NATIONAL TOXICOLOGY PROGRAM

BOARD OF SCIENTIFIC COUNSELORS

March 14, 1989

Summary Minutes

National Toxicology Program
Board of Scientific Counselors Meeting

March 14, 1989

Summary Minutes

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DRAFT SUMMARY MINUTES
NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS MEETING
March 14, 1989

SUMMARY MINUTES

The National Toxicology Program (NTP) Board of Scientific Counselors met on March 14, 1989, at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. (Attachment 1: Federal Register Meeting Announcement; Attachment 2: Agenda and Roster of Members.) Members of the Board are Drs. Michael Gallo (Chairman), John Little, Richard Miller, Frederica Perera, Adrianne Rogers, Robert Scala, and Arthur Upton. Dr. Upton was unable to attend the meeting.

I. Report of the Director, NTP: Dr. David Rall reported that: (1) a conference was held at NIEHS on January 9-11, 1989, dealing with Advances in Lead Research with Related Implications for Environmental Health. Research indicated that human maternal blood lead levels as low as 10 ug/deciliter were shown to result in temporary or permanent defects of neuromuscular and cognitive development of children. With up to two million children being exposed to such levels, this poses a serious public health problem; (2) in response to animal welfare and animal rights concerns, the NIEHS continues to review its procedures to make sure we are dealing with our laboratory animals in the most humane way; (3) the NIEHS is preparing a response to the article in the December 15, 1988, issue of Nature by Lave et.al. that disputes the value and cost-effectiveness of the rodent bioassay for predicting carcinogenic potential in humans (copies of the article were given to Board members); (4) with regard to the 1990 NIEHS budget, the allocation in the Bush administration budget is the same as in the Reagan budget. However, spending reductions have not yet been allocated, and although there are increases for basic research, and many Institute programs are keeping apace of inflation, funding for research and development contracts, so vital to NTP programs, continues to lose ground in terms of 1981 dollars. Dr. Rall presented certificates to retiring Board members Dr. Michael Gallo and Dr. Frederica Perera and an expression of appreciation from himself and the Program for their service on the Board and Peer Review Panel. He also presented a certificate and thanks to Dr. Richard Griesemer for his service on the Board in 1987-1988 prior to becoming Director, Division of Toxicology Research and Testing (DTRT), NIEHS.

II. Concept Reviews- NIEHS-DTRT- Research Support Contracts: Dr. Griesemer introduced the reviews by stating that about 80 % of the NIEHS/NTP's basic and applied research activities are conducted through contracts and interagency agreements. Prior to issuance of a Request for Proposal (RFP), a project concept review is required by Public Health Service regulations. These project concepts often consist of more than one contract or interagency agreement. Concept reviews are needed for new projects, for recompetitions with changes in statements of work, and for projects ongoing for five years or more since the last concept review. The DTRT currently has 150 research and resource contracts and interagency agreements, and 35 concepts in force to enable planning and allocating of resources. Dr. William Johnston, Contracting Officer, reported that project concept reviews are conducted by the NTP Board, whose members are asked to review the concepts for overall value and scientific

relevance as well as for fulfilling the program goal of protecting public health. Reviews are open to the public so long as discussions are limited to review of the general project purposes, scopes, goals, and various optional approaches.

Dr. Griesemer noted that the first concept to be reviewed concerned the 'centerpiece' of the Program-- toxicity and carcinogenicity studies in rodents. This would be followed by seven resource support contract concepts and then seven concepts having to do with primarily testing, methods development and validation activities. Three of the latter were new concepts. He reported that eight more concepts would be reviewed by the Board in November, and then, eight more over the next few years.

(1) Toxicity and Carcinogenicity Studies in Animals -- (Attachment 3-Review of Project Concepts, p.4) Dr. Griesemer said the objective was to continue to characterize the toxicologic effects of chemical and physical substances through studies in animals. The typical approach is repeated administration of the agent to groups of animals for variable periods up to two years or more with clinical, toxicologic and pathologic evaluation of the adverse health effects by comparison with groups of control animals.

The Board affirmed the need for continuing short and long-term toxicology and carcinogenesis studies in animals while noting modifications or additions that could improve the utility or ability of these studies to predict toxicologic and carcinogenic potential. A general suggestion was to add test groups to enable better assessment of hazard to aid regulators. Dr. Griesemer responded that modifications could be included under the broad statements of the concept. Dr. Gallo moved that the concept be accepted as written giving the NTP latitude to add new ideas as they evolve. Dr. Scala seconded the motion, which was accepted unanimously (six yes votes) by the Board.

(2) Chemical Repository and Safety Support -- (Attachment 3, p.5) Dr. Griesemer said this project would continue to provide safe handling, storage, and shipping of chemicals studied by the Program; currently, there were in excess of 1900 chemicals in the repository. As well, the repository provides industrial hygiene support including information on chemical hazard. In addition, the NTP has established procedures for disposing of older bulk test chemicals.

The Board noted that meeting the objectives of this concept were essential to the reproducibility and quality of NTP studies. Inquiries concerned whether the industrial hygiene support was provided by certified safety professionals, and whether hazardous waste disposal was by environmentally correct means. Dr. Douglas Walters, Project Officer, responded that certified professionals were used. Waste disposal was by incineration where possible, otherwise in an approved land fill. Dr. Scala moved that the concept be accepted. Dr. Rogers seconded the motion, which was accepted unanimously (six yes votes) by the Board.

(3) Chemistry Support Services -- (Attachment 3, p.6) Dr. Griesemer said the objective was to continue to provide analytical support services for the NTP toxicity and carcinogenicity studies, which includes chemical procurement and synthesis, bulk chemical characterization, and dosage formulation and

analysis, as well as analysis for chemicals and metabolites in tissues and biological fluids.

The Board affirmed the importance of the chemistry support services for the proper charcterization and analysis of chemicals studied by the NTP. The question was raised as to whether all chemicals used in the Program were characterized. Dr. C.W. Jameson, Project Officer, said all chemicals supplied to laboratories under the master agreement were completely characterized. A major addition to the current work statement is inclusion of a capability for performing pilot studies in rodents to determine basic pharmacokinetic parameters. In response to a question, Dr. Jameson said one of the current laboratories had such capability, and in the future some of this work would be subcontracted. Dr. Scala moved that the concept be accepted. Dr. Rogers seconded the motion, which was accepted unanimously (six yes votes) by the Board.

(4) Rodent Disease Diagnostic Laboratories -- (Attachment 3, p.7) Dr. Griesemer said the objective of this project is to ensure that the animals used are disease free and infection free, and this has been pretty much the case over the past several years. He commented that this was a typical animal disease surveillance program.

Dr. Rogers moved that the concept be accepted. Dr. Scala seconded the motion, which was accepted unanimously (six yes votes) by the Board.

(5) Genetic Monitoring of Inbred Rodents -- (Attachment 3, p. 8) Dr. Griesemer said the objective is to provide genetic monitoring of inbred stocks at rodent production centers. This is necessary to maintain genetic integrity of the rodent production colonies as well as the animals supplied for toxicity and carcinogenicity studies. He said that genetic drift was not now a problem.

The Board asked whether the large amount of data from the Sentinel animals and genetic monitoring was being used or mainly just collected and filed. Dr. Rao, Project Officer, responded that data from these programs were being analyzed and published in appropriate journals. In response to a question about the use of DNA fingerprinting, Dr. Rao said new procedures would be considered. Currently, genetic integrity of inbred rodents was being evaluated by skin grafting and the use of DNA probes. Dr. Rogers moved that the concept be accepted. Dr. Scala seconded the motion, which was accepted unanimously (six yes votes) by the Board.

(6) Pathology Support -- (Attachment 3, p. 9) Dr. Griesemer said this project is intended to provide uniformity and consistent application of diagnostic procedures to the pathology process. This includes necropsy assistance and tissue section preparation for studies conducted in-house as well as for suppplemental studies on pathology specimens generated through contracted studies; reviewing and assessing the pathology performed by contractors; serving on NTP Pathology Working Group reviews; and technical support for quality assessment of pathology evaluations.

The Board affirmed the high quality of the pathology program. Dr. Gary Boorman, NIEHS, said the overall quality of the pathology done at the laboratories had improved over the past few years and the pathology support had

contributed to a more efficient process. Dr. Rogers moved that the concept be accepted. Dr. Scala seconded the motion, which was accepted unanimously (six yes votes) by the Board.

(7) Pathology Archive -- (Attachment 3, p.10) Dr. Griesemer said the objective of this project was to continue to provide storage and retrieval for all of the histological slides, paraffin blocks, formalin-fixed wet tissues, paper data, and microfiche/microfilm for the toxicity and carcinogenicity studies conducted by the Program. Greater emphasis was to be given to reducing paper data to microfiche and to collecting frozen tissues for oncogene analysis.

Dr. Rogers moved that the concept be accepted. Dr. Scala seconded the motion, which was accepted unanimously (six yes votes) by the Board.

(8) Statistical Analysis of Laboratory Studies -- (Attachment 3, pp.11-12) Dr. Griesemer said this project will continue to provide statistical and computational expertise and resources to summarize, analyze, and aid in the interpretation of data from the various laboratory animal experiments conducted by the NTP. Increasing emphasis will be given to handling and assessing data from non-cancer toxicity endpoints especially that related to the new short-term study Technical Reports.

Board discussion centered on clarification of some minor proposed changes in the statement of work. Dr. Perera moved that the concept be accepted. Dr. Rogers seconded the motion, which was accepted unanimously, (six yes votes) by the Board.

III. Concept Reviews- NIEHS-DTRT- Testing/Methods Development/Validation Contracts:

(1) Immunotoxicity of Environmental Chemicals and Therapeutics -- (Attachment 3, p. 16) Dr. Michael Luster, NIEHS Project Officer, reviewed the background on development by the NTP of a panel of assays for measuring and characterizing immunotoxicity of chemicals. The current project concept proposes to continue studying effects of selected chemicals, drugs or biologics on immune response, including immunomodulation, alteration to challenge, or induction of hypersensitivity. Objectives are to test four chemicals/year for immunomodulatory effects and four chemicals for induction of hypersensitivity. A small research component is included which will be devoted to examining methods to test for autoimmunity. Modifications include adding a prescreen partially for dose ranging, evaluation of a second mouse strain or rats for selected compounds, and keeping the panel state-of-the-art by adding new assays.

The Board asked how chemicals were selected for study. Dr. Luster replied that the majority were NTP compounds. In collaboration with the NCI, several AIDS therapeutic agents were being evaluated under a newer contract. The Board stressed the importance of this area and urged the NTP to be more aware of signs of immunotoxicity when reviewing chemicals for study by the Program. Dr. Miller moved that the concept be accepted. Dr. Scala seconded the motion, which was accepted unanimously (six yes votes) by the Board.

(2) Expired Breath Analysis in Chemical Toxicity Assessment -- (Attachment 3, pp. 13-15) Dr. John Bucher, NIEHS Project Officer, noted this was a new

concept and the objective was to evaluate whether changes in components of expired breath in laboratory animals could be a useful part of an overall toxicologic assessment of a chemical. The objectives of the research are to determine: (1) the identity and consistency of breath components in untreated rats; (2) changes in breath components in response to chemical exposure; and (3) mechanisms involved in the toxic processes. This concept is in accordance with NTP objectives to develop and apply new inovative technology, in this case a non-invasive technology.

The Board expressed reservations that the concept might be too ambitious in that there is a lack of baseline data in animals for expired endogenous chemicals. They recommended that the concept be split with the first phase a feasibility study to determine animal to animal variability for endogenous chemicals in the breath and establish a baseline. A second phase could examine the relationship of changes in breath components to expired chemicals. Dr. Bucher agreed. Dr. Scala moved that the concept be accepted. Dr. Rogers seconded the motion, which was accepted unanimously (six yes votes) by the Board.

(3) Neurotoxicology Methods Validation -- (Attachment 3, pp. 17-19) Dr. Dennis Dietz, NIEHS Project Officer, acknowledged his expert in-house collaborator, Dr. Hugh Tilson, NIEHS, on this new concept. This project represents an international collaborative study on methods for the identification of chemicals having potential neurotoxicity in humans. The study will use a functional observational battery and automated measures of motor activity to : (i) evaluate reliability, sensitivity, and specificity of neurobehavioral methods; (ii) begin to develop a data base for neurobehavioral effects of known neurotoxins as reference points; and (iii) analyze data from 10-12 participating laboratories for influence of interlaboratory variability and dose- and time-dependent effects.

The Board inquired as to the stage of development of the methodology and the NTP's role in the study. Dr. Tilson said the NIEHS was involved from the start in 1984 and the methods were well enough established so this would be primarily a validation study. Chemicals chosen were those which measure unique neurobehavioral effects and for which there are good data. High priority needs to be given to developing more negative control chemicals. Dr. Miller moved that the concept be accepted. Dr. Rogers seconded the motion, which was accepted unanimously (six yes votes) by the Board.

(4) Mutagenicity Studies With Salmonella -- (Attachment 3, p. 20) Dr. Errol Zeiger, NIEHS Project Officer, said this was a continuation of a program designed to evaluate the mutagenicity of pure chemicals and complex mixture with Salmonella typhimurium using two types of contracts: (i) those using standard protocols to test for coded chemicals, and (ii) a contract using specially designed protocols for substances that cannot be studied with standard protocols, e.g., ozone, wood dusts.

The Board commented on the need for creative methods development for the study of complex mixtures. Dr. Perera moved that the concept be accepted. Dr. Little seconded the motion, which was accepted unanimously (six yes votes) by the Board.

(5) In Vivo Cytogenetics -- (Attachment 3, pp. 21-22) Dr. Michael Shelby, NIEHS Project Officer, said the objective of the concept was to continue improving, evaluating and applying short-term in vivo cytogenetic assays with the aim of developing information on which of the chemicals mutagenic in vitro might be noncarcinogens and which of the chemicals nonmutagenic in vitro might be carcinogens. Efforts will include completing assessment of the 73 chemicals evaluated in the comprehensive study of four in vitro assays for induction of chromosome aberrations and sister-chromatid exchanges in vivo, characterizing performance of the mouse bone marrow micronucleus assay with 50 of the 73 with the aim of increasing use of this assay as a primary screen for in vivo genetic toxicity.

The Board asked whether the micronucleus assay could completely replace in vivo cytogenetic tests. Dr. Shelby said there was not enough information to make such a decision. Plans for the coming year are to reduce cytogenetic testing capacity and increase micronucleus testing while maintaining the capacity to conduct in-depth chromosome aberration studies on chemicals of interest. Dr. Little moved that the concept be accepted. Dr. Perera seconded the motion, which was accepted unanimously (six yes votes) by the Board.

(6) Mammalian Germ Cell Mutagenesis -- (Attachment 3, pp. 23-24) Dr. Shelby said the NIEHS program was the primary source in the country for data on mammalian germ cell mutagens. The concept proposes to pursue an assay incorporating several endpoints into a single test system which can detect both dominant and recessive mutations and in which selected and unselected genes are screened for mutations. This should provide a more efficient assay and test data more suited for estimating genetic risk. The proposed assay will build on the mouse electrophoretic germinal mutation assay.

The Board affirmed the importance of the concept. Dr. Little moved that the concept be accepted. Dr. Perera seconded the motion, which was accepted unanimously (six yes votes) by the Board.

(7) Identification of Rodent Tumor Suppressor Genes -- (Attachment 3, pp. 25-27) Dr. William Caspary, NIEHS Project Officer, introduced this new concept by noting the involvement of two classes of genes in carcinogenesis - proto-oncogenes and tumor suppressor genes. The latter provide signals for control of cell proliferation and loss or inhibition of their activity is believed to be associated with development of certain tumors in humans; there is less evidence for a role in chemically-induced tumors in animals. The concept proposal is to initiate a study of the loss of tumor suppressor genes in chemically-induced tumors of rodents. Loss is associated with concomitant loss of other genes on the same chromosome as shown by loss of heterozygosity of chromosomal markers. The approach will take advantage of the heterozygous nature of the B6C3F1 mouse. DNA probes which detect restriction fragment length polymorphisms (RFLPs) will be identified and mapped to specific chromosomes. If non-random losses of heterozygosity on a specific chromosome are found for a given tumor type, this would suggest that tumor suppressor genes may be linked to these RFLP markers. Additionally, since the RFLPs can be reprobed, it is also proposed to examine samples for amplified or translocated oncogenes.

The Board expressed concern as to likelihood of locating chromosomal deletions especially lacking cytogenetic data. Dr. Carl Barrett, NIEHS, commented that

this was a powerful technique which would show 75% of the genome. DNA probes have already been identified that detect RFLPs on 12 different chromosomes of the B6C3F1 mouse. A first priority will be to develop more probes. Dr. Perera moved that the concept be accepted. Dr. Little seconded the motion, which was accepted by the Board with five yes votes with one abstention (Dr. Scala).

IV. Update on Activities of the Technical Reports Review Subcommittee: Dr. James Huff, NIEHS, gave the Board a progress report on recent and upcoming activities of the Technical Reports Review Subcommittee and associated ad hoc Panel of Experts (Peer Review Panel). Dr. Huff summarized the findings for carcinogenicity from the Panel's meetings on November 6, 1987, April 18-19, 1988, October 3-4, 1988, and March 13, 1989. He noted that of 30 chemicals reviewed, there was a positive finding (clear evidence or some evidence of carcinogenic activity) in at least one experiment for 17 of the chemicals (57%). Looking at all of the experiments (each sex group of each species used is an experiment) for the 30 chemicals, there were positive findings in 36 of 114 experiments (32%). The Panel concurred with NTP staff recommendations on 94% of the levels of evidence. Dr. Huff summarized the Technical Reports scheduled for review at the next meeting of the Panel on June 27, 1989. He handed out a listing of draft Technical Reports projected for review from November 1989 through October or November 1990.

Dr. Scala, Chair of the Panel, reported on the Panel meeting of the previous day, March 13. He noted that the Panel concurred with the staff on six of the seven chemicals. On one report, that for alpha-methylbenzyl alcohol, there was lengthy discussion by the Panel as to the adequacy of the two-year studies in rats. There was high mortality due apparently to a cluster of gavage accidents midway through the studies. On that basis, the Panel considered the rat studies to be inadequate (NTP staff considered the studies to be adequate and the levels of evidence to be some evidence of carcinogenic activity in male rats and no evidence of carcinogenic activity in female rats). Dr. Scala read a consensus statement from the Panel recommending that the NTP review the technical conduct of the studies in rats with two possible outcomes: (1) if the review confirms the technical adequacy of the overall study procedures, the levels of evidence as written in the report should be affirmed; or (2) if the NTP concludes the rat studies are flawed, then the studies should be reclassified as inadequate and future repeat studies should be considered.

Dr. Scala also reported on the initial peer review on March 13 of three draft Technical Reports on short-term toxicity studies. He concluded by recommending that the NTP consider using caloric restriction to improve animal model viability. This sparked discussion about dietary restriction studies ongoing at the NIEHS and the NCTR.

V. Carcinogenicity of 1,3-Butadiene: An Update: Dr. Ronald Melnick, NIEHS, reported that the NTP had performed two long-term inhalation exposure studies in B6C3F1 mice. The first study was terminated after 60-61 weeks in both sexes due to high mortality from tumors at multiple sites (Attachment 4). The experimental design for the more recent study included doses ranging from 6.25 ppm to 625 ppm (which was the low dose in the first study), and interim sacrifices at 40 and 65 weeks. The same tumors shown as significantly increased in the earlier studies were increased in the newer studies often with a dose response at the higher doses, 62.5, 200 and 625 ppm (Attachment 4). In

female mice, lung neoplasms were increased even at the lowest dose, 6.25 ppm. Dr. Melnick described the results of stop exposure studies with differing exposure concentrations and durations of exposure in male mice. He noted that increases in several types of tumors were observed even after only 13 weeks of exposure, and for some neoplasms, notably lymphocytic and malignant lymphomas, exposure concentration was more critical than duration of exposure.

VI. Update on Activities of the Reproductive and Developmental Toxicology Program Review Subcommittee and Report on Scientific Efforts in NTP Reproductive and Developmental Toxicology Programs: Dr. Miller, Board representative to the Subcommittee, reported on the last meeting, held in Cincinnati, Ohio, November 17-18, 1989. He noted the diversity of the members with expertise ranging from clinical studies to basic science. The purpose of this meeting was to review research projects in progress or planned at NIEHS and NIOSH, specifically in areas of male and female fertility and developmental toxicology. With regard to male fertility, he commented on the close interaction between researchers at the two agencies on development and evaluation of the rabbit as a model for human seminal characteristics. In the area of female fertility, a newer area for the Program, studies on mechanisms of toxicity of phthalates to the granulosa cell are counterparts to similar studies with Sertoli cells in male animals. Dr. Miller commented on a NIOSH study to measure the association between occupational (telephone operators) video display terminal exposure and adverse pregnancy outcomes, particularly fetal loss. He concluded with comments on NIEHS studies in developmental immunotoxicology and postnatal toxicology reviewed at the meeting. (Minutes for the Subcommittee meeting, November 17-18, 1989, are available on request by writing Dr. L.G. Hart, P.O. Box 12233, NIEHS, Research Triangle Park, N.C. 27709; telephone- (919)541-3971, FTS 629-3971).

Dr. Bernard Schwetz, NIEHS, introduced a progress report by stating that the NTP program is a collaborative effort conducted by NIEHS, NIOSH, and NCTR. All three agencies are performing reproductive toxicology studies in animals, with NIOSH also conducting studies in humans. NIEHS and NIOSH both perform developmental toxicology studies, but NIEHS is the only agency doing research on the neonatal animal. The developmental toxicology effort focuses on the conceptus and neonate and includes examination for structural and functional alterations. All three agencies are performing in vivo studies; NIEHS is also conducting in vitro studies. Collectively, the three agencies are testing chemicals for reproductive and developmental toxicity, are engaging in methods development and validation, and conducting research into basic mechanisms.

Dr. Janet Haartz, NIOSH, reported that the toxicology studies at NIOSH are done primarily by the Division of Biomedical and Behavioral Science. Field studies (such as with VDT operators)are done in the Division of Surveillance, Hazard Evaluation and Field Studies. She concentrated her remarks on the work with humans being done by NIOSH. The work in humans consists of methods development projects and field studies in males. Male reproductive potential is being assessed on the basis of semen profiles, endocrine profiles and genetic evaluations, including DNA stability studies and karyotyping of sperm. She discussed a longitudinal study of 45 males to obtain baseline data on semen characteristics. Studies on semen characteristics in chemically exposed males included two different studies on workers exposed to ethylene dibromide and a study on workers exposed to glycol ethers. Dr. Haartz said efforts were

underway to develop methods for evaluating effects of chemical exposure on reproductive potential in females. Initially, this will include neuroendocrine levels in blood, urine and saliva, and some measure of ovarian function.

Dr. Jerrold Heindel, NIEHS, introduced descriptions of the Developmental and Reproductive Toxicology (DART) program at NIEHS. He stated that DART is responsible for determining the reproductive and developmental toxicity of NTP chemicals, primarily through contracts, and for improving the toxicological basis for assessing potential toxicants both through development of methods and through mechanism studies, primarily in-house. With respect to reproductive toxicology, DART performs toxicity testing using the reproductive assessment by continuous breeding protocol (RACB). In assessing the mechanism of action of a reproductive toxicant, DART utilizes a vertical integration approach. RACB studies yield information on the sex(es) affected, dose response, and occasionally the site of action. "Development of the lesion" is followed by determining the most sensitive cells. Chemical disposition studies are performed to identify whether the chemical per se or a metabolite is the active agent. Finally, in vitro studies are performed to investigate the mechanism of action. Dr. Heindel illustrated the use of this approach with several phthalate esters. In attempting to develop models for reproductive toxicity in females, DART is cooperating with NCTR in an investigation of ovarian morphology as an endpoint of female reproductive toxicity.

Dr. Robert Chapin, NIEHS, discussed the rabbit model for assessing the effect of altered semen characteristics on fertility, and some of the problems involved in its development. It is hoped that this system can be used to determine whether altered semen characteristics noted in the human will actually affect fertility.

Dr. Rick Morrissey, NIEHS, described current initiatives in developmental toxicology which encompass toxicity testing, research into the site and mechanism of toxicant action, and development of model systems. Chemicals are screened for toxicity in a short-term in vivo assay; those showing activity are then tested in a definitive teratology assay such as a Segment II (FDA) study. Dr. Morrissey described a system involving transfer of toxicants via lactation. The rat is being investigated as to its adequacy as a model for humans for the excretion of chemicals into milk. On the basis of literature values and data from compounds tested, the rat does appear to be a good model. This system will also be used to study effects of chemicals on the lactation process and subsequent effects on pup development.

VI. Review of Chemicals Nominated for NTP Studies: There were five chemical nominations considered by the Board. All had been reviewed previously by the NTP Chemical Evaluation Committee (CEC). (Summary data on the chemicals including CEC recommendations are provided in Attachment 5.) Dr. Gallo chaired the review. Dr. William Allaben, NCTR, Dr. Dorothy Canter, NIEHS, and Dr. Janet Haartz, NIOSH, CEC members, and Dr. Victor Fung, NTP Chemical Selection Coordinator, served as resource persons. Board members served as principal reviewers for one chemical each, and following the presentation and discussion of each chemical, motions were made and voted on. The Board's recommendations for the five chemicals are summarized in Attachment 6.

[BILLING CODE 4140-01]

PUBLIC HEALTH SERVICE

National Toxicology Program, Board of Scientific Counselors' Meeting

Pursuant to Public Law 92-463, notice is hereby given of a meeting of the National Toxicology Program (NTP) