National Toxicology Program
Board of Scientific Counselors' Meeting
March 27 and 28, 1984

SUMMARY MINUTES

Contents

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Summary Minutes

The National Toxicology Program (NTP) Board of Scientific Counselors met on March 27 and 28, 1984, in the Conference Room, Building 13, National Center for Toxicological Research (NCTR), Jefferson, Arkansas (Attachment 1: Federal Register Meeting Announcement; Attachment 2: Agenda and Roster of Board Members). Members of the Board are Drs. Mortimer Mendelsohn (Chairperson), Norman Breslow, Leila Diamond, Curtis Harper, Jerry Hook, Jeanne Manson, Henry Pitot, and James Swenberg. Dr. Breslow was unable to attend the meeting.

The minutes of the September 27, 1983, Board of Scientific Counselors' meeting were approved unanimously. Dr. Ronald W. Hart, Director of NCTR, welcomed the Board in their first visit to the Center.

I. Report of the Director, NTP: Dr. David P. Rall reported that: (a) the recruitment period had ended for applications of candidates for filling the vacant Directorship of the Toxicology Research and Testing Program, NIEHS; (b) the FY 1984 NTP Annual Plan had been approved in late February and, along with the accompanying FY 1984 Review of Current DHHS, DOE and EPA Research Related to Toxicology, would be distributed in April; (c) Mr. William Ruckelshaus, EPA Administrator, had been elected to a one-year term as Chairman of the NTP Executive Committee; (d) five new members had been appointed to the Peer Review Panel, serving for the first time at the Panel meeting on March 23 in Washington, D.C. They were Drs. Thomas C. Jones, Harvard; Richard Kociba, Dow Chemical; David Kotelchuck, United Electrical, Radio and Machine Workers Union; Steven Tannenbaum, M.I.T.; and Bruce Turnbull, Cornell; (e) the data audits continued with the finding that most of the contract laboratories were performing satisfactorily; (f) comments were being received on the draft report of the Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation, and after meeting to consider the comments, the Panel would submit a final report to the Board at their August meeting; (g) the Testing Needs Study done under the auspices of the National Academy of Sciences was completed and the final report was available; and (h) the third task force on environmental health and research needs (Task Force III) is under way and will meet at NIEHS from June 11 to 22. Drs. Arthur Upton, NYU, and Bernard Goldstein, EPA, will co-chair the task force.

II. Advisory Review Panel for NTP Reproductive and Developmental Toxicology Programs: Dr. Manson stated that the formation of this Panel had been approved previously by the Board to: (a) review concept statements for new contract efforts prior to Board review; (b) aid in peer review of experimental protocols and reports; (c) review the program annually and provide it with advice on its direction and scope of efforts; and (d) advise the Board concerning the progress and quality of the program. The Panel will be chartered as a standing subcommittee of the Board. Dr. Hook moved that the Panel as described be approved. Dr. Swenberg seconded the motion and it was approved unanimously.

III. NIOSH/NTP Concept Reviews - Interagency Agreements: Two proposed interagency agreements were reviewed by the Board.

(1) Inhalation Development and Reproductive Toxicology: (Attachment 3) Dr. Bryan Hardin, NIOSH, stated that the agreement will provide the NTP with a capability for testing chemicals for developmental and reproductive toxicity where inhalation is the appropriate route of exposure. This capability is currently lacking within the NTP. The tasks include review of available literature and identification of specific testing needs and a number of preliminary studies; among them are physical-chemical characterization, vapor generation studies, development of analytical methods for sampling chamber concentrations of chemicals, and dose range-finding studies for the various reproductive tests to be used. The definitive studies would follow and include conventional teratology, behavioral teratology, male and female fertility assessment, or sperm head morphology, as appropriate to specific testing needs. All definitive studies would be carried out in one or more species and at two exposure concentrations. The high dose would be that which produces minimal toxicity in pregnant female animals.

Drs. Manson and Swenberg strongly recommended that the NTP use three exposure concentrations in addition to controls. In response to Drs. Pitot and Manson, Dr. Hardin said the Department of Energy's Battelle Pacific Northwest Laboratory was chosen because this facility had the appropriate staff and the special capabilities to carry out the range of tests using the inhalation route, and because the interagency agreement would permit wide latitude in designing test protocols specific to the testing needs of each chemical. Dr. Swenberg noted that the Board previously had endorsed the need for this capability. There was discussion as to types of chemicals to be tested and how priorities would be set. Dr. Bernard Schwetz, NIEHS, said that in addition to the NTP chemical nomination and selection process, the NTP Toxicology Design Committee would recommend chemicals, although other means would also be used in selection of chemicals. Dr. Manson moved for acceptance, Dr. Hook seconded, and the concept proposal was approved unanimously.

(2) Evaluation of Drosophila for Teratogen Screening: (Attachment 4) Dr. Hardin described development at NIOSH of a rapid teratology screen using Drosophila (fruit flies) larvae in culture followed by testing of the system using 15 known teratogens and two non-teratogens. Based on the results, a peer review group had recommended that the Drosophila system be evaluated further in a second laboratory to confirm the initial observations, to optimize test conditions and procedures, and to develop a broader data base on the test's responsiveness to known mammalian teratogens and nonteratogens. The study will be done through an interagency agreement with the Department of Energy at the Brookhaven National Laboratories. A total of 90 tests will be conducted on as many as 90 or as few as 18 chemicals, depending upon the number of replications required.

Dr. Mendelsohn expressed concern that there had been no measure of the false positive rate. Dr. Hardin concurred but said this was why there were several nonteratogens to be tested under the agreement. Also, he said the typical lesions observed had a background rate in Drosophila ranging from 0 to 6%, depending on the lesion. Dr. Harper asked whether some toxic nonteratogens could be tested first. Dr. Hardin said this would be done although some of the known teratogens would be tested initially to assure there was reproducibility between previous NIOSH results and those from the test laboratory. Dr. Manson urged that candidates for testing be drawn from a list of 47 chemicals chosen by the NTP-sponsored "Consensus Workshop on In Vitro Teratogenesis Testing." This list includes known teratogens that are also mutagens and known teratogens that are not mutagens. Dr. Hardin said this would be done. Dr. Manson moved that the concept be accepted. Dr. Hook seconded the motion and the concept proposal was approved unanimously.

IV. NIEHS/NTP Concept Reviews - Contracts: Three proposed contracts were reviewed by the Board.

(1) Testing the Urine of Rats on Prechronic Tests for Mutagenic Activity: (Attachment 5) Dr. Errol Zeiger, NIEHS, said the objective is to evaluate the metabolism of chemicals to mutagens in vivo by assaying the urine of rats on test. This will provide information that can be evaluated with respect to chemical disposition studies, and eventually, as a predictor of carcinogenicity. Dr. Swenberg stated that the emphasis should be placed on examining urine from animals already in two-year carcinogenesis testing, preferably during the last six months so the results could be better and sooner correlated with carcinogenicity findings. Dr. Zeiger said the long range goal of the project would be to validate the urine assay to establish levels of detectability. This information would be useful in assessing the utility and sensitivity of urine assays for monitoring exposures of workers to chemicals. Dr. Hook said this was a valid objective but needed to be stated in the proposal. Dr. Swenberg moved that the concept be accepted with the modifications that: (1) the chemicals selected for study be chosen from those in the chronic testing phase, and (2) the validation should be stated as an objective in the proposal. Dr. Manson seconded the motion and the concept proposal was approved unanimously.

(2) Examination of Immunotoxicity by Chemical Xenobiotics: (Attachment 6) Dr. Michael Luster, NIEHS, described prior contract initiatives which encompassed development and validation of a sensitive screening panel for evaluation of chemical-induced immunotoxicity. Under the proposed contract, this panel would be used to assess altered host resistance and immunological impairment in rodents exposed to chemicals of specific interest to the NTP. Included will be measures of immunopathology, host resistance, cellular- and humoral-mediated immunity, non-specific immunity, and allergenicity. Four chemicals per year will be tested. One of the chemicals will also be selected

for a more detailed evaluation to determine the cellular and molecular events involved in the immunotoxicity. Dr. Swenberg inquired how doses and chemicals would be selected. Dr. Luster replied that many of the chemicals chosen would be those in long-term toxicity and carcinogenesis studies, especially where clinical or pathologic signs observed in vivo suggest chemical effects on lymphoid or other aspects of immune function, e.g., 1,3-butadiene. Doses selected will be similar to those in long-term studies with the high dose of three usually a minimally toxic dose. Drs. Swenberg and Mendelsohn noted the need for tests to measure hypersensitivity reactions in view of the magnitude of such problems in humans. Dr. Luster said that the screening panel will include an assay for hypersensitivity but the significance of the results may be hampered due to the lack of predictive models for hypersensitivity. Dr. Swenberg moved that the concept be approved. Dr. Diamond seconded the motion and the concept proposal was accepted unanimously.

(3) Data Auditing Support Resources Contract: (Attachment 7) Dr. Scot Eustis, NIEHS, said the objective of the contract is to provide audit support for the NTP to assure that data collected at NTP testing facilities satisfy Good Laboratory Practices and are recorded adequately to allow reporting the data in NTP Technical Reports. The contract would support three teams to do audits. Dr. Eustis projected about 75 audits in 1985, 50 audits in 1986, and 30 in 1987. Dr. Swenberg inquired as to how the competency of an audit team would be assured. Dr. Eustis replied that there would be continuing review of audit reports and some of the raw data by appropriate NTP staff including the chemical manager and the project officer for a contract. Dr. Ernest McConnell, NIEHS, noted the outcome of the audit can affect future contract awards. Dr. Rall said summaries of all audit reports are included in the technical report and available to the public upon request, and the full report is accessible to public viewing when completed. Dr. Swenberg moved that the concept be approved. Dr. Hook seconded the motion and the concept proposal was accepted unanimously.

NCTR PROGRAM PRESENTATIONS (Attachment 8)

V. Introduction and Overview: Mr. Arthur Norris, NCTR, said the rest of the agenda would give the Board an orientation to NCTR and perspective on selected NCTR science programs which may or may not relate directly to NTP-related activities. The NCTR opened in 1971 and until 1983 received some direction and resources from the Environmental Protection Agency as well as from the Food and Drug Administration (FDA). The Center's overall mission is to serve as a national resource to carry out toxicology research and testing in support of the FDA and other agencies. He commented on the new diet preparation facility and pilot facilities for investigating thermal destruction of toxic wastes and for waste water treatment. He mentioned programs not on the agenda in pathology, cell biology and immunotoxicology. Finally, Mr. Norris described the various peer review processes used to ensure quality in NCTR programs.

VI. Developmental and Reproductive Toxicology: Dr. John Young, Director, Division of Teratogenesis Research, said the division goals focus on (a) exposure/pharmacokinetics, (b) measure of toxic effects on the fetus and neonate, and (c) mechanisms of developmental effects. His own studies in the area of pharmacokinetics derive from the hypothesis that there is a predictable relationship between some pharmacokinetics parameter(s) and some teratogenic endpoint(s). As an example, he mentioned studies with salicylic acid. Dr. Young described work in the Developmental Mechanisms Branch, headed by Dr. Gary Kimmel, on cellular mechanisms dealing with estrogen receptors and on Dr. Kimmel's role in in vitro test system development including the use of the embryo culture system. He talked about postnatal evaluation activities in the Prenatal and Postnatal Evaluation Branch, headed by Dr. Carole Kimmel, including development and routine use of a double staining procedure, evaluation of abnormalities of physiologic functions such as blood pressure or blood flow as toxicologic endpoints, evaluation of measures of behavioral teratologic effects, and special studies for the FDA and other agencies.

(1) Pharmacodynamics Branch: Dr. William Slikker, Branch Chief, said their basic approach involved use of the Rhesus monkey as an animal model for studying chemical disposition of teratogens, especially the role of placental transfer, and measuring effects of chemicals on physiological functions in vivo by implanting monitoring devices in the fetal-placental unit. Dr. Slikker described studies on: (a) ethanol disposition; (b) comparative disposition of endogenous estrogenic and glucocorticord hormones (estradiol and cortisol) versus synthetic analogs (diethylstilbestrol and triamcinolone) aimed at explaining the greater teratogenic effects of the synthetic analogs; and (c) the metabolic pathways for doxylamine succinate in the Rhesus monkey.

