regulatory acceptance process; and a chapter on future directions and implementation. He displayed a flow chart of the process by which new methods evolve beginning with identifying the need for a new method all the way through to regulatory acceptance and implementation by users. The permanent ICCVAM has been meeting since May 1997. The committee, which has representatives from 14 Federal regulatory and research agencies and receives operational support from NIEHS, will be primarily concerned with methods of multiagency interest. Interagency working groups will coordinate reviews of a new method and provide recommendations to the agencies. However, regulatory acceptance of a new method would remain under the purview of each agency in relation to their regulatory mandates. Dr. Stokes discussed the NTP Interagency Center for the Evaluation of Alternative Methods which will be located at the NIEHS, will support ICCVAM operations, conduct peer reviews and workshops (from 3 up to 9/year), serve to disseminate information, and serve as a center for communication with stakeholders such as industry. The peer review panels will be comprised of national/international experts, meetings will be announced in the *Federal Register* and be open for public comment. The peer review panels will be charged with developing scientific consensus on the usefulness of test methods for specific human health or ecological risk assessment purposes, with the product being a peer review report. ICCVAM workshops will be held to evaluate the adequacy of current methods, to evaluate the validation status of methods, and to identify needed research, development, and validation studies. Workshop recommendations would be made available to appropriate agencies or organizations outside the government. Dr. Stokes discussed the Federal Advisory Committee on Alternative Toxicological Methods, which will have representation from academia, industry and public interest organizations as well as liaison with

international organizations. The Committee will provide advice on Center/ICCVAM activities including processes, priorities, peer reviews and workshops, and advice on fostering public-private partnerships. He showed a flow diagram of how the Committee and the Center will function to communicate with test sponsors, hopefully early in the test method development process so that the best and most complete information about a method can be provided to a regulatory agency to aid them in deciding whether or not to accept a method. Dr. Stokes provided an update on current Center/ICCVAM activities. He said a peer review of the Mouse Local Lymph Node Assay was planned for late spring or early summer. An ICCVAM working group has been established composed of immunotoxicologists from various agencies. Corrositex® is an *in vitro* method for assessing dermal corrosivity and a peer review of that is projected for later in the year in response to requests from CPSC and EPA. Several members of the committee have been involved in reviewing a proposed OECD test guideline on *in vitro* percutaneous absorption methods. Future activities may include evaluations of the Frog Embryo Teratogenesis Assay in *Xenopus* (FETAX), transgenic models, and test methods being considered for the endocrine disruptor screening and testing program at EPA.

Discussion: Dr. Peterson suggested the consideration of egg laying vertebrates such as fish and amphibians as alternative species for test methods. His concern was that the peer review panels would not have individuals with expertise in this area and thus, these species might be overlooked. Dr. Stegeman commented that the major impetus for finding alternative tests derived from drug and cosmetic industry testing procedures, with the Draize oocular irritation test in rabbits being a prime example. He asked for comment on the NTP's use of fish species, e.g., Medaka, in carcinogenesis testing. Dr. Stokes said the Medaka and FETAX systems were being intensively evaluated in collaboration with EPA. He noted the NIEHS Marine and Freshwater Biomedical Research Centers program with regard to evaluating the use of fish species. Dr. Peterson said another species of value were zebra fish. Dr. Stokes reported that an NIEHS-sponsored workshop on aquatic models, including zebra fish, would be held in Research Triangle Park, April 20-21.

V. Concept Review:

Pathology Support for the National Toxicology Program -- (Attachment 3) Dr. Ronald Herbert,NIEHS, presented the concept, and Dr. Henry, Board Member, served as principal reviewer. Dr. Herbert said that under the mandates of the NTP large amounts of pathology materials are generated both through contract and inhouse studies and pathology support is needed to ensure high quality of pathology data, to ensure accuracy and consistency, to provide capabilities for conducting supplemental studies, and to provide support for other investigators in the NIEHS intramural research program. Types of more routine support include necropsy assistance, histology and histopathological evaluation, while more sophisticated support includes application of special procedures, e.g., immunohistochemistry, and application of cellular and molecular biology techniques. In addition, support for the peer review process is provided. Dr. Herbert said there are two level of effort contracts with a duration of five years due to expire in September 1999.

Dr. Henry stated that this obviously is a very important contract operation for the success of the Program. Her major comment was that with the increasing need for application of cellular and molecular biology techniques, those issues need to be highlighted to make them criteria for responders to the RFP. Dr. Henry moved that the concept proposal be approved. Dr. Hooper seconded the motion. In discussion, Dr. Frederick agreed that the molecular biology aspects be emphasized and saw some of this becoming more routine in future years. Dr. Stegeman said we should not undervalue collection of pathology materials as an important aspect of this effort. Dr. Bucher commented that most of the newer techniques have been developed and/or evaluated in-house, which serves as a form of quality control. Dr. Maronpot said that most contractors can't afford to have staff to perform all of the newer techniques so the capability is built into the RFP for awarding of subcontracts for some of these tasks. The motion was approved unanimously by the Board.

VI. Chemicals Nominated and Reviewed for NTP Study by the Interagency Committee for Chemical Evaluation and Coordination (ICCEC) on August 15 and December 11, 1997:
Dr. H.B. Matthews, NIEHS, noted that one of the NTP goals is to provide toxicological evaluation on substances of public health concern. Accordingly, the purpose of the NIEHS/NTP Office of Nominations, which he heads, is to identify, select and recommend the most relevant substances for study by the NTP. He said that we have become proactive in recent years in seeking quality nominations from all sectors of society. In the past a majority of nominations tended to come from other agencies, particularly the NCI. Dr. Matthews went through the process leading to selection for study noting the Board's role as part of the opportunity for public comments. Dr. Matthews then proceeded to present each of the chemicals or mixtures recommended for study by the ICCEC (Attachment 4, A). With regard to asphalt fumes, Dr. Bucher noted that this project will be conducted under an Interagency Agreement between NIEHS and NIOSH and that identifying biomarkers will be an important aspect. Dr. Douglas Sharpnack, NIOSH, said there had been no chronic inhalation studies done with asphalt fumes. Dr. Henry said the asphalt industry is part of the petroleum industry and there will be much activity this year concerned with asphalt fumes including a major symposium at the Society of Toxicology meeting. A major issue will be trying to reproduce fume composition in the laboratory as it occurs in the field. Several of the chemicals recommended for study were medicinal herbal supplements and a rationale for study was because of extensive human exposure. Dr. Matthews then presented chemicals for which no further study was recommended (Attachment 4, C). There was considerable discussion around one of these chemicals, *trans*- 1,4-dichloro-2-butene, which was recommended for no further testing because there was an adequate industry study showing it to be a carcinogen. The question had to do with whether the carcinogenesis data were in suitable form or had sufficient peer review to support nomination for listing in the *Report on Carcinogens* . Though they were not discussed in the meeting, a listing of chemicals recommended for deferral pending receipt of additional information was provided in Attachment 4, B.

Discussion: There was a generic discussion about the nomination and selection process. Dr. Hooper asked how labor and public interest groups get input into the process. Dr.

Matthews responded that Dr. Frank Mirer, a Board member currently and in the past who was not present, has provided considerable input through his affiliation with the UAW and some of these nominations have been accepted and are under study. Dr. Lucier said we try to identify contact persons and have reached out to the various state health agencies seeking nominations. Dr. Matthews asked Dr. Hooper for his suggestions on persons that we might not be reaching. Dr. Rodier thought there was too much emphasis on nominations for cancer testing and not enough for other toxicity endpoints.

[Billing Code 4140-01-P]

Public Health Service

National Toxicology Program

Board of Scientific Counselors' Meeting

Pursuant to Public Law 92-463, notice is hereby given of a meeting of the National Toxicology Program (NTP) Board of Scientific Counselors, U.S. Public Health Service, in the Conference Center, Building 101, South Campus, National Institute of Environmental Health Sciences (NIEHS), 111 Alexander Drive, Research Triangle Park, North Carolina, on February 5 and 6, 1998.

Agenda

The meeting will be open to the public from 8:45 a.m. to 4:00 p.m. on February 5, and from 8:45 a.m. to adjournment on February 6, with attendance limited only by space available. The primary agenda topic on February 5 will be a comprehensive evaluation of the strategies for use of transgenic mouse models in bioassays for carcinogenesis. Included will be an introduction, history and rationale for using transgenic animals to identify carcinogens, sharing of results from three mouse models -- $p53^{def}$, Tg.AC, and *rasH2 --,* NTP strategies for evaluating models, utility of transgenic model results for risk assessment, and regulatory agency perspectives. There are four issues that the Board will be asked to advise the NTP on, being: (1) Is the NTP approach to evaluation and validation of transgenic models for use in cancer bioassays sufficient and appropriate?;?; (2) How can existing models be best utilized? What are their limitations?; (3) What new models are needed, i.e., should the NTP seek to develop organ-specific tumor models; and (4) Are the scientific needs of regulatory agencies being adequately addressed? Background materials pertaining to the evaluation of transgenic

models will be available on request from the Executive Secretary after January 12, 1998.

Among several agenda topics on February 6 will be a discussion of and opportunity for public comment on chemicals nominated for NTP studies that were reviewed by the NTP Interagency Committee for Chemical Evaluation and Coordination on August 15, 1997, and December 11, 1997. The Committee recommended nine chemicals and four mixtures for toxicological studies, recommended four chemicals be deferred for additional information, and recommended six chemicals not be studied. The chemicals/classes with CAS Nos. in parentheses are -- Recommended for Study: (1) 2-Acetylpyridine (1122-62-9); (2) Asphalt Fumes (8052-42-4); (3) 2-Chloropyridine (109-09-1); (4) Comfrey (72698-57-8) with Symphytine (22571-95-5); (5) Glycoluril (496-46-8); (6) Goldenseal (--) containing Berberine (2086-83-1) and Hydrastine (118-08-1; (7) Luminol (*o*--Aminophthalic Hydrazide) (521-31-3); (8) 4-Methoxy-N-methyl-1,8-naphthalimide (3271-05-4); (9) Myristicin (607-91-0); (10) 7-(2H-Naphthol[1,2-d] triazol-2-yl)-3-phenylcoumarin; (11) Orthanilic Acid (88-21-1); (12) Phenothiazine (92-84-2); and (13) Saw Palmetto containing β-Sitosterol (83-46-5). The chemicals for which No Study is Recommended are: (1) *trans*- 1,4-Dichloro-2-butene; (2) Dicyclopentadiene (77-73-6); (3) C.I. Direct Black 80 (8003-69-8); (4) Ethyl Cyanoacrylate (7085-85-0); (5) Isoamyl Acetate; and (6) 2,4,6-Tribromophenol. Chemicals Deferred for Additional Information are: (1) 3-Amino-5-mercapto-1,2,4-triazole (16691-43-3); (2) Diethylamine (109-89-7); (3) Isopropylamine (75-31-0); and (4) Triethylamine (121-44-8).

Also, on February 6 will be reports of recent meetings of the Report on Carcinogens and Technical Reports Review Subcommittees. The Board will review concept proposals on (1) genetic susceptibility of the pregastrulation embryo to

environmental exposures, (2) molecular detection of aneuploidy in rodent germ cells, and (3) pathology support for the National Toxicology Program.

Public Input Encouraged

In order to facilitate planning for the meeting, persons wanting to make a formal presentation during the public comment period must notify the Executive Secretary, Dr. Larry G. Hart, P.O. Box 12233, Research Triangle Park, NC 27709 (telephone 919/541-3971; FAX 919/541-0295; or email at hart@niehs.nih.gov) by no later than February 2, 1997, and, if possible, provide a written copy in advance of the meeting. Written statemenmts should supplement and may expand on the oral presentation, or may be submitted in lieu of an oral presentation, and should be received by February 2 so copies can be made for distribution to Subcommittee members, staff, and the public. Oral presentations should be limited to no more than five minutes.

The Executive Secretary will furnish agenda and a roster of Board members and *ad hoc* expert reviewers prior to the meeting. Summary minutes subsequent to the meeting will be available upon request.

Dated:

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Samuel H. Wilson, M.D.

