

NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS MEETING
OCTOBER 31 AND NOVEMBER 1, 1984

Summary Minutes

The National Toxicology Program (NTP) Board of Scientific Counselors met on October 31 and November 1, 1984, in the Conference Center, Building 101, South Campus, 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 and Expert Consultants). Members of the Board are Drs. Mortimer Mendelsohn (Chairperson), Norman Breslow, Leila Diamond, Curtis Harper, Jerry Hook, Jeanne Manson, Henry Pitot, and James Swenberg. All were in attendance.

Review of NIEHS/NTP Cellular and Genetic Toxicology Branch Programs
(Attachment 3)

I. Overview: Dr. Raymond Tennant, Branch Chief, described the organizational structure of the Toxicology Research and Testing Program (TRTP), the NIEHS component of the NTP. The Cellular and Genetic Toxicology Branch (CGTB) is one of five branches within the TRTP. He stated that the ultimate goal of the Branch was to identify and characterize chemical mutagens and carcinogens. The specific aims were: (1) to develop reliable and sensitive systems for analyzing various endpoints of genetic toxicity; (2) to apply these systems to actual testing for genetic toxicity; (3) to analyze responses obtained for predictability of carcinogenesis and other diseases; and (4) to maintain a state of the art research program which addresses mechanisms of inheritance, genetic stability and processes which are the causes of genetic changes. Dr. Tennant noted that there was an inadequate data base across chemical classes and test systems, and for non-carcinogens in any test system. Thus, the CGTB is attempting to ensure fidelity in the large test data base it is generating by use of coded compounds, interlaboratory comparisons with the same chemicals, in-depth auditing of results, and frequent site visits to the testing laboratories.

Functionally, the Branch is divided into three areas; one being concerned with somatic cell effects especially as they relate to carcinogen identification; secondly, a similar effort in identifying and evaluating germ cell mutagens; and thirdly, conducting an inhouse basic research program looking at underlying mechanisms. The first area is conducted primarily through contracts and interagency agreements, covering about 40 projects. The Salmonella mutagenesis test is the core of the system based on its reliability and large data base. He briefly described the other types of assays being developed or used in a testing mode. Dr. Tennant discussed how the data base from the two-year toxicology and carcinogenesis studies in F344 rats and B6C3F₁ mice was used in comparison of test results from short-term genetic toxicology assays and as a source of data on non-carcinogens. He presented an analysis of spontaneous tumor patterns in the two species, and patterns of chemically-induced tumors for recent NTP long-term studies of 75 chemicals. He noted that with chemical studies where there

were increased tumor incidences at only one site and in one sex of one species, there were few, if any, positive effects of these chemicals in in vitro short-term genetic toxicology systems. Conversely, for chemicals shown to induce tumors at more than one site and in more than one sex/species, there were usually positive responses in several of the in vitro systems. Thus, the CGTB programs were in a position to use this unique rodent carcinogen data base to not just test chemicals but also test hypotheses, and help characterize and distinguish between carcinogens.

Discussion: In response to a query by Dr. Haynes, Dr. Tennant said the Branch objectives represented a mixture of carrying out studies as part of their mission within the NTP and internally generated interests. Dr. Pitot asked whether there were projects specifically aimed at altering the spontaneous incidences of tumors. Dr. Ernest McConnell, NIEHS, said NTP had new initiatives examining the effects of diet and reduced caloric intake on tumor incidence. There was some discussion on what is known about the spontaneous incidences of and types of tumors in wild animal strains versus the usual inbred strains used for laboratory studies.

TESTING AND TEST DEVELOPMENT PROGRAM

II. Evaluation of Mutagenicity Results: Dr. Errol Zeiger, Head, Environmental Mutagenesis Section, described the testing initiatives in the Section, some of which began prior to the NTP. Their goals were to test chemicals, develop new systems, and investigate the biological bases for effects observed. Cumulatively, there have been 1300 samples (1063 unique chemicals) tested for gene mutations in the Salmonella typhimurium system, 298 (264) tested for cytogenetic effects in Chinese hamster ovary (CHO) cells, and 191 (174) for heritable genetic effects in Drosophila melanogaster. Most chemicals being tested in CHO cells or Drosophila have been or are being tested in Salmonella. As to uses for the data, Dr. Zeiger said the first aim was to make the data available to the public by publishing them in peer reviewed literature. Uses of the data were: to aid in selection of chemicals for further testing; to aid in evaluation and interpretation of carcinogenesis and toxicology results; for use by regulatory agencies; and as a data base for comparative mutagenesis and structure activity relationship (SAR) correlations. Dr. Zeiger amplified on use of this large data base for comparative mutagenesis and SAR correlations. All of the chemicals for which long-term bioassays were completed by the National Cancer Institute or the NTP were being tested, with mutagenesis data (at least in Salmonella) now available on 175, about 65%, of these chemicals. Contrasted with the primal findings from McCann and Ames, and Sugimura, which reported 95% of carcinogens tested were mutagens in Salmonella, only about 47% of the carcinogens tested were mutagens. This reflects the preponderance of potent carcinogens and mutagens, e.g., alkylating agents, nitrosamines, in the earlier studies, while in the NTP data base about 50% of the 'non-mutagenic carcinogens' were chlorinated chemicals, and there were no polycyclic aromatic hydrocarbons or nitrosamines. Thus, the Salmonella test does have predictive value but not as broadly as originally believed.

Dr. Zeiger outlined the current Salmonella testing protocol which includes four strains (TA-97, TA-98, TA-100, and TA-1535) with activation by liver S9 fractions from rats or hamsters, or no activation. He then discussed studies done using permutations of the full protocol, and one permutation that was as

sensitive which would be proposed for use in the testing contracts. The resources saved would be used for more in-depth studies on selected mutagens and non-mutagens. Future studies will include contracts to improve capabilities for evaluating chemicals in Salmonella that require reductive metabolism to the active mutagens, e.g., benzidine dyes. Also, other tester strains and customized activation systems, including the use of organs other than liver, will be investigated.

Discussion: Dr. Mendelsohn wanted to know how to reconcile needs for customizing the assays to enhance sensitivity for detecting mutagenicity with the needs for a standardized data base which can be used to make comparisons and correlations. Dr. Zeiger replied that both aspects were charges of the program and both would continue. Obviously, with the unique data base available for rodent carcinogenicity more emphasis would be given to making structural-activity and other correlations relating to carcinogenicity and mutagenicity. There was further discussion on what types of correlations should be done and would be most useful.

III. Mammalian Cell Mutagenesis: Dr. William Caspary, Cancer Biology Section, described the experimental conditions for the L5178Y mouse lymphoma cell mutagenesis assay. Currently, 100 chemicals/year are being tested in two contracts for mutagenic activity. He discussed the three primary objectives of the project: (1) to optimize all phases of the assay - including measures of reproducibility in two laboratories and under varying conditions, and establishing quality control criteria and methods of analysis. He reported a high concordance (90%) between the two laboratories. He discussed the phenomenon of small colony formation and how modification of the culture medium could accent their formation; (2) to understand the mechanism of action of the chemical with the host cell. This includes characterizing the type of chromosomal aberration (CA) induced as another endpoint of genetic toxicity, and confirming correlation of CAs with small colonies; (3) to confirm that mutagenesis at the TK locus in these cells is representative of mutagenesis in other mammalian cells.

Discussion: Dr. Pitot asked whether chemicals tested in the mouse lymphoma system would be tested in another mammalian cell gene mutation assay. Dr. Caspary said the same chemicals would also be tested in the Chinese hamster ovary (CHO) cell system. Dr. Ray seconded the need for evaluating chemicals presumed to be chromosomal mutagens in mouse lymphoma in a more established system, whether it be CHO or another system. Dr. Mendelsohn noted that of 10 chemicals reported as non-carcinogens in the long-term bioassay, nine were positive in mouse lymphoma, and expressed concern as to possible problems with false positives. Dr. Caspary acknowledged the concern and said more emphasis was being given to looking at chemicals negative in other mutagenesis assays as well as testing them for comparative purposes in the CHO system.

IV. Cytogenetics and Aneuploidy Systems: Dr. Michael Resnick, Environmental Mutagenesis Section, described the goals of the in vitro cytogenetics testing project as: (1) development of reliable and sensitive protocols and systems for doing efficient testing; and (2) development of a sound decision making scheme. He outlined the protocol used, and the statistical characteristics and criteria for evaluating sister chromatid exchange incidences in Chinese hamster ovary (CHO) cells. Because many chemicals induce only modest effects in systems such as CHO, the concept of a weak positive response was introduced. This involves using a low dose of a chemical positive for effects in CHO cells to delineate the sensitivity of the system. Dr. Resnick discussed future activities to

include: (1) examination of the relation between chromosome aberrations (CAs), sister chromatid exchanges (SCEs), and unique cytogenetic effects such as polyploidy, endoreduplication, and X-chromosome specific aberrations; (2) induction kinetics of aberrations, especially in regard to the relationship between simple and complex aberrations; and (3) the relation of cytogenetic responses to other genotoxic endpoints and carcinogenesis.

Dr. Resnick then described the developmental activities on contracts in the aneuploidy program. The primary purpose of two contracts for studies in yeast has been to develop mitotic and meiotic systems which will enable the routine testing of chemicals for their ability to induce aneuploidy, and one contract for studies in Drosophila to develop a meiotic system. Ongoing and future activities include: (1) further development of aneuploidy detection systems; (2) studies relating haploid to diploid rates of aneuploidy; (3) participation of Dr. Resnick (and Dr. James Mason, Project officer for the Drosophila contract) in a major interagency program that is evaluating aneuploidy-inducing agents and detection systems; (4) analyze responses in terms of other genetic endpoints; and (5) relating induction of aneuploidy in yeast and specific effects at the centromeres in yeast.

Discussion: There was discussion of the weak positive response in CHO cells and differentiating strong mutagens at low doses from weak mutagens at high doses. Dr. Resnick said use of the weak positive aided the resolving power of the assay. Concern was expressed about false positive rates in the CHO cell system.

V. Mammalian Cell Transformation Test Evaluation: Dr. Raymond Tennant, Branch Chief, described the background and rationale for this program. An objective is to evaluate and compare three assays for neoplastic transformation to determine which system may be most useful in identifying or distinguishing chemical carcinogens. Neoplastic transformation assays included Syrian hamster embryo (SHE); SHE infected with Simian adenovirus (SA7); and rat cells infected with Rauscher leukemia virus. Aims are to develop a standardized test protocol, establish sources of inter- and interlaboratory variability, and establish interlaboratory reproducibility. Dr. Tennant presented data comparing results with known carcinogens and other compounds in the three assays plus the BALB/c 3T3 cell line previously evaluated by the NTP. All assays distinguish between carcinogenic and non-carcinogenic polycyclic aromatic hydrocarbons. His proposal for the next generation of study was to continue development of the four transformation systems. At present there is no one assay which appears to be better than the others.

Discussion: Dr. Stanbridge questioned advisability of doing interlaboratory comparisons when there have been shown to be problems with interlaboratory reproducibility. There was discussion about the pros and cons of using these in vitro systems for studying promotion.

VI. Coordinated Testing Program: Dr. Judson Spalding, Cancer Biology Section, said this program was established to test the hypothesis that a battery of genetic toxicity tests could predict chemical activity in the rodent carcinogenicity assay. Two objectives are to help set priorities for long-term toxicology and carcinogenicity studies, and to evaluate, retrospectively, chemicals already studied in rodents. Up to eight individual assays in four test categories are being used. He described results in the different assays for 19 chemicals nominated in 1982 for long-term studies. Dr. Spalding then talked about the retrospective study. Criteria for selection of chemicals included different

chemical classes, different patterns of tumor induction, multiple sites, unusual tumors, and some single sex or species tumors. Of 50 chemicals chosen, 20 had been non-carcinogenic in rodents. He discussed in depth the extent of correlations between the in vitro tests and the rodent study for four chemicals (one strong positive, one equivocal, one positive in one species at one site, and one clearly negative in vivo). Although the current data base is the most comprehensive available, it is not yet possible to tell which group of assays will be most predictive for in vivo carcinogenicity. In the future, it may be possible to select a battery of tests which will be most appropriate for each chemical.

Discussion: Dr. Nesnow suggested that with the large and growing data base being developed, there should be emphasis directed toward multiple dimensional analysis. Dr. Mendelsohn seconded this. Dr. Tennant responded that there had been data management problems but these were being resolved so such analyses could be done.

VII. Human Cell Studies: Dr. Robert Langenbach, Head, Carcinogen Metabolism Section, stated the goals as: (1) to determine the advantages and disadvantages of using human cells in place of or in combination with routine rodent cell systems; (2) to investigate approaches for improving the metabolic capabilities of human cells used. There will be two contracts to carry out two tasks, namely: Task I - to cocultivate primary human hepatocytes to metabolically activate the chemical with human target cells in which genetic endpoints can be measured; and Task II - to modify metabolic capabilities of human cell lines so activation and genetic endpoints can be measured in the same cell line. He outlined the specific goals for each task. These studies should help provide insights into the usefulness of human cells in genotoxicity assays.

In Vitro Assay for Promoters: Dr. Langenbach said the V79 cell metabolic cooperation assay is being evaluated through an interagency agreement with NIOSH. The assay is based on the ability of chemicals with promoter activity to interrupt cell-to-cell transfer of low molecular weight metabolites. Twenty-five chemicals of known promoting or nonpromoting activity were coded and tested in a dual laboratory study to determine the predictiveness of the system. In new contracts, attention will be given to protocol improvement followed by dual laboratory evaluation of 50 coded chemicals. There have been NCI/NTP long-term carcinogenesis studies on most of these chemicals.

Discussion: Dr. Ray inquired as to how many non-carcinogens/nonpromoters were tested in the initial validation of the V79 assay, and what the concordance was with previous results. Dr. Langenbach said that about 20% of the original 25 were negatives, and there was concordance for at least 24 of 25. Suggestions were made by the peer reviewers as to other chemicals which should be tested and that other cell lines should be considered.

VIII. Germ Cell Mutagenesis: Dr. Michael Shelby, Head, Mammalian Mutagenesis Section, noted there has never been a clear indication in vivo in either animals or humans that increased frequencies of germ cell mutations lead to health effects in subsequent generations. The reason may be that there are no effects or, more likely, adequate tests have not been done. He said there were four assay systems which have been undergoing evaluation: the dominant lethal test and the heritable translocation assay which reflect chromosome damage, and the morphological and biochemical specific locus assays which measure gene mutational effects. Dr. Shelby described findings in these assays with a number of chemicals, some of which are known to have germ cell effects, and others which

had not been studied, including ethylnitrosourea, ethylene oxide, urethane, and ethylene dibromide. Future directions include: (1) development of a unified scheme for determining both gene and chromosomal effects in pre- and post-meiotic stages and in both sexes, to give a good profile of the germ cell mutagenicity of chemicals, and (2) development of a stable data base which can be used in genetic risk estimation, and for assessing predictiveness of current and newer assays.

Discussion: There were questions about the duration of animal exposure to the various chemicals, and about the relative sensitivity to germ cell mutagens of males versus females.

IX. Concept Review - Development of an In Vitro Assay for Chemically Induced Neoplastic Transformation Using Oncogene-Primed Target Cells: (Attachment 4) Dr. Lawrence Boone, Cancer Biology Section, said the objective of the proposed project was to develop target cell cultures that are primed for detecting chemically-induced transformation and will be more sensitive than available in vitro transformation systems. He reported the approach would be to obtain clones of several normal proto-oncogenes, engineer the genes into a retrovirus vector, and introduce the vector into potential mammalian target cells (mouse embryo). An increase in frequency of carcinogen-induced transformation in cells with the proto-oncogenes relative to cells not given the genes would form the basis for assay development. Several types of proto-oncogenes would be examined.