VII. Biochemical Toxicology: Dr. Fred Kadlubar, Director, Division of Carcinogenesis Research, listed the primary research areas in the Division: (a) metabolic activation of chemical toxicants; (b) identification of the nature of damage to cellular constitutents by activated toxicants; (c) effects of cellular repair mechanisms on the toxic response; (d) determination of critical detoxification pathways; (e) elucidation of biochemical mechanisms by which chemical carcinogenesis is initiated and expressed; and (f) evaluation of specific toxicants and classes of toxicants in relation to mechanisms of action. Dr. Kadlubar described some of the principal research activities of Division scientists including: (1) metabolism and toxicology of acetaminophen and phenacetin; (2) metabolic activation of benzene to phenol through an epoxide intermediate; (3) comparative metabolism of sulfamethazine and gentian violet, the latter by intestinal material to an aromatic amine; (4) interaction of sulfamethazine with nitrites to form toxic metabolites; (5) metabolic activation and carcinogenesis of nitroaromatic hydrocarbons; (6) structure-activity-relationships of halogen-substituted polycyclic aromatic hydrocarbons; (7) genetic toxicology of dinitropyrenes; (8) chemically-induced changes in DNA structure, using force field calculations; (9) biochemical mechanisms of liver, urinary bladder, and colon carcinogenesis; and (10) saccharin as a tumor promoter.

(1) Comparative Metabolism of Aromatic Amines: Dr. Kadlubar reported on recent studies in his own laboratory done in conjunction with NIOSH comparing metabolic activation of two carcinogens, 2-naphthylamine (2-NA) and 2-aminofluorene, which are N-oxidized in rat and human liver, as contrasted with the non-carcinogen, 1-naphthylamine, which is not N-oxidized. A related project has shown the urinary bladder may have metabolic activation capability. Conversion of 2-NA to reactive metabolites is effected by the enzyme, prostaglandin endoperoxide synthetase, to the extent that perhaps 20% of the 2-NA binding in the bladder is due to metabolism by the enzyme.

(2) Nitropolycyclic Aromatic Hydrocarbons, DNA Adducts, and Mutagenicity: Dr. Frederick Beland discussed metabolic activation studies with nitropyrenes, found in diesel exhaust emissions, some of which are potent mutagens as measured in Salmonella. Using 1-nitropyrene, it was found that activation occurs through N-oxidation to the aryl hydroxylamine. As with other aromatic amines, the site of substitution on DNA is at the C-8 position of deoxyguanosine. He described adduct formation with 1-nitropyrene in mammalian cells (Chinese hamster ovary).

(3) Saccharin: Mr. Robert West described four experiments designed to characterize the biological activity of saccharin. The first was a two-year study in rats with several dose levels up to five percent in the diet and with methylnitrosourea (MNU) as an initiator. These treatments resulted in an increase in bladder tumors and a decrease in times-to-tumor. The second were studies in mice using 2-acetylaminofluorene as initiator for bladder tumors. No promotional activity was demonstrated for saccharin. Third were studies in mice with saccharin and N-methyl-4-aminoazobenzene (MAB), a potent liver and bladder carcinogen. No promotional effects were shown and, at the highest dose of saccharin, there was an inhibition of MAB-induced liver tumorigenesis. Fourth, were studies in cultured human cells (fibroblasts) with saccharin using MNU or ethylnitrosourea as an initiator. Results suggested a co-carcinogenic action for saccharin. In summary, saccharin appears to be a biologically active molecule. There was some discussion with the Board as to additional experiments currently under way to distinguish between possible promotional and co-carcinogenic effects of saccharin.

VIII. Microbiology Division: Dr. Carl Cerniglia described the role intestinal microorganisms play in metabolism of various xenobiotics to activated or inactivated products. Projects include: (a) characterization of the activation of azo dyes, expecially benzidine congener dyes, through cleavage of the azo linkage by anaerobic gut bacteria from different species including humans; (b) role of the gut microflora in reductive metabolism of nitropolycyclic aromatic hydrocarbons, e.g., 1-nitropyrene to 1-aminopyrene; (c) reduction by microflora of gentian violet to leuco gentian violet; (d) studies on metabolism of polycylic aromatic hydrocarbons by microorganisms showing metabolic attack does not occur at the K-region but microorganisms will catalyze epoxidation and diol formation at sites

such as the 7, 8, 9, 10-position of benzo(a)pyrene. He contrasted the differences in microbial metabolism of polycyclic aromatic hydrocarbons in prokaryotic versus eukaryotic organisms. Dr. Cerniglia briefly described his group's environmental program wherein a simulated microcosm had been developed to help bridge the gap between in vitro culture and the complexities of an environmental ecosystem.

IX. Mutagenesis Research Program: Dr. David Casciano, Program Director, said his group's goals were to develop in vivo systems to provide information for genetic risk assessment, and to develop short-term assays for measuring effects associated with the carcinogenic process. He commented on in vivo test systems, especially the heritable translocation assay where current emphasis is on characterizing false negative and positive error rates, the biochemical specific locus test, and systems measuring single gene mutations in eukaryotes. He discussed several of the short-term in vitro tests being used including collaborative studies with the NIEHS using hepatocytes to measure unscheduled DNA synthesis (UDS) in response to chemicals. Dr. Casciano reported on UDS studies with five antihistamines, and the cytogenetic activity of 1-, 3- and 6-nitrobenzo(a)pyrenes in Chinese hamster ovary (CHO) cells.

(1) Genetic Toxicology: Dr. Suzanne Morris reported that her laboratory's aim was to identify types of DNA damage that are important in biological responses caused by chemicals. She discussed studies with four alkylating agents where the endpoints measured were mutation induction at the HGPRT locus, cell survival, and sister chromatid exchanges (SCE)/chromosome. Methylating agents were more toxic than the ethylating agents studied. As expected, there was a strong inverse correlation between increased frequency of SCE/chromosome and decreased cell survival for all four chemicals. DNA-adduct formation in CHO cells was quantified and correlations were made with the biological responses observed for the alkylating agents. Similar studies were described for 1-aminopyrene, 2-aminofluorene, and 3, 3'-dimethylbenzidine. Conclusions were that little correlation is seen between either SCE formation or cytotoxicity and mutation but there is a predictive relationship between alkyl guanine formation and mutation induction.

(2) Metal Toxicity: Dr. Joel Pounds described his group's studies on the effects of lead (Pb) on the cellular metabolism and function of calcium (Ca) in cultured cells. He said the factors which influence Ca metabolism also to a large degree regulate Pb metabolism and he commented on the key role of Ca-mediated processes in all compartments of the cells. Dr. Pounds said the group's hypothesis is that Pb ion intoxication alters the homeostasis of cellular Ca thereby modifying the second messenger function of Ca. The general experimental design was to incubate cultured rat hepatocytes with Pb for 20 hours after which time steady state fluxes of Pb and Ca were achieved. Levels of Pb were used that did not produce overt intoxication of the cells. The studies showed the Pb treatment resulted in an increase in size of all three Ca pools, a finding which correlated with reported in vivo effects of Pb. Whether these

effects were due to increased cell permeability to Ca or decreased efflux is not clear. It could be concluded that cellular metabolisms of Pb and Ca are similar. Dr. Pounds mentioned that ongoing and future studies include Pb-zinc interactions, Pb-essential element interaction in primates and Pb-calmodulin interactions.

X. Progress Report on TDMS (Toxicology Data Management System): (Attachment 8) Mr. Gary Green, Director, Division of Toxicology Data Management Systems, stated that major objectives of the Division are to develop new ways to collect, store, and look at data, and to serve as a national resource for support of toxicology systems. He stressed the importance of interagency cooperation and how their working with other agencies reduced redundancy, enhanced efforts in standardization of computer systems and data, and maximized use of limited resources. Mr. Green decribed types of hardware being developed, including a microprocessor developed to meet the joint needs of NCTR and NTP. He related how the history of the TDMS started with the initial National Cancer Institute/NCTR discussions in 1976, and continued with installation of the first data terminals in a contract laboratory (Southern Research Institute) in 1981, and the first entry of data from a chronic test in November, 1981.

(1) Objectives and Philosophy of the TDMS: Mr. E. B. Fernstrom listed the goals of automating toxicology data collection and reporting: (a) ensuring consistency of data collection across laboratories; (b) ensuring data quality; (c) allowing for timely monitoring of data from a study; (d) flexibility in handling of data from a variety of study types; (e) enabling the experimental protocol to drive data collection; (f) facilitating Good Laboratory Practice compliance; and (g) serving as an information resource for management of research and testing data. Mr. Fernstrom discussed some of the specific premises around which the TDMS was developed. He then discussed the major components of the system: the protocol analysis system (PAS); the experimental information system (EIS), which has to do with in-life data collection; and the post-experimental information system (PEIS), which is focused on micropathology data processing as well as the central data base and reporting system.

(2) Implementation of TDMS at NTP Sites: Ms. Pat Straw, Systems Development Corporation, decribed production support provided for the TDMS by her and her staff. This includes: (a) applications support to the PAS, EIS, PEIS, and administrative data systems; (b) system setup and training at the contract laboratories performing long-term studies as well as at NIEHS laboratories; (c) providing experimental support during chronic and subchronic testing; and (d) providing site liaison. Ms. Straw and her staff demonstrated the data terminals now used in the laboratories.

(3) Future Plans for TDMS: Mr. Green reported on several new initiatives which are underway or planned. Among these are: (a) increasing the ease of terminal use; (b) increasing terminal

speed; (c) devising a better means of storing and recalling recurring observations; (d) incorporating voice recognition; and (e) developing a data system for gross pathology observations. Dr. Swenberg asked how TDMS was being received by the pathologists in the contract laboratories. Dr. McConnell replied that it was being received positively. At the first laboratory where TDMS was installed there was an estimated 30% savings in time realized compared with the previous data management system.

XI. Overview of the NCTR Director's Laboratory: Dr. Angelo Turturro said the main objective was to elucidate the molecular parameters important in extrapolation from mouse to humans and in the modulation of agent toxicity.

(1) Modulation of Asbestos Cytotoxicity: He discussed the basis for detoxification by heating, noting the hypothesis that heat may alter the electronic state of the asbestos fiber. Among alterations of the biological effects of chrysotile asbestos caused by heating were decreased binding to bovine serum albumin, decreased inhibition of phagocytosis by pulmonary macrophages, decreased LDH release by cultured trachea, and decreased selenium release by tracheal epithelial cells. Irradiation at least partially reversed the effects of heating on the endpoints measured. In examining possible mechanisms for the effects of heating, Dr. Turturro said there was no change in the physical state of asbestos including length to width ratio of the fibers. There did seem to be a change in electronic configuration allowing a freeing up of free radicals, especially peroxyl and hydroxyl species. In response to a question by Dr. McConnell, Dr. Turturro said no correlations had yet been made with *in vivo* effects of asbestos, e.g., whether heating would alter fibrogenic potential.

(2) Male versus Female Expression of Toxicity: Dr. Rick Charlton described studies aimed at explaining differences in spontaneous tumor formation between sexes of $B6C3F_1$ mice. In confirmation of literature values, he showed a 2.5 to 3.5 fold greater incidence of liver tumors in males than in females and with tumors developing at an earlier age. Their postulate was that the difference derived from a differential rate of liver development. Using flow cytometry to determine ploidy, it was shown that in the liver, males develop a higher ploidy rate, a higher percentage of cells with higher ploidy, and a faster rate of DNA replication, while females have a higher percentage of cells with lower ploidy. Thus, factors which may account for the differential rates of liver tumor formation could include: (a) a faster rate of DNA replication in males; (b) DNA repair difference with more time for repair in females due to slower replication; and (c) differences in metabolism in cells of differing ploidy. Dr. Mendelsohn said he couldn't see a connection between ploidy and DNA replication. Dr. Hart noted they were in the process now of determining total numbers of liver cells and total DNA.

XII. Overview of Biometry: Dr. David Gaylor, Division Director, said about 60% of the Division's efforts were devoted to collaborative research with other groups at NCTR, about 30% were in biometry research, primarily methods development, and 10% were with interagency programs. Collaborative activities were primarily in the areas of mutagenesis, teratogenesis, carcinogenesis, chemical toxicity, and animal husbandry. Specific projects in biometry research included development of computer programs for the analysis of chronic studies, and an evaluation of optimal experimental designs for the chronic bioassay which produced similar results to those obtained by Drs. Hoel and Poitier at NIEHS.

(1) Cause of Death Considerations: Dr. Ralph Kodell said that current information generally available from a chronic toxicity study includes age at death and types of tumors present, while in some studies a cause of death is assigned, e.g., studies at NCTR require this, and cause of death assignment will be required by the NTP. Cause of death information permits determination of a disease/death estimation adjusted by censoring for competing risks. Problems with assigning a cause of death relate to disagreement among pathologists as to the reliability of such diagnoses and experimental evidence that misclassification or equivocation can occur. One approach to these problems is to allow more flexibility in diagnosis so that there is an equivocal category. Dr. Kodell described some estimation techniques designed for handling survivorship type data, all nonparametric.

XIII. Overview of Chemical Toxicology (Gentian Violet and Sulfamethazine) and Chemistry Including Microencapsulation: Time constraints did not allow for presentations of these programs as planned although those interested were able to discuss them over lunch.

Prior to adjournment, Dr. Mendelsohn thanked Dr. Hart and the NCTR staff for stimulating and well designed presentations. The next meeting of the Board of Scientific Counselors will be held in Research Triangle Park, N.C., on August 16 and 17, 1984.

February 16, 1984
NOTICE OF MEETING
NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS

Pursuant to Public Law 92-463, notice is hereby given of the meeting of the National Toxicology Program (NTP) Board of Scientific Counselors, U.S. Public Health Service, in the Conference Room, Building 13, National Center for Toxicological Research (NCTR), Jefferson, Arkansas, on March 27 and 28, 1984.

The meeting will be open to the public from 8:30 a.m. to adjournment on March 27. The preliminary agenda with approximate times are as follows:

8:30 a.m. - 9:00 a.m.	Report of the Director, NTP
9:00 a.m. - 9:20 a.m.	Advisory Review Panel for NTP Reproductive and Developmental Toxicology Programs
9:20 a.m. - 10:00 a.m.	NIOSH/NTP Concept Reviews: a. Testing of Chemicals for Teratogenicity by Inhalation Exposure b. Review of a Study to Validate Drosophila as An In Vitro Method for Screening of Chemicals for Teratogenic Effects
10:15 a.m. - 12:00 p.m.	NIEHS/NTP Concept Reviews: a. Mutagens in the Urine of Rodents on Subchronic Toxicity Tests b. Mutagens from the Cooking of Food c. Mutagenicity Testing in Salmonella d. Chemical Induced Immunotoxicity e. NTP Repository and Archives f. NTP Quality Assurance Data Auditing Support

NCTR Programs

1:00 p.m. - 1:20 p.m.	Introduction and Overview
1:20 p.m. - 2:30 p.m.	Developmental and Reproductive Toxicology a. NCTR Research Program b. Pharmacodynamics
2:45 p.m. - 4:00 p.m.	Biochemical Toxicology - Overview a. Saccharin b. Promotion c. Aromatic Amines
4:00 p.m. - 4:20 p.m.	Microbial Toxicology
4:20 p.m. - 4:45 p.m.	Metal Toxicity

The meeting on March 28 will be open to the public from 8:30 a.m. to adjournment. The preliminary agenda with approximate times are as follows:

NCTR Programs (Continued)

8:30 a.m. - 10:00 a.m.	Progress Report on TDMS a. Objectives/Philosophy b. Implementation at NTP Sites c. Demonstration d. Future of TDMS
10:00 a.m. - 10:20 a.m.	Identification of Genetic Modulation of Toxicity
10:20 a.m. - 10:40 a.m.	Male vs. Female Expression of Toxicity
10:40 a.m. - 11:00 a.m.	Biometry - Maximizing Numbers of Animals
11:00 a.m. - 11:20 a.m.	Chemical Toxicology - Gentian Violet and Sulfamethazine
11:20 a.m. - 11:40 a.m.	Microencapsulation
12:45 p.m. - 2:30 p.m.	Tour of NCTR

The Executive Secretary, Dr. Larry G. Hart, Office of the Director, National Toxicology Program, P. O. Box 12233, Research Triangle Park, North Carolina 27709, telephone (919) 541-3971, FTS 629-3971, will furnish a roster of Board members and other program information prior to the meeting, and summary minutes subsequent to the meeting. For information on hotels and directions to the Center contact Mrs. Linda Vetsch at NCTR, telephone (501) 541-4516, FTS 542-4516.

Date

David P. Rall, M.D., Ph.D.
Director
National Toxicology Program

AGENDA

Board of Scientific Counselors
National Toxicology Program
March 27-28, 1984

Conference Room, Building 13
National Center for Toxicological Research
Jefferson, Arkansas

TUESDAY, MARCH 27, 1984

8:30 - 9:00 a.m.	Report of the Director, NTP	Dr. David Rall
9:00 - 9:20	Advisory Review Panel for NTP Reproductive and Developmental Toxicology Programs	Dr. Jeanne Manson
9:20 - 10:00	NIOSH/NTP Concept Reviews:	
	(1) Inhalation Development and Reproductive Toxicology	Dr. Bryan Hardin
	(2) Evaluation of Drosophila for Teratogen Screening	Dr. Bryan Hardin
10:30 - 12:00	NIEHS/NTP Concept Reviews:	
	(1) Testing the Urine of Rats on the 14-Day Prechronic Test for Mutagenic Activity	Dr. Errol Zeiger
	(2) Examination of Immunotoxicity by Chemical Xenobiotics	Dr. Michael Luster
	(3) Data Auditing Support Resources Contract	Dr. Scot Eustis
12:00 - 1:00 p.m.	LUNCH	

NCTR PROGRAMS

1:00 - 1:20	Introduction and Overview	Mr. Arthur Norris
1:20 - 2:20	Developmental and Reproductive Toxicology	Dr. John Young
	. Pharmacodynamics	-- Dr. William Slikker
2:20 - 2:35	BREAK	

Tuesday, March 27, 1984 (continued)

2:35 - 3:40	Biochemical Toxicology Overview	Dr. Fred Kadlubar
	• Comparative Metabolism of Aromatic Amines (NIOSH Study)	-- Dr. Kadlubar
	• Nitropolycyclic Aromatic Hydrocarbons, DNA Adducts, Mutagenicity	-- Dr. Frederick Beland
	• Saccharin	-- Mr. Robert West
3:40 - 4:00	Microbiology Overview	Dr. Carl Cerniglia
	• Microbial Toxicology	-- Dr. Cerniglia
4:00 - 5:00	Mutagenesis Research	Dr. Daniel Casciano
	• Metal Toxicity	-- Dr. Joel Pounds
	• Genetic Toxicology	-- Dr. Suzanne Morris

WEDNESDAY, MARCH 28, 1984

8:30 - 10:00 a.m.	Progress Report on TDMS	Mr. Gary Green
	• Objectives/Philosophy	-- Mr. E. B. Fernstrom
	• Implementation at NTP Sites	-- Ms. Pat Straw
	• Demonstration (with break)	-- Ms. Pat Straw
	• Future of TDMS	-- Mr. Gary Green
10:00 - 10:40 a.m.	Overview of Director's Laboratory	Dr. Angelo Turturro
	• Modulation of Asbestos Cytotoxicity	-- Dr. Turturro
	• Male vs. Female Expression of Toxicity	-- Dr. Rick Charlton
10:40 - 11:05	Overview of Biometry	Dr. David Gaylor
	• Cause of Death Considerations	-- Dr. Ralph Kodell
11:05 - 11:25	Overview of Chemical Toxicology (Gentian Violet; Sulfamethazine)	Dr. Neil Littlefield

Wednesday, March 28, 1984 (continued)

11:25 - 11:45	Overview of Chemistry	Dr. Ronald Mitchum
	. Microencapsulation	-- Mr. Harold Thompson
11:45 - 12:45	LUNCH	
12:45 - 2:30 p.m.	Tour of Facility	Dr. Hart/Mr. Norris
	. Bldg. 14 . Bldg. 6 . Pathology . Chemistry . Mutagenesis/Teratogenesis (Bldg. 53)	
2:30 -	Vans Depart for Airport	

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National Toxicology Program
Concept Review

Concept Title: Inhalation Development and Reproductive Toxicology

Period and Type of Award: 5 years, Interagency Agreement with the Department of Energy, Pacific Northwest Laboratories

Funding ($ in thousands): FY 1984 = $987; FY 1985 = $1037; FY 1986 = $1089; FY 1987 = $1143; FY 1988 = $1200; Total = $5456

Funding Mechanism: Interagency Agreement

A. Objective: The purpose of this agreement is to provide the National Toxicology Program (NTP) with a mechanism to test chemicals for developmental and reproductive toxicity using inhalation as the route of exposure.

B. History and Need: Until FY 1980, NIOSH annually awarded contracts for inhalation teratology/reproductive toxicology studies, and these served as the NTP effort in this area. No contract or other efforts of this type, however, have been undertaken by NIOSH or other NTP agencies since FY 1980, and the NTP Board of Scientific Counselors in their March 1983 review of the NTP Reproductive Toxicology Program considered the lack of inhalation studies to be a major deficiency in the overall program. The Board strongly recommended that activity in inhalation reproductive toxicology be resumed at the earliest possible time. This proposed Interagency Agreement has been prepared in response to the Board's direction.

C. Relevance: This project is an integral part of the overall Reproductive Toxicology Program. It is essential to the proper testing of many chemicals, inhalation being the predominant or only route of exposure for many industrial and environmental agents. For many of these compounds, tests performed by other routes of exposure are not acceptable substitutes for inhalation studies and cannot serve as adequate or appropriate estimations of the possible human health hazard.

D. Priority: Due to the importance of inhalation as a route of exposure in reproductive toxicity testing, as reflected in the comments and recommendations of the NTP Board of Scientific Counselors, this should be regarded as a high-priority effort.

E. Approach: Under the provisions of the proposal (copy attached), NTP will select chemicals for testing and supply copies of NTP literature reviews and safety and health guidelines. Ordinarily test chemicals will also be supplied by the NTP. The Department of Energy (DOE) Pacific Northwest Laboratories will review the relevant literature and recommend to NTP appropriate reproductive toxicity testing. The agreement will be flexible so that a wide variety of testing procedures can be followed, selecting those systems most appropriate to addressing specific research needs identified for each chemical. Upon selection by

the NTP Project Officer of the tests to be performed, the DOE laboratories will perform the necessary preliminary studies, to include preparing project protocols and SOPs tailored to the specific testing needs, developing appropriate systems to generate exposures and analytical methods to monitor chamber atmospheres, conducting acute toxicity testing and dose range-finding studies for the various reproductive tests selected, etc. Upon completion of these studies, a report will be prepared from which the NTP Project Officer will select exposure conditions for the definitive studies. Studies will then be conducted and a draft final report submitted for each test performed. Following a review of draft reports by the NTP Project Officer, members of the NTP Reproductive and Developmental Toxicology Work Group, the NTP chemical manager or other NTP scientists as appropriate, or members of the NTP Board of Scientific Counselors Ad Hoc Advisory Panel on Reproductive Toxicology, the final report will be prepared by the DOE scientists and submitted to the NTP.

It is anticipated that the Memorandum of Understanding between the NTP and NIOSH will be modified to provide for Dr. Bryan D. Hardin of NIOSH to serve as a Co-Project Officer, along with Dr. Bernard Schwetz of NIEHS, for this NTP/DOE Interagency Agreement. A wide variety of test systems is expected to be employed at one time or another and thorough monitoring of all aspects of the project will be critically important. This will require frequent site visits by the Project Officers or other NTP personnel, such as NIOSH or NTP quality assurance teams and various members of the NTP Reproductive and Developmental Toxicology Work Group. Travel orders for these site visits should be issued by the DOE and charged to the project. This will conserve the limited travel budgets of the NTP agencies and encourage participation as needed by appropriate staff members from all of those agencies.

National Toxicology Program
Concept Review

Concept Title: Evaluation of Drosophila for Teratogen Screening

Period and Type of Award: 28 months--Interagency Agreement

Funding ($ in thousands, assuming June, 1984 initiation): FY 1984 = $25.4, FY 1985 = $76.2, FY 1986 = $76.2, (Total = $177.8)

Funding Mechanism: Interagency Agreement

A. History and Need: The need for rapid toxicologic assessment test systems is great. This need is especially important in the area of reproductive effects where large numbers of new chemicals are introduced into commerce annually and each one cannot undergo vigorous examination by more definitive testing due to time, money, and personnel limitations.

In FY 81 NIOSH developed a teratology screen using the fruit fly. Early test results revealed a dramatic response of the adult fly following treatment during developmental (larval) stages. This work was presented at the NTP-sponsored "Consensus Workshop On In Vitro Teratogenesis Testing" held August 2-5, 1981 in Arkadelphia, Arkansas (Schuler, Hardin, and Niemeier, Drosophila as a Tool for the Rapid Assessment of Chemicals for Teratogenicity, Terat Carcin Mutag 2:293-301, 1982). A reprint of this paper is attached. FY 82 and FY 83 efforts investigated a series of 17 chemicals including 15 known teratogens and 2 "nonteratogens" (coumarin and colchicine) The results of this study showed 14 of the 15 known teratogens exhibited "strong" effects in the flies, while the remaining teratogen (ethanol - to which the fly has a known resistance) and the two "nonteratogens" provided significantly "weaker" activity. These results were presented at the "Fourth Annual Meeting of the American College of Toxicology" held November 30 to December 2, 1984 in Arlington, Virginia, and will be published in the American College of Toxicology Journal.

B. Objective: Based upon the results of the NIOSH methods development and testing, the Drosophila test system appeared to be promising as an "in vitro" (i.e., nonmammalian) teratogenesis screening system. A Project Peer Review Group recommended that the test system be applied in a second laboratory to confirm the observations made by NIOSH, to optimize test conditions and procedures, and to develop a much broader data base on the responsiveness of Drosophila to known mammalian teratogens and nonteratogens. The objective of the proposed project is to address those recommendations.

C. Approach: Dr. P. G. Kale, of the Brookhaven National Laboratories (D.O.E. - Upton, New York), will be the Principal Investigator in an Interagency Agreement established between the NTP and Brookhaven to evaluate the Drosophila test system developed by NIOSH. Dr. Kale is an experienced researcher whose work has employed a variety of mutation assays using the fruit fly. The project, to be monitored by NIOSH, will use this system to

test a series of up to 47 chemicals. These chemicals were selected by the ad hoc committee formed during the Arkansas workshop and were chosen as standards with which to validate in vitro teratology screens (Smith, et al., A Selection of Candidate Compounds for In Vitro Teratogenesis Test Validation, Terat Carcin Mutag 3:461-480, 1983).

A total of 90 screening tests will be performed. Each test will employ a range of six chemical concentrations including a control. Thus dose-response relationships will be investigated. These concentrations will be determined during preliminary range-finding tests. For each test a single pair (male and female) of adult virgin flies is introduced into a culture vial. Within each vial is a standard Drosophila medium containing the test chemical. The flies mate and the female deposits fertilized eggs upon the surface of the medium. After 20 hours these parent flies are discarded. The eggs begin to hatch approximately 24 hours after deposition and the emerging larvae begin to feed upon the medium. Following metamorphosis through three larval stages and one pupal stage (in about 10 days), the adult flies are scored for abnormalities. Flies are viewed at 25X magnification under a binocular microscope and inspected for gross morphological deformities. The incidences of specific types of abnormalities are recorded and statistically compared to control values. Screening tests for each chemical will be performed until statistically significant differences are recorded between treated and control groups, for up to 5 times if no effects are detected. This will be done in order to generate a large data base and to establish the numbers of flies that may be required for routine testing. Therefore, based upon the requirement of performing 90 tests, as many as 90 or as few as 18 chemicals will be investigated using this test system. The attached RFC contains the details and requirements (protocol and SOP) for project performance.

Attachment 5

March 27, 1984
Dr. Errol Zeiger
Environmental Mutagenesis
Section, CGTB

National Toxicology Program
Concept Review

Title: Testing the Urine of Rats on the 14-Day Prechronic Test for Mutagenic Activity

Period of Award: 4 year Contract

Funding ($ in 000): FY 1985 $30; FY 1986 $32; FY 1987 $34; FY 1988 $36 (Total $132)

Funding Mechanism: Competitive Award

Objective:

The objective is to test approximately 48 chemicals (12 chemicals per year) for mutagenicity by sampling the urines from rats in 14-day prechronic (repeated dose) tests. The urines will be collected once, after 7 days of treatment, and tested for direct-acting mutagens, S-9 requiring mutagens, β-glucuronidase-requiring mutagens, and for mutagens that require both β-glucuronidase and S-9. This will permit an assessment of whether rats on the 14 day prechronic tests excrete mutagens in their urine, and an evaluation of the urine mutagenicity assay with respect to chemical disposition studies, and eventually, as a predictor of carcinogenesis.

Background:

The measurement of mutagenicity of urine has been used to study the in vivo metabolism of chemicals; to compare in vitro to in vivo metabolism of chemicals; to monitor exposure of laboratory animals to chemicals; and to monitor humans exposed to cigarette smoke, drugs, and to chemicals in the workplace. The literature through 1979 has recently been reviewed (Legator et al., 1982), and an additional 67 references (where Salmonella is the test organism) have been published since 1979 (EMIC, 1984). Chemicals administered to laboratory animals (and humans) can appear in the urine as the free parent chemical, as one or more metabolites, or conjugated with glucuronic acid, cysteine, or other substances. Because chemicals administered to laboratory animals in the diet or by other routes are subject to metabolism and detoxification by the intact, in situ organs, it is likely that many of the metabolites formed would be different, or in different proportions, than in the in vitro 9000xg liver homogenate routinely used in the Salmonella test. Additionally, there is the possibility that some chemicals that are not mutagenic in vitro might be metabolized to mutagens in vivo.

Although not all metabolites are excreted in the urine, urine assays have been used successfully in laboratory animals to test the in vivo metabolism and mutagenicity of aromatic amines, benzidine and dimethylaminoazobenzene dyes, cigarette smoke, antineoplastic drugs, food mutagens, and other chemicals. The urine mutagenicity procedure has not previously been used by the NTP.

Approach:

The Salmonella test using rat urine offers an opportunity to determine whether or not mutagenic urinary metabolites are produced from chemicals being administered to rats daily, for one week. If positive results are obtained in the urine assay, these results will be compared to the in vitro Salmonella preincubation test and evaluated with respect to whether or not the urinary mutagen(s) are direct acting or require β-glucuronidase (or other deconjugating enzymes) and/or rat liver S-9 for activity. (Negative results in the urine assay cannot be extrapolated further.) The mutagenicity results will also be evaluated against whatever chemical disposition data are available on the test chemicals. This will provide information on the ability of the rat to metabolize the test chemical to a mutagen in vivo and preliminary information regarding the activation and/or detoxification of the chemical. The results from positive urine assays will eventually be compared to the results from lifetime bioassays in the rats in order to determine whether or not the presence of mutagenic substances in the urine are an accurate predictor of carcinogenesis.

Reference:

Legator, M.S., E. Bueding, R. Batzinger, T.H. Conner, E. Eisenstadt, M.G. Farrow, G. Ficsor, A. Hsie, J. Seed, and R.S. Stafford (1982). An evaluation of the host-mediated assay and body fluid analysis. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutation Res. 98: 319-374.

February 22, 1984
Dr, Michael Luster
Head, Immunotoxicology Group
Systemic Toxicology Branch

Attachment 6

NATIONAL TOXICOLOGY PROGRAM

CONCEPT REVIEW

Title: Examination of Immunotoxicity by Chemical Xenobiotics

Period of Award: Five (5) years

Funding (in thousands): FY 1985, $400; FY 1986, $420; FY 1987, $440; FY 1988, $460; FY 1989, $480 (TOTAL: $2,200)

Funding Mechanism: Contract

Objective:

To measure altered host resistence and immunological impairment in rodents exposed to chemical xenobiotics of specific interest to the NTP. Plans are to provide one contract award.

Background:

Correlations have been established between the administration of chemical immunosuppressants and an increased incidence of infectious diseases and neoplasia. The evidence for increased bacterial, viral, fungal and parasitic diseases in patients on chronic therapy with immunosuppressive chemicals has been well documented by Allen (1). Likewise, McKhann (2) and more recently Penn (3) and Calne (4) observed that the incidence of cancer in renal transplant recipients on prolonged immunosuppressive chemotherapy was higher than in the general population. In another respect, epidemiological evidence has indicated that xenobiotics may act as predisposing agents in patients who develop Acquired Immunodeficiency Syndrome (e.g. 5) or development of hypersensitivity disease (rev. in 10).

Studies in laboratory animals have supported these clinical observations and demonstrated that extended periods of treatment with immuno-suppressants enhance the incidence of UV, viral or chemically induced tumors in rodents (e.g. 6-8). The mechanisms and relationships between altered host resistance/immune dysfunction and chemical-induced injury are complex, poorly defined, and of biological relevance. Chemicals of environmental concern which have been reported to be immunotoxic in experimental animals include halogenated aromatic hydrocarbons, polycyclic aromatic hydrocarbons, benzene, diethylstilbestrol, certain organo and heavy metals, dimethylnitrosamine, etc. from experimental studies (rev. by 9). Certain of these compounds, including diethylstilbestrol and halogenated aromatic hydrocarbons, may produce similar immunological effects in humans following inadvertent or therapeutic exposure (10). Of major concern is the determination of potentially immunotoxic chemicals and the role of the ensuing immunosuppression on tumorigenesis or infectious disease processes.

In addition, a number of industrial chemicals have been shown to induce hypersensitivity or autoimmunity in humans and laboratory animals such as TDI (rev. in 10). To extrapolate these chemically-induced immunologic effects from experimental studies to humans, no effect levels, structure activity relationships, and mechanisms immunomodulation of need to be more clearly defined.

Approach:

Previous NIEHS contracts awarded to Medical College of Virginia and IIT Research Institute have provided a comprehensive screening panel for measuring altered host resistance and immune modulation in rodents exposed to xenobiotic chemicals. The tasks in this previous project over a four-year period included: (a) evaluating host resistance assays to bacteria, viruses, animal parasites and transplantable tumors; (b) establishment and proficient demonstration of a standardized set of immunologic tests; and (c) integration and validation of the test systems for altered host resistance and immunological function using at least five chemicals.

Utilizing this screening panel (Table 1), five chemicals per year will be examined (total 25). Chemical nominations will be provided by NIEHS, regulatory agencies or private organizations. Final chemicals selected will be determined by an *ad hoc* committee chaired by the Project Officer. Data obtained from this screen will allow for extrapolation with a reasonable degree of confidence regarding the safety of the drug or chemical for the immune system under the conditions defined. Two chemicals per year (total of 10) that are positive (immunotoxic) in the screen will be evaluated in detail as part of this contract using additional *in vitro* and *in vivo* assays to determine the cellular and molecular events associated with the onset of immunomodulation. Depending upon results obtained in the primary screen, this may involve combinations of any of the following: (a) hypersensitivity testing; (b) enumeration and function of lymphocyte subpopulations; (c) expanded host resistance studies; (d) tests for autoimmunity (e) bone marrow function; (f) evaluation of structure-activity relationships; and (g) employment of various co-culture systems. All of these methods are well defined in mice, and many are used in clinical evaluation of humans.

This combination of testing and elucidation of mechanism should provide the best possible estimate of chemical safety relative to the human immune system, with respect to suppression, hypersensitivity or autoimmunity in the absence of clinical studies.

References:

1. Allen, J,C. (1976): In: Injection and the Compromised Host, Williams and Wilkins Co., Baltimore.

2. McKhann, R.S, (1971): Transplantation, 8: 209,

3. Penn, I. (1982): In: Current Problems in Cancer, Year Book Medical Publisher, New York, pp. 5-64.

4. Calne, R, (1979): Immunol. Rev., 46: 113.

5. Goedert, J,L. et al. (1982): Lancet, 20: 412.

6. Allison, A,C. and Friedman, R,M. (1966): J. Natl. Cancer Inst., 36: 859.

7, Outzen, H,C, (1980): Int. 1. Cancer, 26: 87,

8. Kalland, T, and Fonsberg (1981): Cancer Res., 41: 5134.

9. Dean, J.H. and Luster, M.I. (1982): In: Immunopharmacology, Elsevier Biomed. Press, New York, pp. 349-377,

10. Dean, J.H., Luster, M.I., and Munson, A.E. (editors)(1984): Target Organ Toxicology Series: Immunotoxicology, in press.