Deputy Director

National Toxicology Program

AGENDA
NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS

February 5-6, 1998

Building 101, Conference Center, South Campus
National Institute of Environmental Health Sciences (NIEHS)
Research Triangle Park, North Carolina

February 5

8:45 - 9:00 a.m	Welcome and Director's Report	Dr. K. Olden, NIEHS
9:00 - 9:15 a.m.	Introduction - purpose of meeting	Dr. G. Lucier, NIEHS
9:15 - 9:45 a.m.	History and rationale for using transgenic animals to identify carcinogens	Dr. R. Tennant, NIEHS
9:45 - 10:15 a.m.	Studies with the p53^{def} mouse model	Dr. J. French, NIEHS
10:15 - 10:35 a.m.	*Break*	
10:35 - 10:55 a.m.	Studies with the Tg.AC mouse model	Dr. J. Spalding, NIEHS
10:55 - 11:20 a.m.	Molecular and cellular Tg.AC studies	Dr. R. Cannon, NIEHS
11:20 - 11:50 a.m.	Results of NTP transgenic evaluations	Dr. W. Eastin, NIEHS
11:50 - 12:50 p.m.	*Lunch*	
12:50 - 1:30 p.m.	Studies with the *ras*H2 mouse model	Dr. R. Maronpot, NIEHS Dr. K. Mitsumori, National Institute of Health Sciences, Tokyo
1:30 - 2.00 p.m.	Summary of model studies evaluation	Dr. J. Bucher, NIEHS
2:00 - 2:30 p.m.	Transgenic studies and risk assessment	Dr. C. Portier, NIEHS
2:30 - 2:50 p.m.	*Break*	
2:50 - 3:10 p.m.	FDA perspective on transgenic models	Dr. J. Contrera, FDA
3:10 - 3:30 p.m.	EPA perspective on transgenic models	Dr. V. Dellarco, EPA
3:30 - 4:00 p.m.	Public comments	

February 6

8:45 - 9:20 a.m.	Report of the Director, ETP	Dr. G. Lucier
9:20 - 10:00 a.m.	Reports of Committee Activities:	
	- Report on Carcinogens Subcommittee	Dr. C. Jameson, NIEHS
	- Technical Reports Review Subcommittee	Dr. R. Hailey, NIEHS
	- Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) - Meeting to Discuss NTP Center for the Evaluation of Alternative Toxicological Methods	Dr. W. Stokes, NIEHS
10:00 - 10:20 a.m.	*Break*	

10:20 - 10:40 a.m.	Concept Review - Pathology Support for the National Toxicology Program	Dr. R. Herbert, NIEHS
10:40 - 11:50 a.m.	Chemicals Nominated and Reviewed For NTP Study by the Interagency Committee for Chemical Evaluation and Coordination (ICCEC) on August 15 and December 11, 1997	Dr. H. Matthews, NIEHS
Adjournment		

1/26/98

Eula Bingham, Ph.D. (6/99)
Professor
Department of Environmental Health, M.L. 056
University of Cincinnati
College of Medicine
Cincinnati, OH 45267-0001
(Occupational and Environmental Health)

James S. Bus, Ph.D. (6/99)
Technical Director
Health & Environmental Sciences
Dow Chemical Company
1803 Building
Midland, MI 48674
(Toxicology, Chemical Disposition)

Gary P. Carlson, Ph.D. (6/99)
Professor
School of Health Sciences
Purdue University Building
1338 Civil Engineering
West Lafayette, IN 47906-1338
(Pharmacology, Toxicology, Chemical Metabolism)

Susan Fischer, Ph.D. (6/00)
Professor of Carcinogenesis
The University of Texas
M.D. Anderson Cancer Center
Science Park - Research Division
Box 116
P.O. Box 389
Smithville, TX 78957
(Experimental Carcinogenesis)

Clay Frederick, Ph.D. (6/00)
Senior Research Fellow
Mechanistic Toxicology Group
Toxicology
Rohm and Haas Company
727 Norristown Road
Spring House, PA 19477
(Toxicology, Animal Models)

George Friedman-Jimenez, M.D., DrPH. (6/98)
Director, School of Public Health
HHC/Bellevue Occupational & Env. Medicine Clinic
Bellevue Hospital, Room CD349
462 First Avenue
New York, NY 10016-9198
(Occupational Epidemiology, Environmental Justice)

Carol J. Henry, Ph.D. (6/97)
Director
Health and Environmental Science Department
American Petroleum Institute
1220 L Street, NW
Washington, DC 20005-4070
(Toxicology, Risk Assessment)

Nicholas K. Hooper, Ph.D. (6/00)
Head, Research & Methods Development
Hazardous Materials Laboratory
Department of Toxic Substances Control
California Department of Health Services
2151 Berkeley Way, Annex 11
Berkeley, CA 94704
(Cancer, Genetic Hazard Assessment)

Franklin E. Mirer, Ph.D. (6/97) *
Director
Health and Safety Department
UAW International
8000 East Jefferson Avenue
Detroit, MI 48214-2699
(Toxicology, Occupational Health)

John J. Mulvihill, M.D. (6/98)
Professor of Human Genetics
University of Pittsburgh
Graduate School of Public Health
A 302 Crabtree Hall
Pittsburgh, PA 15261-0001
(Genetic Epidemiology)

Curtis Parker, Ph.D. (6/00)
Professor and Chairman
Department of Anatomy
Morehouse School of Medicine
9720 Westview Drive
Atlanta, GA 30310
(Developmental Biology)

Richard E. Peterson, Ph.D. (6/99)
Professor, Div of Pharmacology & Toxicology
School of Pharmacy
University of Wisconsin
425 North Charter Street
Madison, WI 53706
(Reproductive & Developmental Toxicology)

Patricia M. Rodier, Ph.D. (6/00)
Senior Scientist, Dept of OB/GYN, Box 668
University of Rochester Medical Center
601 Elmwood Avenue
Rochester, NY 14642
(Developmental Neuroscience)

John J. Stegeman, Ph.D. (6/97) **(Chair)**
Senior Scientist
Biology Department
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
(Alternatives, Xenobiotic Metabolism)

Expert Consultants

Hiroshi Yamasaki, Ph.D. (6/98)
Chief, Unit of Multistage Carcinogenesis
International Agency for Res on Cancer
150 Cours Albert-Thomas
69372 Lyon Cedex
FRANCE
(Experimental Carcinogenesis)

Dr. William Farland
Director Office of Health
and Environmental Assessment (RD-689)
Office of Research and Development
U.S. Environmental Protection Agency
Mail Drop 8601
401 M Street, S.W.
Washington, D.C. 20460-0003

Dr. Michael Lieberman
Chairman, Department of Pathology
Baylor College of Medicine
Department of Pathology
1 Baylor Plaza
Houston, TX 77030

Dr. James MacDonald
Senior Vice President
Drug Safety and Metabolism
Schering-Plough Research Institute
2015 Galloping Hill Road
Kenilworth, NJ 07033-0539

Dr. Bernard Schwetz
Director
National Center for Toxicological Research
Food and Drug Administration
FDA HF-32
Room 17-35
Parklawn Building
5600 Fishers Lane
Rockville, MD 20857

Environmental Toxicology Program
Division of Intramural Research
National Institute of Environmental Health Sciences

CONCEPT. REVIEW
Prepared for:
National Toxicology Program
Board of Scientific Counselors
February 6, 1998

CONCEPT REVIEW
National Toxicology
Program
Board of Scientific
Counselors

02/06/98

Table of Contents

Background on Concept Reviews

Title:	Pathology Support for the National Toxicology Program
Presenter:	Dr. R.A. Herbert
Primary Reviewer:	Dr. C. Henry

BACKGROUND ON CONCEPT REVIEWS

NTP contracts, interagency agreements, and grants support a variety of activities—toxicologic characterization, testing, methods development, and program resources (i.e., chemistry, occupational health and safety, animal production, pathology, quality assurance, archives, etc.).

Prior to issuance of a Request for Proposal (RFP) or a Request for Application (RFA), a project concept review is required. These project concepts in many instances may consist of more than one contract, interagency agreement, or grant. Concept reviews are needed for new projects, recompetitions with changes in statements of work, and projects ongoing for five years or more since the last concept review.

The project concept reviews are conducted by the NTP Board of Scientific Counselors and are open to the public so long as discussions are limited to review of the general project purposes, scopes, goals, and various optional approaches to pursue the overall program objectives. The meeting will be closed to the public, however, if the concept discussions turn to the development or selection of details of the projects or RFPs/RFAs, such as specific technical approaches, protocols, statements of work, data formats, or product specifications. Closing the session is intended to protect the free exchange of the advisory group members' opinions and to avoid premature release of details of proposed contract projects or RFPs/RFAs.

The Board members are asked to review the project concepts for overall value and scientific relevance as well as for fulfilling the program goal of protecting public health. Specific areas should include:

a. scientific, technical or program significance of the proposed activity;

b. availability of the technology and other resources necessary to achieve required goals;

c. extent to which there are identified, practical scientific or clinical uses for the anticipated results; and

d. where pertinent, adequacy of the methodology to be used in performing the activity.

NATIONAL TOXICOLOGY PROGRAM CONCEPT PROPOSAL

Contract Title: Pathology Support for the National Toxicology Program

Presenter: Ron A. Herbert, Laboratory of Experimental Pathology, Environmental Toxicology Program

Objectives: The objective of this contract is to provide a broad range of pathology support for studies conducted by the National Toxicology Program and by in-house investigators at NIEHS, as well as for supplemental studies on pathology specimens generated through contracted studies. The support will include: 1) necropsy assistance, 2) tissue processing and slide preparation, 3) histopathological evaluation, 4) the application of specialized qualitative and quantitative morphological procedures such as immunohistochemistry, morphometrics, cell proliferation and apoptosis, 5) the adaptation, development, refinement and application of new techniques in cellular and molecular biology, 6) peer review and assessment of pathology evaluations from NTP toxicity/carcinogenicity studies performed by contractors through NTP Pathology Working Group reviews, and 7) providing technical support as needed for quality assessment of pathology evaluations and Pathology Working Group reviews.

Concept Statement: Studies designed to characterize the toxicity and carcinogenicity of chemicals, and biological or physical agents are conducted through contracts or at NIEHS under the auspices of the NTP. These studies provide important data for risk assessment relating to potential human exposure to toxic substances in the environment. A program of the magnitude and diversification of the NTP requires the cooperation and collaboration of numerous testing facilities. For these studies, there is a need to assure uniformity, consistency, accuracy of diagnostic criteria and pathology procedures. This is accomplished through a variety of pathology tasks which are performed prior to, during, and after study completion. Furthermore, as study results become available, there is often need for additional studies to further define the toxicity or carcinogenicity and mechanisms involved. This may include additional routine gross and/or histopathological evaluations, or the application of specialized procedures such as immunohistochemistry, electron microscopy, morphometrics, or the measurement of cell replication and apoptosis. In addition, NTP studies conducted by in-house NIEHS investigators frequently require routine and/or specialized pathological support and evaluations before the studies can be completed. This contract will provide critical support for the timely completion of the pathology portion of NTP studies where technical assistance, microscopic review and additional pathology evaluations must be performed before final evaluation of the studies can be completed and the studies reported.

Proposed Changes To The Current Work Statement: The work to be performed under the proposed five-year recompetition is essentially the same as described above. However, it is estimated that additional technical effort will be required to handle an increased workload for NIEHS histology laboratory support and for newer techniques in cellular and molecular biology such as immunohistochemistry, apoptosis, quantitation of cell replication and apoptosis, and the application of *in situ* techniques such as polymerase chain reaction (PCR) to detect molecular events in tissues.

Table A. Chemicals recommended for testing.

Chemical	Nomin. by	Testing for	Reason for selection
Asphalt fumes [8052-42-4]	NIOSH	toxicity studies; immunotoxicity; lung irritation and function; identify biomarkers of exposure	high worker and public exposure; need to identify potential human health effects and biomarkers
Luminol [521-31-3]	Private indivs.	toxicity; carcinogenicity	widely used and uses increasing; large numbers of individuals exposed; insufficient toxicity data available
Orthanilic acid [88-21-1]	NIEHS	short-term toxicity studies	potential for extensive worker and consumer exposure; insufficient toxicity data available
Phenothiazine [92-84-2]	NIEHS	carcinogenicity	extensive worker exposure; potential for wide-spread consumer exposure; present in human and veterinary drugs
2-Acetylpyridine [1122-62-9]	NCI	carcinogenicity	potential for human exposure; suspicion of carcinogenicity
2-Chloropyridine [109-09-1]	NCI	dermal carcinogenicity in transgenic mice	increasing production; occupational and environmental exposure
Comfrey [72698-57-8] **Symphytine** [22571-95-5]	NIEHS	reproductive and developmental toxicity; carcinogenicity	extensive use as a herbal supplement and medicinal; presence of pyrrolizidine alkaloids
Glycoluril [496-46-8]	NCI	in vitro and in vivo nitrosation studies	moderate production; potential human exposure; potential to form nitrosamides
Goldenseal **Berberine** [2086-83-1] **Hydrastine** [118-08-1]	NIEHS	reproductive and developmental toxicity; carcinogenicity	extensive use as a herbal supplement and medicinal; presence of active alkaloids
4-Methoxy-N-methyl-1,8-naphthalimide [3271-05-4]	NCI	chemical disposition	occupational and extensive consumer exposure
Myristicin [607-91-0]	NCI	genetic toxicity; metabolism; carcinogenicity in transgenic animals	widespread natural product; extensive consumer exposure; similarity to known carcinogen safrole
7-(2H-Naphthol[1,2-d]triazol-2-yl)-3-phenylcoumarin [3333-62-8]	NCI	chemical disposition	moderate production; extensive occupational and consumer exposure
Saw palmetto **b-Sitosterol** [83-46-5]	NIEHS	carcinogenicity; multigeneration reproductive toxicity	widely used herbal remedy; contains active sitosterols

Table B. Chemicals deferred pending receipt of additional information.

Chemical	Nomin. by	Testing for	Information needed
5-Amino-5-mercapto-1,2,4-triazole [16691-43-3]	NIEHS	toxicity; carcinogenicity	production, use, and exposure information through the EPA-ITC
Diethylamine [109-89-7]	NIEHS	carcinogenicity	production, use, and exposure information through the EPA-ITC
Isopropylamine [75-31-0]	NIEHS	carcinogenicity	production, use, and exposure information through the EPA-ITC
Triethylamine [121-44-8]	NIEHS	carcinogenicity	production, use, and exposure information through the EPA-ITC
Benzothiazole [95-16-9]	NCI	carcinogenicity	information on other benzothiazoles tested by the NTP
Ethyl silicate [78-10-4]	NCI	subchronic, genetic, and developmental toxicity	request voluntary submission of production, use, and toxicity data through the EPA-ITC
b-Citronellol [106-22-9]	NCI	mechanistic studies; metabolism; carcinogenicity; mutagenicity	await results from testing citral and b-myrcene because of similarity of structures
Linalool [78-70-6]	NCI	mechanistic studies; metabolism; carcinogenicity; mutagenicity	await results from testing citral and b-myrcene because of similarity of structures
Methylal [109-87-5]	NCI	metabolism and disposition	production; use; exposure, and health effects data through the EPA-ITC
Methylolurea class study **Methylolurea** [1000-82-4] **Dimethylolurea** [140-95-4] **Dimethylolurea dimethyl ether** [147-07-1] **1,2-Dimethylol-4,5-dihydroxyethyleneurea** [1854-26-8]	NIEHS	toxicity; carcinogenicity	determination of why 1,2-dimethylol-4,5-dihydroxyethyleneurea was not tested for carcinogenicity by the NTP after its approval in 1980

Table C. Chemicals for which no further testing is recommended.

Chemical	Nomin. by	Testing for	Reason for recommendation
Dicyclopentadiene [77-73-6]	NCI	reproductive toxicity; carcinogenicity	no adverse effects in teratology studies; not mutagenic; low potential for human exposure
C.I. Direct Black 80 [8003-69-8]	NCI	carcinogenicity by dermal route	only 1.3% of applied dose is absorbed through skin
Ethyl cyanoacrylate [7085-85-0]	NCI	inhalational neurotoxicity; carcinogenicity; reproductive and developmental toxicity	rapidly polymerizes in presence of water; stable vapor or aerosol cannot be generated
Isoamyl acetate [123-92-2]	NIEHS	general toxicity; neurotoxicity; carcinogenicity	readily hydrolyzed in blood to isoamyl alcohol, which has been studied, and acetic acid

Table D. Chemicals that will not be tested.

Chemical	Nomin. by	Testing for	Reason for recommendation
***trans*-1,4-Dichloro-2-butene** [110-57-6]	NIEHS	carcinogenicity	adequate industry carcinogenicity studies exist
2,4,6-Tribromophenol [118-79-6]	NIEHS	carcinogenicity	low production; little exposure to the population; little chance for bioaccumulation