Discussion: Dr. Diamond inquired as to why mouse embryo cells were chosen in view of the possibility of spontaneous transformation occurring. Dr. Boone said the large amount of information available on the structure of mouse proto-oncogenes outweighed the drawbacks at this point. He added that if these appeared to be significant problems a cell system from another species such as rat or hamster could be considered. Dr. Tennant proposed that the scope be modified to make it a pilot project looking at three to four retroviral C-onc constructs in three to four cell lines, both mouse and human. Initially analyzed would be any heritable effects on cell phenotypes by the constructs. Dr. Pitot and Dr. Mendelsohn expressed concern about the possibility of there being constructed a new human retrovirus. Dr. Tennant said this could not be ruled out completely. He noted that the study protocol would have to go through the NIH Recombinant DNA Advisory Committee for clearance. Dr. Pitot said that other cell types such as those from hamsters should be evaluated. Because of the concerns expressed and the proposed change in scope, Dr. Rall and Dr. Mendelsohn agreed that a revised concept along with the Request for Proposal (RFP) should be brought to the next Board meeting for further review.

RESEARCH PROGRAM

X. Drosophila Mutagenesis: Dr. James Mason, Environmental Mutagenesis Section, described three research projects with which he has been involved. The first concerned genetic control of mutation rates in Drosophila. The focus was on a mutator gene which was shown to potentiate recovery of terminal deletions in Drosophila. He elaborated on studies trying to determine the mechanisms including the mode of induction of telomeres ("neotelomeres"), structure of the "neotelomeres" and sequence specificity. The second project is the cytogenetic characterization of DNA-repair defective mutants in Drosophila, in particular, mutants at the mei-41 locus, to gain a basic understanding of the genetic

control of sensitivity to mutagenic agents. A genetic fine structure map of the mei-41 region has been constructed using several independently isolated alleles. The third project is an investigation of whether chemicals can induce transposition of mobile genetic elements. Initial experiments suggest that alkylating agents at low levels will induce copia transposition. Further studies will be aimed at improving the sensitivity of the system through use of a wider range of transposable elements, and finding good positive control compounds for use in assay development.

XI. Genetic and Molecular Mechanisms of Meiosis and DNA Repair in Yeast:

Dr. Resnick talked of their studies concerned with determining the nature of DNA damage as produced in cells and the relationship to chromosome structure, an examination of DNA repair processes and genetic control in mitotic cells, and the possibility of DNA repair processes in meiotic cells and their function in normal meiotic recombination. He enumerated various reasons why yeast is preferable to other experimental systems, and discussed in more detail studies on the role of the RAD repair genes in meiosis, and on the nature of DNA damage to chromosome structure.

Discussion: Dr. Swenberg asked why ultraviolet (UV) was being used rather than chemically-induced DNA damage in the yeast studies. Dr. Resnick said they were concerned with basic mechanisms of DNA-damage and repair which meant characterizing the genetics of the system, and UV is a model probe for examining genotoxic issues because the results are not confounded by delivery of the chemical to the DNA, or by its interaction with other cell constituents. Use of chemicals to study DNA-interaction and damage/repair would follow.

XII. Molecular Genetics of Meiosis and Mutagenesis in Yeast: Dr. Craig Giroux, Environmental Mutagenesis Section, said the central area of his research was on the processes which maintain genetic information and ensure faithful passage to future generations. This currently involved two projects. The first and major was focused on meiosis, especially chromosome behavior. The second was concerned with mutagenesis in both somatic and meiotic cells. Both have the goals of understanding molecular mechanisms underlying mutagenic phenomena, and provide information which can be useful in a practical sense for genotoxic studies in yeast, and eukaryotes in general. He elaborated the advantages of using yeast rather than other experimental systems and discussed studies with a meiotic specific gene mutant Spoll-1. Dr. Giroux then described the newer project concerned with understanding the molecular basis for mutagenesis in yeast.

XIII. General Discussion. Dr. Manson commented on the importance of pursuing the differential susceptibility between meiosis and mitoses. Dr. Ashby noted that the NTP already has the most complete data base of genetic toxicology test results in existence yet lacks sufficient data for a number of chemicals from key classes such as nitrosamines, polycyclic aromatic hydrocarbons and alkylating agents. Dr. Zeiger responded that there was sufficiently adequate data in the literature for these classes, most of which would be positive. He said the data base had been driven by the NCI/NTP list of long term studies and by the chemical nominations process. Dr. Mendelsohn wondered whether any attention was being paid to the universe of chemicals in general, who is exposed, how the chemicals are distributed, and how they are represented in the data base. Dr. Rall replied that there was only one such sampling close to a real "universe", that being the list of about 650 chemicals from the NAS Testing Needs Study performed for the NTP. Dr. Mendelsohn asked when the overall genetic toxicology data base will be useful. Dr. Tennant replied that we were

moving toward this but more data for across-test-system-comparisons, data on non-carcinogens, and data from the mutagenesis/carcinogenesis interface were needed. Dr. Haynes expressed concern that there was too much emphasis on the predictiveness of mutagenicity for carcinogenicity, and that other possible health effects of somatic mutations needed also to be considered. The session concluded with an active discussion of the types of statistical tests used and the appropriateness of the tests for analyzing short-term in vitro assay data.

XIV. Molecular Analysis of Gene Toxic/Carcinogenic Events in Mammalian Cells:

Dr. Boone described this project which is focused on the role of endogenous ecotropic provirus in induced and spontaneous hematopoietic neoplasms of the mouse, specifically the RFM/Un mouse. Provirus rearrangements have been studied as a model for oncogenesis by transposition and promoter insertion, and have detected somatically reintegrated copies of the endogenous ecotropic provirus in greater than 85% of spontaneous reticulum cell sarcomas in this mouse strain. He said his group was trying to clone individual rearrangements and examine other independent primary neoplasms to identify common chromosomal integration domains and potential oncogenes.

XV. Organ and Species Differences in Carcinogen Activation: Dr. Langenbach said his laboratory is developing methodology for determining organ and species differences in in vitro metabolic activation. Primary intact cells (rather than S-9 fractions) from various organs are used as activation systems and multiple genetic endpoints are measured, including Chinese hamster V79 cell mutagenesis, sister chromatid exchanges and toxicity as well as Salmonella reversions. Other studies include: (1) correlating mutagenic activity with metabolic profiles; (2) investigating the relative role of the liver versus the bladder in activation of bladder carcinogens; (3) developing methods for maintaining hepatocyte metabolizing capabilities in vitro; and (4) determining structure-activity relationships in carcinogenic and mutagenic activities for a series of short chain nitrosamines. Dr. Langenbach showed data with specific chemicals for some of these studies. Their findings indicate there is less interspecies variation in the ability of the liver to activate various chemicals than in the extrahepatic tissues. He said the laboratory's research goals over the next three years would include: (1) investigating the mechanistic basis for carcinogenicity of NTP chemicals in B6C3F₁ mice and/or F344 rats using metabolic and DNA adduct profiles, in vitro promotion, and effects in human cells; (2) continuing bladder cell studies with possible cocultivation of bladder and liver cells; and (3) developing techniques for maintaining or increasing the ability of primary cells in culture to metabolize chemicals.

Discussion: Dr. Diamond commented on the problem of large variations in metabolic activity of human cells and wondered how this could be overcome.

Dr. Langenbach acknowledged this to be a problem. However, since humans are the ultimate species of interest, information on variability should be valuable in itself. Several reviewers commented on the technical difficulties associated with some of the proposed studies.

XVI. Salmonella Mutagenesis: Dr. Zeiger discussed two directions in the research: (1) studies to better understand the Salmonella test system aiding in better interpretation of results and in refining and improving test protocols; and (2) using the Salmonella system as an endpoint and tool for studying in vivo and in vitro metabolism -- in vivo through the host mediated assay or analysis of urine mutagens, and in vitro through organ homogenates or perfused

organs. He briefly described several projects under the first research area including: (1) studies with sodium bisulfite which have shown that Salmonella strains can survive and be mutagenized under acidic conditions; (2) the effect of prolonged storage of mutagen solutions used for positive controls; and (3) studies that showed, contrary to expectations, DNA repair-deficient strains are mutagenized less than repair-competent strains at low doses of a mutagen. Projects under the second area included: (1) studies on mutagenicity of benzo(a)pyrene and benzidine in Salmonella in the presence of S-9 fractions from liver, prostate and testes of control rats and rats treated with TCDD; and (2) studies comparing electron-accepting and electron-donating substituents on the mutagenicity of phenanthrene imines. New studies include: investigation of the organ specific metabolism of a number of mutagens which are organ-specific carcinogens; and the capability for converting chemicals to mutagens by purified cytochrome isozyme.

XVII. General Discussion: Dr. Ashby noted the existence of a contract for studying unscheduled DNA synthesis (UDS) in vivo in rat liver, and asked how this fit into the program. Dr. Tennant said the assay was still in an evaluation phase. Both known animal hepatocarcinogens and chemicals going into long-term studies are being assayed, under code. Dr. Ashby and Dr. Ray supported a thorough evaluation of the UDS assay for its usefulness in a battery for detecting hepatocarcinogenic activity.

XVIII. Report of the Director, NTP: Dr. David P. Rall reported that: (1) the report of the Board's Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation had been received favorably, and the NTP has or is beginning to follow many of the recommendations and will give a detailed report to the Board on the Program's responses to the recommendations; (2) the NTP will hold a science workshop at NIEHS on January 29 and 30, 1985 which will involve staff from all the component agencies of the NTP, and to which the Board was invited. The theme will be Chemical Class Studies, specifically, glycol ethers, antihistamines, ketone solvents, halogenated short chain aliphatics, and benzidine dyes; (3) the NIEHS budget has been approved by Congress and sent to the President. It allows for an increase to \$194 million from \$183 million in FY 1984; and (4) the report of the third task force on environmental health and research needs (Task Force III) is in final form. The report points out that the new biology/molecular biology can be applied to environmental health problems.

XIX. NIEHS/NTP Concept Review-Contract - Systematic Study of Recognized Human Carcinogens in Rodents: (Attachment 5) Dr. Richard Irwin, Carcinogenesis and Toxicology Evaluation Branch, stated the concept proposal was to systematically evaluate the effectiveness of the test methods currently used by the NTP for detecting chemicals with carcinogenic potential, and to evaluate the reliability of rodent studies for detecting carcinogenic hazards of chemicals to humans. In other words, are chronic animal studies reliable predictors of carcinogenic hazard to humans? He cited the 23 chemicals/chemical mixtures recognized by the International Agency for Research on Cancer (IARC) as human carcinogens and said that where animal data was adequate there was concordance. For some of these chemicals, the design and/or conduct of the animal studies was inadequate for comparisons to be made. The NTP proposes to select and test with current NTP protocols these recognized human carcinogens and then make detailed comparisons with available human data. Two other important types of information which might be derived are: (1) identification of target sites and characterization of dose-response; and (2) a broadening and deepening of the data base of animal testing results. At the outset, decisions will be made as to which chemicals on

the IARC list require further animal testing and then a priority order will be established based on the criteria given on page 2 of Attachment 5.

Dr. Swenberg commented that the concept has merit but noted no budgetary information time frames had been provided, and since adequate bioassay data were available for some of the chemicals, he thought some priority setting could have already been done. Dr. Irwin responded that not all of the chemicals would be tested and input would be sought from the scientific community. Dr. James Huff, NTP, said the Board would be consulted after priorities were set. Dr. Pitot stated that the NTP's goal should be disease prevention and there were other more important questions in relating animal data to humans that should receive the resources. Dr. Mendelsohn said the concept should be simplified to focus on the issue of analyzing the IARC list, the longer list not just the 23, and setting priorities from this list. The actual NTP testing would be a separate issue and there were already mechanisms in place for deciding what chemicals to test. Dr. McConnell said there was not adequate data for some of the chemicals in the NTP rodent strains, especially the B6C3F₁ mouse. Dr. Pitot said the analysis should determine the existence of dose-response relationships both in human and animal data. Dr. Rall then proposed that the NTP go ahead and do the extensive analyses of the IARC lists, paying special attention to dose response data, set priorities, and then return to the Board for review. Dr. Mendelsohn agreed and said this seemed to reflect the wishes of the Board.

XX. Discussion of Peer Review for Reports on the Data from Remaining Long-Term Studies Performed at Gulf South Research Institute: (Attachment 6) Dr. E. McConnell provided the Board with an update of the NTP activities regarding the auditing of studies performed at Gulf South Research Institute(GSRI), and proposed future plans for reporting the findings. He noted that all studies at GSRI were terminated in the first half of 1983, and data audits were begun on GSRI studies and studies from other laboratories in late 1983. Dr. McConnell described the kinds of problems identified in audits of the GSRI studies (page 2 of Attachment 6), emphasizing that not all of the problems were observed in every study. The status of the 28 studies at GSRI were indicated (pages 3 to 6 of Attachment 6), and of these, eight have been restarted as new studies. He emphasized that studies from eight other laboratories have been audited and no flaws were found sufficient to invalidate any study (pages 7 and 8 of Attachment 6).

Dr. McConnell then discussed the proposed NTP strategy for disposition of the GSRI studies, and asked for comments and suggestions from the Board. He said a new audit contractor will be assigned to work solely on the GSRI studies. Those studies judged to be adequate will be brought before the Peer Review Panel with one possibility being to devote an entire meeting to GSRI studies. He said the Panel would be told that the study is worth reporting and the flaws would be highlighted. The Board will also be briefed. For studies identified as being severely flawed so they should not be reported, the Board and Panel will be informed and there will be a notice to this effect in the Federal Register. In response to a question by Dr. Swenberg, Dr. McConnell said restarted studies would take into account experiences gained from the initial study and, for the most part, a different mode of dosing would be used, e.g., microencapsulation instead of gavage.

XXI. Peer Review and Priority Ranking of Chemicals Nominated for NTP Testing: (Attachment 7) The Board considered 11 chemical nominations (five azo and nitro dyes, and six organometallic compounds) which had been reviewed previously by the NTP Chemical Evaluation Committee (CEC). Dr. Mendelsohn chaired the review and Dr. Dorothy Canter, NIEHS, member of the CEC, and Dr. Victor Fung, NTP Chemical Selection Coordinator, served as resource persons. Each Board member had been asked to serve as principal reviewer for one or two chemicals. Following the principal reviewer's presentation and discussion by the Board members, a motion was made and voted on.

The group of five azo and nitro dyes (C.I. Acid Yellow 151, C.I. Basic Red 18, C.I. Direct Red 80, C.I. Direct Yellow 4, and C.I. Disperse Brown 1) had been reviewed by the Board on August 16, 1984, and deferred until the next meeting to allow for a presentation on the rationale for the nomination of these dyes as representative of the azo and nitro dyes class. In the interim, the NTP CHEMTRACK computerized data base was searched for all chemicals in the NTP carcinogenesis studies program with primary or secondary use categories as dyes, either intermediates or finished dyes, inks or pigments. The 110 chemicals identified are shown in Attachment 8. Dr. Tucker Helmes, SRI International, discussed the chemical class study on azo and nitro dyes that was conducted for the National Cancer Institute (NCI) with assistance from the Dyes Environmental and Toxicology Organization, Inc. (DETO). Dr. Barry Bochner, representing DETO, was present as a resource person. Dr. Helmes said the dye class study was initiated because chemicals in this class have a significant potential for human exposure and are regarded with some suspicion of carcinogenicity. Since no chronic data was available for many of the low-volume dyes, one of the objectives of the study was to provide a framework for obtaining data for establishing a set of reference compounds. These compounds would represent each major structural category and identify both positive and negative chemicals in each category. The ultimate goal would be to develop predictive test methods for over 2000 dyes. In evaluating the azo and nitro dyes, weight was given to potential human exposure, suspicion of carcinogenicity, and structure-activity interest. A series of meetings involving SRI staff, DETO representatives, staff from the NCI and the various relevant Federal agencies, and, in some cases, labor union representatives, led to a list of 37 dyes that were considered prime candidates for carcinogenesis bioassay. A subsequent meeting reduced the list to 14 out of which the five nominated dyes were given high priority. Dr. Helmes concluded his presentation by giving rationales for each of the five dyes. The Board then discussed the five dyes, and recommendations were made and voted upon.

Dr. Canter presented background information on the remaining six compounds to be reviewed, which were nominated by the NCI as a result of a class study on organometallic compounds. She said this study complimented an NCI class study of inorganic metallics. The compounds were evaluated by the CEC on February 28, 1984 (Attachment 7). The Board then discussed the six organometallics, and recommendations were made and voted upon.

The Board's recommendations, priority for testing, and additional remarks and/or caveats for the five dyes and the six organometallic compounds are summarized in Attachment 9.