TABLE 1

Screening Panel for Detecting Immune Alteration

Following Chemical and Drug Exposure in Rodents

Parameter	Procedures Performed
Immunopathology	Hematology - complete blood count with differential Weights - body, spleen, thymus, kidney, liver. Cellularity - spleen Histology - spleen, thymus, bone marrow, lymph node
Host Resistance	Susceptibility to transplantable syngeneic tumor (TDg.20 of PYB6 sarcoma), bacterial; (LDQ.20 of Listeria monocytogenes), viral (LDQ.20 of EMC or influenza) and parasite (Plasmodium yoellii-parasitemia)
Cell-Mediated Immunity	Lymphocyte blastogenesis to mitogens (PHA or Con A and LPS) and allogeneic leukocytes in mixed leukocyte culture; delayed hypersensitivity response and B- and T-cell enumeration
Humoral-Mediated Immunity	Enumerate IgM and IgG antibody plaque-forming cells to T-dependent antigen (SRBC)
Non-Specific Immunity	Quantitation of cell number, phagocytic ability, and basal and activated ectoenzyme levels of peritoneal cells; natural killer cell activity in spleen

March 27, 1984
Dr. Scot Eustis
Quality Assurance

Attachment 7

National Toxicology Program

Concept Review

Title: Data Auditing Support Resources Contract

Period of Award:

The present contract will be awarded for three (3) years. However, it is requested that the concept review be for a period of ten years as this will be a continuing need of the NTP testing program. The present three year contract is based on the high level of effort required to audit studies given high priority by Executive Committee agencies, all GSRI studies, and all studies being completed within the next three years. By making the contract for three years it will be easier to adjust the level of effort required as the backlog of studies diminishes.

Funding ($ in 000): FY 1985 - $926; FY 1986 - $919; FY 1987 - $965. (Total - $2,810)

Funding Mechanism: Resource Contract.

Objective:

The objective is to provide an audit support resource for NTP to assure that data collected at NTP testing facilities satisfy Good Laboratory Practices and are scientifically adequate to support the interpretative conclusions in the NTP Technical Report series. Through this auditing resource NTP will be better able (1) to assure the integrity and scientific quality of the data, (2) to validate the data presented in the Technical Reports and other publications, and (3) to determine procedures which need to be incorporated in the present testing program to avert future problems. Areas of concern are adherence to protocols and standard operating procedures, adequacy of data collection, specimen collection and preservation, thoroughness of pathological evaluation, and accuracy of reporting.

Background:

The National Toxicology Program provides information about potentially toxic chemicals to regulatory and research agencies and to the public. Toxicological and carcinogenicity data are derived from chemical testing conducted both in-house and through research contracts. Experience has shown that monitoring for GLP compliance through laboratory inspections, site visits, and evaluation of routine laboratory reports are insufficient to ensure the scientific validity of the data. Auditing of the raw data (laboratory records of procedures, chemical analyses, dose preparation and administration, clinical evaluation and data collection, pathological evaluation and data recording, etc.) is necessary for this assurance.

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

Attached is an overview of the Toxicology Data Management System now being used for data collection in the NTP chronic rodent testing program.

Following that is a summary of the research effort by each division at NCTR. The summaries are extracted from NCTR's Ongoing Research compilation which details each project and is printed annually. If the reader desires specifics of the research projects, the entire volume will be available at the Board meeting.

Toxicology Data Management Systems

OVERVIEW

The Toxicology Data Management Systems (TDMS) are the result of two federal entities with a common problem working together toward a single solution. The two entities are the National Toxicology Program (NTP) and the National Center for Toxicological Research (NCTR). The problem was how best to capture, maintain, report, and ensure the integrity of data resulting from toxicological testing.

General Information

TDMS was designed to provide a mechanism whereby all studies can be defined using a common set of primitives which, when combined, allow the construction of automated interpretable study parameters. This study definition process takes place on a centralized IBM mainframe computer.

A study defined through this process can be transmitted to the intelligent data collection terminal (IDCT). The IDCT is a Z80 microcomputer-based terminal specifically constructed to meet the rigors of laboratory use. It includes such features as touch screen, digital balance interface, multiple communication protocol support, and on-board disk storage.

The application software which resides in the IDCT is designed to provide user prompts and data verification based on the contents of the protocol "download" from the central mainframe. Study data is collected on the IDCT; then it is transmitted to the central mainframe for posting to the master archival data bases.

After the central mainframe receives the data, the data bases are available for reporting. A prospective user must obtain a TDMS operator ID number and password to access data which resides on the master data bases. Via the Experiment Status System (ESS), users at diverse locations can browse stored reports. This eliminates the need for generating and distributing multiple hard copies of reports.

The following are descriptions of the application units which comprise the flow of information through the TDMS.

Protocol Analysis System

Studies are defined to TDMS via the Protocol Analysis System (PAS) which resides on the mainframe. The PAS is responsible for recording study parameters such as:

- compound on test;
- route of administration;
- frequency of administration;
- identification of lab conducting study;
- identification of responsible parties associated with the study;
- procedures to be followed in the animal room and in pathology;
- terminology to be made available to the animal room technician and pathologist for recording findings;
- environmental conditions under which the test will be conducted; and
- architecture of the study (number of cages and their contents).

Parameters such as these make up the PAS data base (PEX). These parameters serve three main functions:

1. They drive the user-prompting and data validation processes on the IDCT.

2. They provide the information necessary for the reporting program to interpret collected data.

3. They allow later users to determine the exact methods used in conducting a given study.

Reports are available which validate the logical relationships among the components of the PAS defined study and present them in an understandable format.

Since the IDCT is not permanently connected to the central mainframe, the PAS parameters must be transferred to the IDCT through a process called "download." Download accesses the PEX data base, formats the parameters needed by the Experiment Information System (EIS) or Post Experiment Information System (PEIS), and transfers the parameters to the IDCT. The download process can be executed at the remote laboratory; or it can be carried out at the central site, and the resulting floppy disks (recording media used by the IDCT) can then be mailed to the laboratory. The download process not only transfers information from one machine to the other, but also it resolves differences between the internal codes used by the two computers.

Experiment Information System

The EIS is the automated system designed to collect, store, and report information resulting from in-life studies. Based on the downloaded protocol, the EIS provides user prompting and data collection for such items as:

- data entry operator ID;
- old/new feeder/bottle weights;
- cage conditions;
- animal conditions;

- dose volumes administered (injection/gavage); and
- cage and animal notes.

The EIS provides prompts based on a study-relative calendar. This is possible because all animal room experiment activities are day-dependent in the protocol.

Data elements are validated in at least three ways as they are entered:

1. Current data and previously collected data undergo logical comparison. For example, if an animal was reported as dead in a previous observation period, then it cannot be reported as alive today.

2. Data is screened for percentile changes which fall outside percentage windows established in the protocol. For example, current animal weight is compared to previously collected animal weight to identify unrealistic gains or losses.

3. Data is subjected to specific value checks based on the downloaded protocol; therefore, a user cannot enter a value which is not part of the protocol. (This check usually concerns vocabulary items such as clinical observations.)

Collected data can be shipped immediately to the central mainframe, or it can be reviewed at the laboratory via EIS reports which reside on the IDCT. The reports available on site provide limited data review and are geared toward study management. They include summaries of animal removal, cages not observed, dosing information, and transaction information.

New EIS-related capabilities to be introduced in the future include additional dose route options, remote error correction, and a major new component in support of animal quarantine and allocation.

Post Experiment Information System

The PEIS is the automated system designed to collect, store, and report information resulting from those activities which occur after an animal has been removed from test. A Microscopic Pathology system is already on line. Eventually, the PEIS will include Gross Pathology and Clinical Pathology systems also.

The PEIS receives two major components in the download: the study architecture (cages, animals, treatment groups, etc.), and the terms to be used. The central mainframe has a highly structured Pathology Code Table (PCT) which lists items such as systems, organs, sites, morphologies, and qualifiers. The PCT also establishes the valid relationships among these items. Terms are selected from the PCT for inclusion in the PAS download. These items make up the vocabulary which will be available to pathologists at the PEIS terminal. The

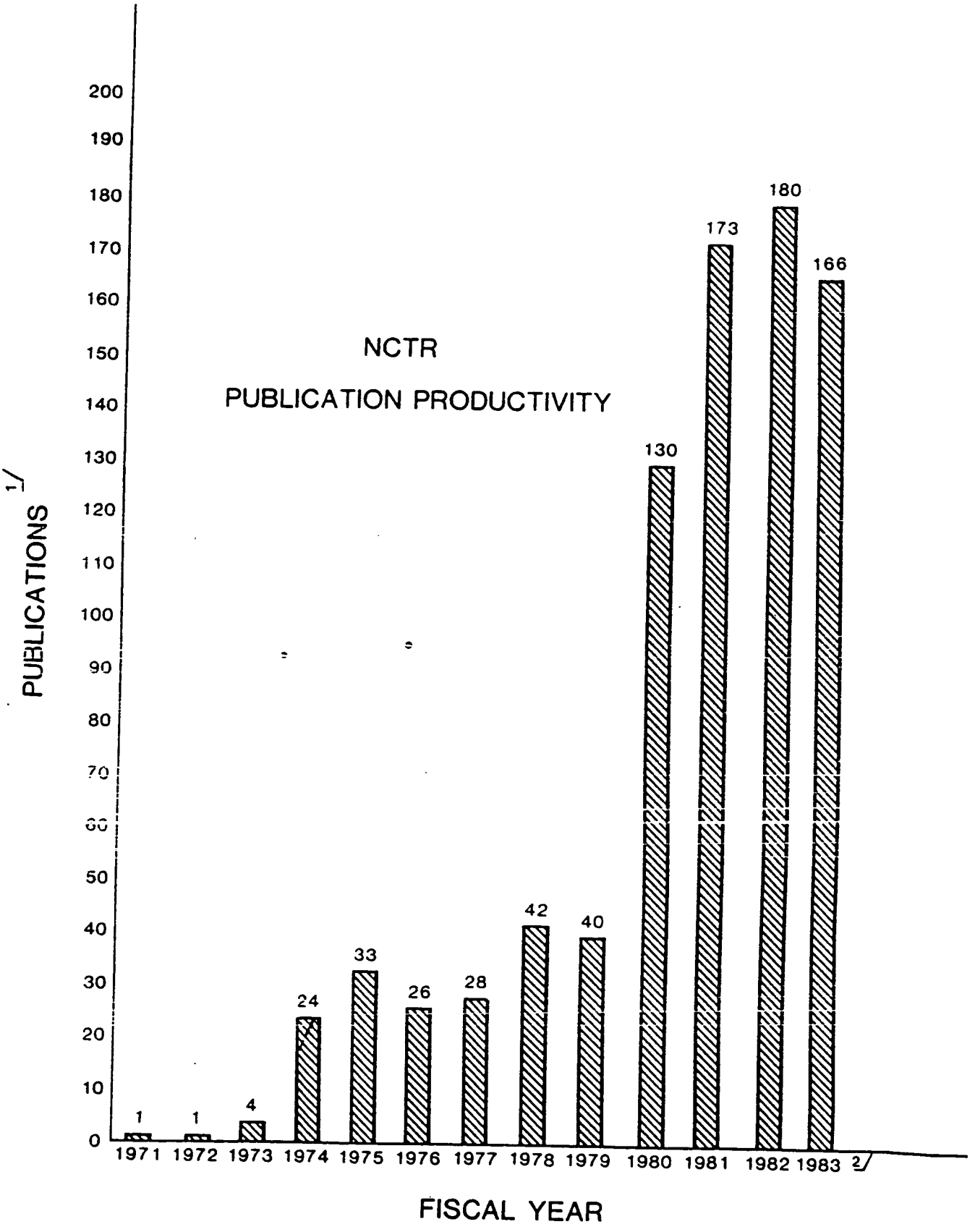
primary function of the Microscopic Pathology system is to record histopathological findings from reading the slides of test animals. The system provides the pathologist a tool which will construct observations using the pre-defined set of downloaded base terms. In the event a term is needed which was not downloaded, the pathologist may add that term via a mechanism which ensures only valid terms are added and that they are used correctly.

Some other data collection features available through PEIS are:

- tracing grossly observed lesions;
- recording status of accountable sites;
- establishing organ accountability/status;
- forced qualification of specific morphologies;
- definition and use of a constructed observation; and
- target organ definition.

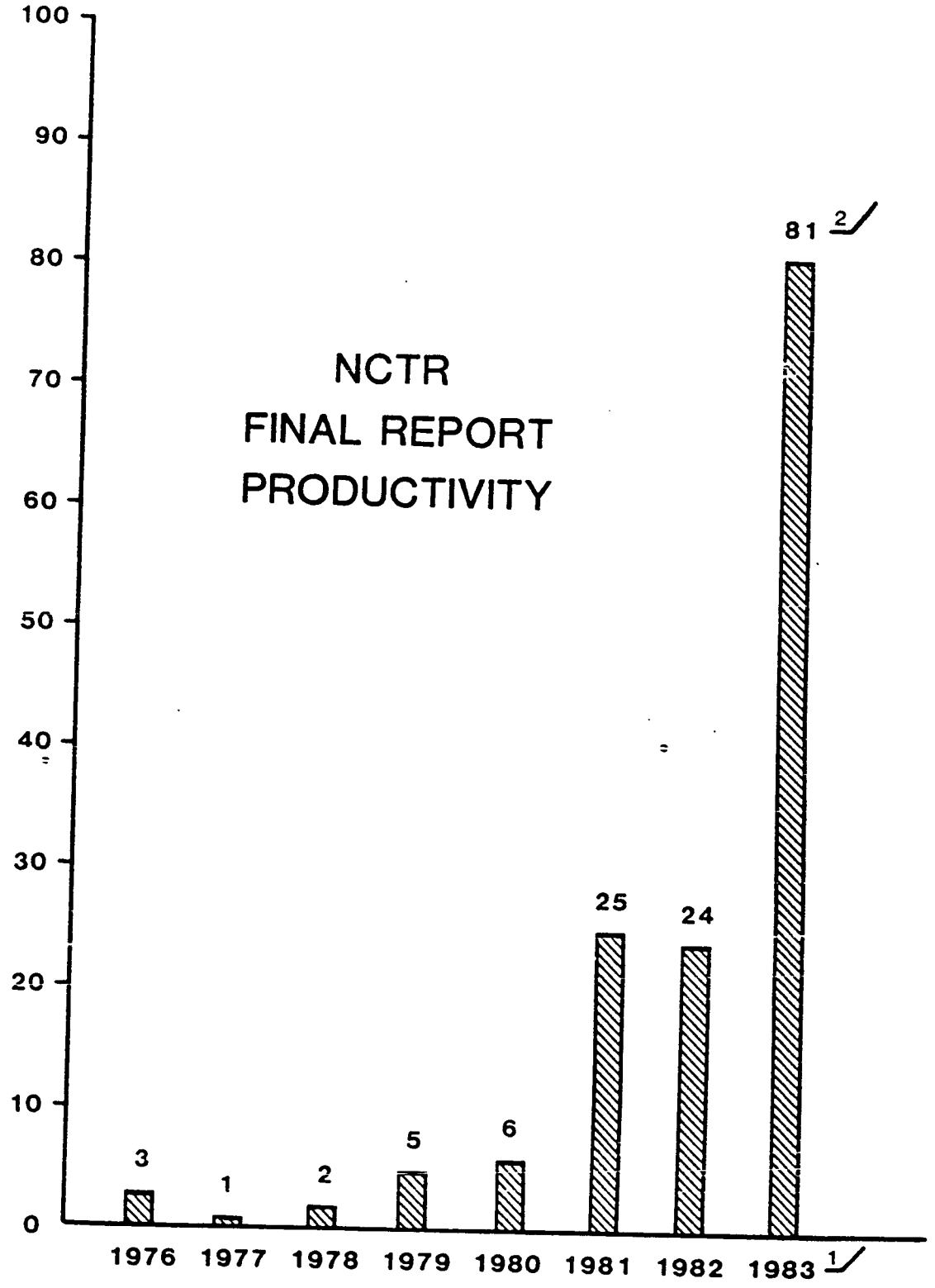
In addition to data collection, the PEIS terminal may be used to generate basic reports at the laboratory before data is transmitted to the mainframe. These include summary reports which tabulate by treatment group or experiment such items as organ status and morphologic findings. A detailed report, by animal, of all current Microscopic Pathology data collected also is available.

EIS and PEIS provide powerful, flexible, structured tools for the collection of data resulting from the conduct of animal studies. After data is collected on either of the systems, it is transmitted to the mainframe for inclusion in the master data bases. When the laboratory is notified that data was received successfully, it then can request mainframe reports to be generated and placed on ESS for review, or it can request hard copies of reports--or both.



1/ DOES NOT INCLUDE ABSTRACTS, PRESENTATIONS, OR FINAL REPORTS

2/ THRU SEPT 1983



1/ THRU SEP 1983

2/ DOES NOT INCLUDE 37 FINAL REPORTS FOR CPSC INTERAGENCY AGREEMENT

EXTRAPOLATION PROGRAM

Ronald W. Hart, Ph.D., Director

The primary goal of toxicology is to predict the potential health impact of deleterious substances on the human population. In order to achieve this goal, toxicology operates within two paradigms for research on the action and effect of toxic substances. One, which we can call the ontogenic, seeks to account for or describe as a function of dose, time, or route of administration, the effect of a toxic substance on cells and tissues of an organism. The other, which can be called the evolutionary/-comparative approach, looks for differences (or causes for such differences) in the genetically defined population exposed to toxic materials.

The former approach dominates the contemporary research scene in toxicology and will continue to do so for it produces important descriptive information which is useful in the extrapolation of high-dose to low-dose effects (existence of thresholds) within biological systems. However, this approach is not sufficient by itself to solve the problem of extrapolation between species, since the ontogenic approach to toxicology focuses primarily on effect rather than cause and difference. An observed toxic effect, no matter how close to the fundamental biosynthetic or operative processes it may be, is still a species-specific effect for which there must be an antecedent cause or difference between species to explain differences in response. Thus, while extrapolation from high-dose to low-dose effects generally utilizes the ontogenic paradigm, extrapolation between species relies primarily upon the evolutionary/comparative paradigm. This approach, while the least studied, is obviously the most critical in the establishment of risk in human populations since most toxic substances are studied in laboratory animals rather than in human populations due to the inherent difficulties and ethical considerations involved in the latter studies.

The chemical evaluation mission of NCTR concentrates, at present, predominately on the ontogenic approach in the determination of threshold doses of compounds of major sociological or economic importance. The methods development and basic science missions of NCTR operate across both paradigms, supplying new techniques and approaches to the elucidation of both thresholds and interspecies and target tissue comparisons. These three missions, therefore, all culminate in the development of better data bases and methods for determination of risk within human populations.

There are many important factors in the extrapolation of animal to man, including DNA repair (especially in different species), longevity, proliferative potential, gene expression, either in normal development or in cancer, fidelity of DNA replication. Methods are being developed in areas such as these to allow proper analysis of these factors. Information is also being obtained on basic mechanisms in teratology and whole animal variables, such as circadian rhythm to deepen the understanding at all levels of organization, from subcellular to whole organism. Comprehensive studies will be necessary before data obtained from model systems will be applied at the many levels in biological organisms, as is needed, to truly provide surrogate information for humans. This application is the organizing principle of many of the studies in this division, and in the NCTR as a whole.

OFFICE OF SCIENTIFIC INTELLIGENCE

Lawrence Fishbein, Ph.D., Director

The Office of Scientific Intelligence (OSI) plays a vital role in the National Toxicology Program (NTP) for testing potential chemical carcinogens. OSI provides the expertise and technical assistance for reviewing and evaluating the published literature on chemicals which have been nominated for consideration as candidates for testing. An executive summary is prepared on each chemical which provides the informational basis for chemical selection and for setting priorities on chemicals. These reviews prepared by OSI also are used in making decisions pertaining to what kinds of toxicological tests are needed on a particular chemical.

NCTR provides a significant portion of the actual chemical testing of the NTP. Protocols are prepared under NTP guidelines to assess the toxicology of each selected chemical. These include acute, subchronic, and chronic studies. When the need for special studies, such as reproduction or metabolism studies, is indicated they are included in the overall experimental approach. Results of the experimental studies are presented in the form of final reports to NTP and significant findings are communicated to the appropriate regulatory agency. OSI serves as a focal point for evaluating the results and coordinating the needs of the various regulatory agencies pertaining to evaluating the results and identifying the need for additional needs.

MAMMALIAN GENETICS IN TOXICOLOGY PROGRAM

George L. Wolff, Ph.D., Director

Environmental toxicants damage the genetic constitution directly (mutations), alter its phenotypic expression (birth defects), or exert either or both effects in different instances (cancer). In each case the response of the organism and its constituent tissues to the presence of a toxicant, especially at environmental dose levels, is determined by the expression of the characteristic gene patterns comprising its genetic constitution. The activation or detoxification of a chemical and DNA repair are examples of metabolic processes, intimately involved in determining the tissue response to environmental chemicals, which are controlled by particular gene patterns.

In general, differences between gene patterns controlling particular groups of metabolic processes are due to mutations which have occurred in the past and have become incorporated in the gene pools of different individuals or different inbred strains of laboratory animals. These mutations alter the response of the organism to exposure to environmental chemicals by increasing or decreasing its sensitivity to the toxic effects of particular classes of chemicals or physical agents.

The purpose of this program is to utilize gene patterns and specific mutations which increase the test animal's sensitivity to low dose levels of environmental substances in the development of *in vivo* toxicologic assay systems which are more efficient and more relevant for hazard evaluation and risk assessment than the presently available systems.

Initially, a mutation (viable yellow, A^{vy}) at the agouti locus on Chromosome 2 of the house mouse is being used in the development of more sensitive carcinogenicity assays. This mutation decreases the latent period to the formation of hyperplastic and neoplastic lesions. It also induces a maturity-onset type of obesity. The former characteristic appears to result from a tissue microenvironment which favors multiplication of transformed cells; there is no evidence that the mutation favors transformation itself. The obesity appears to result from interference with a developmentally programmed decrease in the rate of carcass lipogenesis at puberty.

This mutation is particularly useful since it is inherited as an autosomal dominant with a visible coat color marker (yellow). Therefore, it can be easily incorporated in any type of population, e.g. inbred, F-1 hybrid, outbred, and its carriers can be identified visually.

The particular tissues in which tumor formation is enhanced are determined by the background strain genome. Therefore, tissue specificity can be predetermined by selecting the appropriate inbred strain, crossing it with an inbred strain carrying the mutation and thus producing a F-1 hybrid population with the appropriate tissue sensitivity and two levels of susceptibility to lesion formation. Such a F-1 hybrid population provides its own normal controls since only one-half of the animals carry the mutation. An outbred population can also be established and maintained so that both mutant and normal mice segregate in each generation. In such a population the background genome of each mouse will be more or less different from every other one, much as in the individuals of a human population but, the individuals at higher risk of lesion formation can be identified by their coat color as early as one to two weeks of age.

The alleles at the agouti locus may also be useful for the detection of the alteration of embryonic development by low dose levels of environmental substances since at least some alleles affect the developmental program of the zygote as early as the two-to-four cell stage as well as at later pre-and postnatal stages.

The diverse effects of mutations at the agouti locus suggest that this locus controls a polypeptide which is involved in very basic cellular processes. Identification of this polypeptide and its role in cellular metabolism would provide a useful tool for reaching a better understanding of the mechanisms involved in the induction of toxic effects by environmental substances.

Depending on the availability of the necessary resources, additional genetic loci and gene patterns affecting specific aspects of the toxic response in different mammalian species will be encompassed in the future development of this program. These efforts will be focused on improving the validity of extrapolating the results of animal toxicity tests to man.

DIVISION OF CARCINOGENESIS RESEARCH

Fred F. Kadlubar, Ph.D., Director

The current interest and concern about carcinogens and mutagens in the environment stems not only from their potential pathological, teratological, and economic effects, but also arises from an increasing awareness of the potentially preventable nature of these diseases. The widely quoted estimate that 60-90% of all human cancers have their etiology in environmental factors has contributed greatly to this awareness. Some of the environmental factors have been identified as specific types of radiation, but the majority are probably chemical in nature. While chemically-induced tissue necrosis and organ failure have not received the notoriety associated with carcinogenesis or teratogenesis, they are important health problems that have arisen after exposure to certain industrial solvents, ingestion of foodstuffs containing plant- or fungal-derived toxicants, use or abuse of various drugs, and inhalation of certain anesthetics. A better understanding of some of the biological mechanisms of carcinogenesis, mutagenesis, and tissue toxicity has also developed over the past several years and this understanding has strengthened the belief that, even if not curable, these biological events may be amenable to prevention.

A first line of defense in prevention lies in the accurate detection of toxic materials. Accordingly, the development of short-term test systems for carcinogenicity, mutagenicity, and cytotoxicity has received high research priority. Current test systems yield both false-positive and the potentially more serious false-negative responses, but improvements in these systems could minimize such deficiencies. However, before significant new improvements can be made that will permit an appropriate assessment of human risk, further studies on the effects of chronic low-level exposure to toxicants and on the biochemical mechanisms of mutagenicity, carcinogenicity, and cytotoxicity will be required. The former represents a primary goal of the NCTR and the latter is the basis for research programs within the Division of Carcinogenesis Research.

Toward this end, participants in these programs are currently conducting research in the following areas:1) metabolic activation of chemical toxicants; 2) identification of the nature of the damage to cellular constituents by activated chemical toxicants; 3) effects of cellular repair mechanisms on the toxic response; 4) determination of critical detoxification pathways; 5) elucidation of biochemical mechanisms by which chemical carcinogenesis is initiated and expressed; and 6) evaluation of suspected toxicants in relation to the above mechanisms of action, with special consideration given to those chemical classes which are of regulatory interest (e.g., sodium saccharin). The Ongoing Research compilation lists individual projects in accordance with these categories.

MUTAGENESIS RESEARCH PROGRAM

D.A. Casciano, Ph.D., Director

The Mutagenesis Research Program was developed in response to the need for improved mammalian and non-mammalian systems which can be used to provide reliable and meaningful dose-response data to estimate accurately the relative risk to human populations exposed to various levels of environmental chemicals. The primary objective of this program is to develop *in vivo* germ cell mutagenesis assay systems to detect and quantitate induced microlesions (single gene mutations, small deletions) and macrolesions (gross chromosomal aberrations); a secondary objective is development of short-term bioassays to detect and quantify genotoxic events.

At present, there are few good *in vivo* mammalian systems to detect genetic damage transmitted to subsequent generations via the germ cells. A major effort of this program is oriented toward estimating the mutagenic potential of chemicals in *in vivo* mammalian assays which can detect induced microlesions. This system employs techniques used in the study of inborn errors of metabolism, namely, detection of altered gene products using enzyme activity analysis and electrophoretic mobility analysis. In addition, the heritable translocation assay in mice, an assay which measures chromosomal damage transmitted to subsequent generations, is being refined and validated to assess its merits for routine application in toxicology/safety evaluations.

A second approach involves development of rapid, reliable, and economical methods for estimating a chemical's genotoxic potential using short-term bioassays. These short-term bioassays will include somatic cell systems capable of detecting genotoxic events. These systems initially will be used in an attempt to interrelate DNA adducts with metabolism, repair and mutation induction in order to have a more adequate understanding of the mutagenesis process. This understanding will then be applied to the germ cell in order to quantitate germ cell mutations for eventual assessment and quantification of risks to exposed human populations and applied toward predicting and understanding the carcinogenic potential of a chemical.

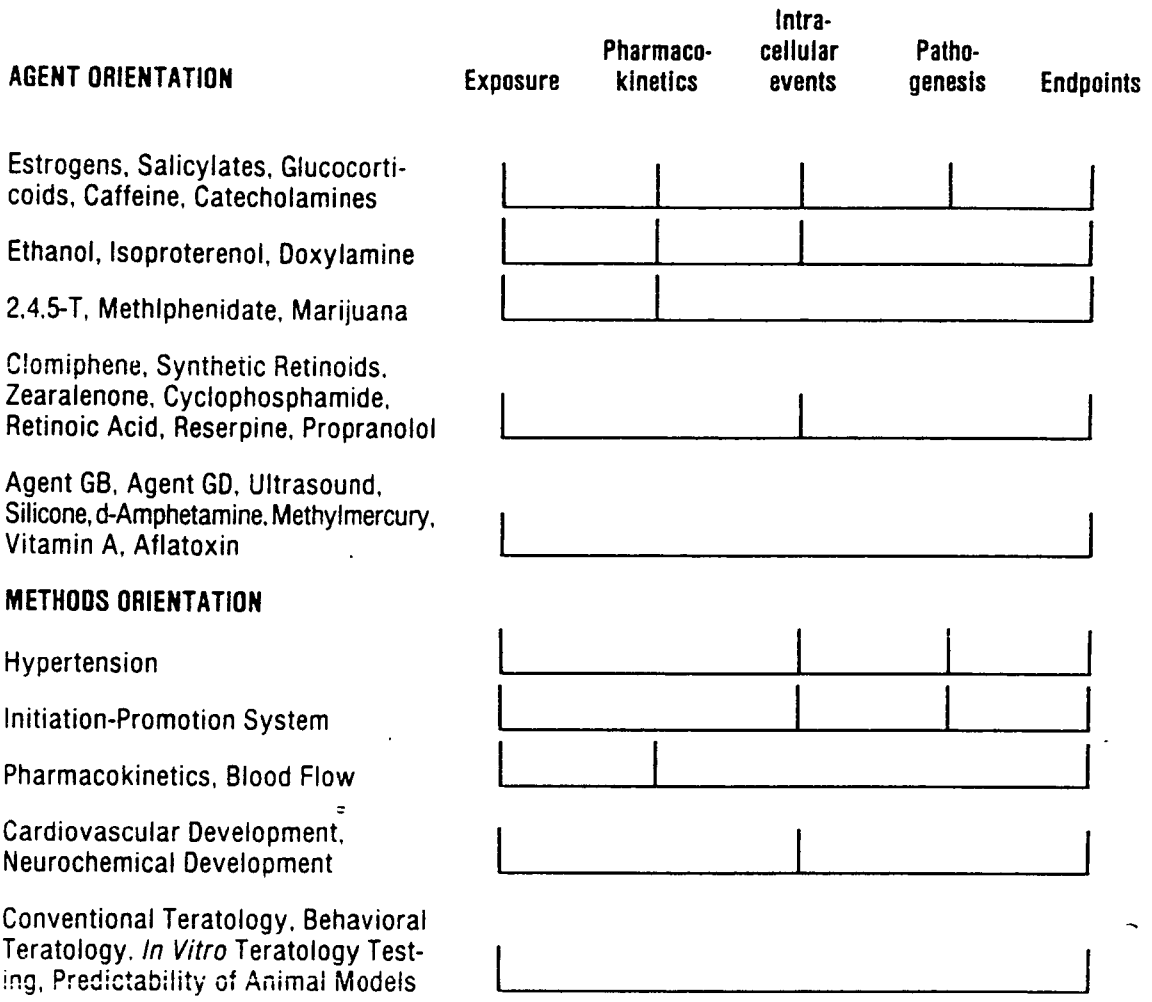
DIVISION OF TERATOGENESIS RESEARCH

John F. Young, Ph.D., Director

The assessment of human risk as a result of exposure to developmental toxicants is the long-range goal of the Division of Teratogenesis Research. The Division's research program is developed around several objectives designed to meet this long-term goal. These objectives include: 1) development of a sound information base of comparative pharmacokinetics and metabolism to define valid mathematical models for extrapolation of animal data to man; 2) expansion of knowledge of basic developmental processes which can be affected by toxicants and definition and understanding of mechanisms of teratogenicity; and 3) development of improved and validated procedures for detecting the full range of possible toxic manifestations throughout the lifespan of the organism. The organizational structure within the Division reflects these objectives in the three Branches: Pharmacodynamics, Developmental Mechanisms, and Perinatal and Postnatal Evaluation.

We have made progress toward these goals over the past several years. Mathematical models have been developed for estrogens, glucocorticoids, salicylates, alcohols, xanthines, and other agents in learning more about comparative metabolism and pharmacokinetics. Prediction of teratogenic outcome in an individual litter has been made based on that maternal animal's pharmacokinetic handling of a specific teratogen. Experimental systems have been designed which allow for more unobstructed views of maternal/fetal interactions with toxicants. Basic characteristics of the estrogen receptor during the perinatal period have been found to be similar among various species; this forms another basis for extrapolation across species. Procedures have been developed to assess new endpoints such as fetal cartilage, behavioral ontogeny, and cardiovascular function.

The importance of interplay among the various components of information which must be brought to bear on understanding events in developmental toxicity demands a multidisciplinary approach. The integration of key components in specific divisional program areas and thus the multidisciplinary nature of these projects are illustrated in the following diagram.


AGENT ORIENTATION
Exposure
Pharmaco-kinetics
Intra-cellular events
Patho-genesis
Endpoints
Estrogens, Salicylates, Glucocorti-coids, Caffeine, Catecholamines
Ethanol, Isoproterenol, Doxylamine
2,4,5-T, Methlphenidate, Marijuana
Clomiphene, Synthetic Retinoids, Zearalenone, Cyclophosphamide, Retinoic Acid, Reserpine, Propranolol
Agent GB, Agent GD, Ultrasound, Silicone, d-Amphetamine, Methylmercury, Vitamin A, Aflatoxin
METHODS ORIENTATION
Hypertension
Initiation-Promotion System
Pharmacokinetics, Blood Flow
Cardiovascular Development, Neurochemical Development
Conventional Teratology, Behavioral Teratology, In Vitro Teratology Test-ing, Predictability of Animal Models

DIVISION OF BIOMETRY

D.W. Gaylor, Ph.D., Director

The primary role of the Division of Biometry is to provide support for the NCTR scientists on the statistical design, analysis and interpretation of toxicological experiments. In addition, assistance is provided scientists for the randomization and placement of animals on experiments, data processing, and preparation of reports and manuscripts. The Division has five professional statisticians with support from three contract statisticians administered by DTDMS.

During the last year, personnel of the Division of Biometry worked on approximately 70 experiments. These experiments included studies in carcinogenesis, mutagenesis, teratogenesis, chemistry, molecular biology, animal husbandry, pathology, and chemical toxicology. Division personnel were senior authors of four publications, co-authors of 10 other publications, and presented three talks at professional meetings. Dr. Ralph Kodell received the annual H.O. Hartley Award from the Institute of Statistics, Texas A&M University, for outstanding contributions to the field of statistics. Dr. David Gaylor was elected Chairman of the Biopharmaceutical Section of the American Statistical Association.

NCTR experiments were statistically designed so that there was a high probability of meeting the objectives with a minimum number of animals. Stratified designs were used to simultaneously study the effects of two or more factors. Randomization schemes and cage configurations for teratogenic, subchronic, and chronic bioassays were devised to minimize bias and the effects of extraneous confounding factors.

Special statistical techniques were required for teratology data where measurements generally are ratios of discontinuous random variables, e.g., proportion of abnormal fetuses in a litter. Such data often required the use of nonparametric statistical techniques. To the extent possible, factorial experiments were analyzed by linear model and analysis of variance procedures. Data from the behavioral teratology pilot studies employing amphetamine and methylmercury have been used to establish standard analyses and reports for interlaboratory studies.

For the Division of Teratogenesis Research, the correlation of pharmacokinetic and teratogenic parameters have been studied. Experiments on the teratologic and reproductive effects of estrogens have been analyzed. Computer programs were developed to edit data and for the conversion of teratology data on Agent GB from the PDP-12 to SAS files for analyses on the IBM computer. The correlation of cholinesterase levels with Agent GB toxicity were studied in order to develop a monitor for exposure of workers. Animals were exposed *in utero* to propranolol and randomization, data collection, retrieval, and analysis systems were devised.

An improved procedure was developed in conjunction with the Division of Mutagenesis Research for detecting mutagens with the hepatocyte DNA repair system by using each animal as its own control. Analyses were conducted on a data base for positive controls in the heritable translocation assay. Analyses were performed on sister chromatid exchange induction. Analyses were conducted for circadian effects of enzymes and the circadian effects of hormones on enzymes. Assistance was provided in designing an experiment to study circadian effects in aged mice.

In conjunction with the Division of Chemistry, techniques were investigated for the validation and calibration of mass spectrometry measurements of 2,3,7,8-tetrachlorodibenzo-p-dioxin in samples. Statistical techniques were incorporated into the mass spectrometer software to estimate the uncertainty associated with dioxin measurements.

Statistical analyses were performed to study the fertility rates and litter sizes as a function of age for various strains of mice in the breeder colony for the Division of Animal Husbandry.

Assistance was provided the Division of Chemical Toxicology on the design of an experiment to study the effects of length of animal feed storage on subsequent weight gain and breeding performance of animals.

An experiment was designed in conjunction with the Division of Carcinogenesis Research to study the cocarcinogenic effects of saccharin and MNU. A chronic study on rotenone was analyzed to determine effects produced by long-term exposure.

Data from large NCTR experiments were analyzed in order to study experimental conditions other than chemical exposure which may influence bioassay results. The effects of these extraneous factors may be minimized by the proper design of experiments or may be adjusted by analytical techniques such as covariance analyses. Animal rack shelf level effects have been demonstrated. Hence, experiments were designed such that each treatment group of animals was represented an equal number of times on each shelf level. The power of Tarone's test of homogeneity of proportions against a beta-binomial alternative was studied for the ED01 experiment where there was a large number of cases with one or two littermates treated alike. No heterogeneity of tumor rates among litters was detected for spontaneously occurring tumor types or for tumors produced by 2-acetylaminofluorene in BALB/c female mice.

The analyses of chronic experiments require the use of complex statistical techniques to adjust for mortality when comparing tumor incidence and prevalence rates. The analysis of the incidence rate for palpable or fatal tumors require the use of life table techniques. The analysis of nonlethal incidentally discovered tumors requires the use of prevalence rate techniques. A distinction is required between fatal and nonfatal tumors because of a difference in the way the number of animals at risk are calculated. The distinction between a fatal and a nonfatal tumor is often a difficult diagnosis. More flexibility has been provided pathologists by modifying our statistical procedures to allow for an equivocal cause of death.

The standard National Toxicology Program bioassay to detect carcinogens consists of administering a chemical at the maximum tolerated dose (MTD), 1/2 MTD, and controls. Extensions to this design were studied to also provide better information for low dose extrapolation. Among the designs investigated, computer simulations indicated that using equal numbers of animals at the MTD, 1/2 MTD, 1/4 MTD, and controls, or at the MTD, 2/3 MTD, 1/3 MTD, and controls also provide adequate information for low dose linear extrapolation for a wide variety of dose response curves likely to be encountered.

The computer program GLOBAL82 utilizes the multistage model to describe tumor rates as a function of dose. The program was modified to provide plausible upper bounds on low dose tumor risk estimates using linear extrapolation below the experimental dose range.

Various techniques are available to estimate tumor rates at specified times, adjusting for death due to competing risks. A procedure was developed which adjusts the tumor rates in a treated group to the rates expected with the same non-tumor mortality pattern as the control animals. These standardized age adjusted tumor rates can be used to estimate the lifetime tumor rates for use in risk assessment.

Nine industrial research centers requested our SAS procedure (CHRONIC) for the estimation and testing of tumor rates from chronic animal bioassays. The procedure was modified to provide an option for the choice of time intervals for estimates and comparisons throughout an experiment.

Risk estimates were performed for dioxin in Great Lakes fish for the FDA and on benzene for OSHA. Principles of risk analyses for terata and reproductive effects were discussed with the EPA Office of Pesticides and Toxic Substances and applied to risk estimates for PCB and ethylene glycol ethers.

A response to the Society of Toxicology Task Group on the ED01 Study was published in *Fundamental and Applied Toxicology*.

A proposal was submitted to Dr. Kodell to the Air Force Office of Scientific Research to investigate the application of survival analysis techniques to equipment reliability.

Assistance in reviewing research proposals has been provided the Veterans Administration Southeast Regional Health Services Research and Development Field Program.

Dr. Gaylor served as a member of the National Academy of Sciences Committee on Toxicology. Emphasis this year has been on devising procedures for setting emergency exposure limits for toxic substances, particularly for airborne exposure. An in-depth review of the toxic effects of ethylene oxide was performed.

Dr. Gaylor served as Chairman of the Risk Estimation Panel for the Workshop on Formaldehyde.

DIVISION OF CHEMISTRY RESEARCH

Ronald K. Mitchum, Ph.D., Director

The Chemistry Program performs highly sophisticated analytical chemical research pertaining to the development and implementation of new or improved procedures for the trace analysis of mutagens, teratogens, carcinogens, toxicants, food additives and other biologically active substances, their analogs and metabolites. Examples of substrates of interest are a variety of consumer products, biological fluids, tissues, air, water and other environmental compartments. Such procedures are prerequisites for initiating and sustaining a broad spectrum of toxicological research programs at the NCTR as well as other high priority experiments in FDA, EPA, NIOSH, CPSC, and the National Toxicology Program (NTP). Good Laboratory Practices (GLP's) mandate that the test chemicals must be controlled from the time they enter the laboratory until their safe disposal; this can be accomplished only by the continuous development of new procedures that are both specific and ultrasensitive. Another ongoing assignment of equal importance is the structural identification of metabolites and other compounds of interest in pharmacokinetic, bioavailability and biochemical mechanism studies.

The analytical expertise of the research scientists in the Division is sustained by staying apprised of advances in their area of specialization and by actually contributing to the state-of-the-art. This sustained effort becomes even more important in light of the increasing complexity of problems related to the protection of consumers from hazardous substances in foods, drugs and the environment. The development of techniques that are more reliable and sensitive is essential to ensure that data of high quality are available for use in making regulatory decisions.

Research experiments that are currently in progress or slated for the future include the development of procedures employing 1/ atmospheric pressure ionization (API) mass spectrometry (MS) for quantitating trace contaminants at the low parts per trillion level, 2/ fast atom bombardment-MS for the analysis of labile polar molecules, 3/ high pressure liquid chromatography with various types of detectors and the use of paired-ion techniques for analysis of polar and/or labile compounds, 4/ gas chromatography employing both packed and capillary columns and a wide variety of detectors used in conjunction with derivatization techniques for enhanced sensitivity and specificity, 5/ centrifugal counter-current chromatography for preparative separation of analytically intractable compounds, 6/ radiotracers for studies of substances which contain a moiety that upon metabolism have the potential of producing a hazardous compound, 7/ MS and nuclear magnetic resonance (NMR) spectrometry performed in all modes to elucidate chemical structures and 8/ atomic absorption (AA) and inductively coupled argon plasma (ICAP) spectrometry to provide a broad spectrum of analytical capability in the area of elemental analysis.

These analytical methodologies will be used to investigate azo dye and antihistamine metabolism, microencapsulation techniques, nitrosamines in rubber products and chinoline dye composition. Other areas of research include Nuclear Magnetic Resonance and Mass Spectrometry studies of DNA adducts, carcinogen metabolites and environmental contaminants. Personnel and waste water surveillance and dose feed certification will also be major efforts in the Division of Chemistry in 1984.

DIVISION OF MOLECULAR BIOLOGY

Joe A. Tortorich, Acting Director

Research in the Division of Molecular Biology involves immunotoxicology, radioimmunoassay development, automated flow cytometry and cell cycle analysis. These efforts are interrelated and focus on the development and validation of new test procedures for application to problems in toxicology. Research within these elements emphasizes the concept that toxicant-induced changes in measures of immune function or events within the cell cycle must be related to adverse health effects in order to be considered deleterious.

Ongoing and developing projects in the Immunotoxicology Program of Molecular Biology Division at NCTR are intended to complement present and future FDA, DPA and NTP programs concerned with food and drug safety, environmental safety, and the evaluation of potentially toxic substances. These projects strike a balance between 1) the immediate need to examine compounds of regulatory importance to the extent currently possible and 2) the continuing need to develop and improve those animal models best able to detect harmful alterations in immune function. Certain drugs, environmental pollutants, and food additives have been shown to depress immune parameters associated with antibody responses, cell mediated immunity and macrophage function. The detection and characterization of immunotoxic compounds are important in the context that they may suppress parasitic, and neoplastic disease; or they may potentiate adverse immune reactions such as hypersensitivity and autoimmune disease. An important premise of immunotoxicology is that the immune system, because of its ontogeny, rapid cell proliferation, constant exposure, and inherent amplification, is particularly vulnerable to toxic substances. These features, combined with the sensitivity of analytical methods available to evaluate immune responses, suggest that immune systems should reflect early changes as a result of exposure to low doses of toxicant which do not cause overt toxicity or clinical symptoms. The major objective of immunotoxicology at NCTR is the development and verification of models for detecting and assessing the impact of chemical compounds on immune defense mechanisms.

The Cell Biology Research Program within the Division of Molecular Biology was created to provide NCTR with the capability to study discrete biological transitions in mammalian cells and tissue culture cell systems which are exposed to carcinogenic and toxic compounds. This will be accomplished via the isolation and biological analysis of pure cell types (e.g., mammalian reproductive cells, exfoliated bladder cells, liver cells, etc.), nuclei and chromosomes.

The program is utilizing the scientific and support capabilities of a number of programs at NCTR in achieving the following goals:1) development of cell sorting parameters for the separation of homogeneous cell populations from toxicologically exposed tissue, chemically induced tumors and transformed cells; 2) cytological, cytochemical and biochemical characterization of these cells will also be undertaken; 3) establishment of sensitive biochemical markers in early hyperplastic cell population and their application in event of "time to tumor"; 4) development of subclinical markers for use in early diagnosis in carcinogenesis and abnormal cellular transitions after chemical exposure; 5) evaluation of differential uptake of carcinogenic and toxic compounds by homogeneous cell populations and associated biological changes during oncogenesis and abnormal cellular growth; 6) analysis and interpretation of the multistep process of carcinogenesis and abnormal cellular growth induced by chemical compounds.

The development and refinement of various test systems, to be utilized by the FDA in a regulatory capacity, should allow the early assessment of ever-so-slight biological changes during the process of chemical insult before they are clinically evident.

DIVISION OF CHEMICAL TOXICOLOGY

Neil A. Littlefield, Ph.D., Director

The Chemical Toxicology Division deals principally with aspects of the toxicological evaluation and characterization of selected chemical agents. This includes studies to determine the hazard/-safety of compounds, design and technique verification, and validation of procedures. Many of the studies are designed to aid in the regulatory processes, such as providing safety information, data to establish tolerances, and experimental technique verification to aid in formulating new regulations. A significant share of the studies are designed to deal with the various methods, techniques and rationale for long-term and low-dose extrapolation.

The primary mission consists of research programs to study biological effects of potentially toxic chemical substances found in the environment. Programs of the Division have emphasized the determination of health effects from long-term, low-level exposures of animals to chemical toxicants and also attempted to define basic biological processes involved in pathological responses to such chemicals in an effort to enable better extrapolation from laboratory animals to man. Other programs are a series of long-term feeding studies to document the extent and limitations to which 2-AAF and hormonally active substances may affect carcinogenesis.

The nutrition program is intended to examine the relationship between nutrition and oncogenesis and general clinical toxicology. The studies are designed to provide an understanding of the etiology of cancer with respect to diet and nutrition. These studies will help document the influence of diet during long-term and low-dose studies and progress will be made in development of a reference diet for carcinogenesis studies.

PATHOLOGY RESEARCH PROGRAM

Thomas J. Bucci, V.M.D., Ph.D., Program Director

The "last stop" in the bioassay procedure is examination of the tissues and fluids of treated animals. The NCTR mission includes study of a large number of chemicals which differ greatly from each other, and most require testing in a variety of doses for different time intervals. Abnormal findings in the treated animals vary from obvious to subtle, and from rare to ubiquitous. All must be catalogued precisely and interpreted for their effect on the animals' health. Finally, inferences must be made about the potential effect of the same chemicals upon the health of human beings.

The Pathology Services Project is currently not staffed to permit a great deal of research. The major part of the resources will necessarily be devoted to provision of careful, prompt evaluation of the animals examined. A significant amount of professional and technical effort is currently spent in collaboration with DTDMS and the NTP to complete development of an automated vocabulary and management system in necropsy and microscopic pathology which will be used nationwide for the National Toxicology Program. The vocabulary will represent a major step to standardize the evaluation of bioassay data.

To provide comprehensive pathology service and also have time for the staff to participate in research, we are continuing the cross-training of our technical staff. We will focus our research program toward methodologies which will increase the sensitivity and specificity of our efforts. We are very interested in minimizing the time required to recognize meaningful deviations from normal, e.g. preneoplastic lesions. The Pathology Staff will evaluate techniques which promise to reveal such changes at earlier times or which promise increased specificity in identifying them. As time permits, we would like to examine systematically a large list of immunoreagents for their ability to reveal altered cells not otherwise recognizable as abnormal, e.g. use of peroxidase-labelled monoclonal antibodies to hormones and enzymes to detect inappropriate synthesis.

If we were able to detect subtle changes in size and shape of cells and cell components with great confidence, we could reduce markedly the number of animals or tissues examined and still have very meaningful data. Simultaneously, since the subtle changes occur earliest, bioassay could be shorter. Equipment and techniques to quantify such tissue changes rapidly at the ultrastructural level are now becoming available. During 1983, one such computerized planimetry device was acquired and placed into service to support NCTR work. We expect it to assist us to make meaningful contributions.

DIVISION OF MICROBIOLOGICAL SERVICES

Joe A. Tortorich, Director

Microbiology programs at NCTR are currently concentrated in two areas of applied research and development:(a) diagnostic methods testing and validation and, (b) development of new procedures of microbiological analyses from concepts and preliminary reports in the research literature. The former supports an extensive surveillance and monitoring effort on NCTR quarantine, breeding and barrier operations and has as its goal the cost-effective assessment of laboratory animal status. The latter concentrates on the preliminary experimentation in the literature or incomplete methodologies under development and brings these to a practical use state for assay verification. Research on the biodegradation of xenobiotics including polycyclic and nitropolycyclic aromatic amines and azo dyes is also being conducted in the Microbiology Division. The biodegradation research program is performed with collaborative research support of the Chemistry and Carcinogenesis Divisions.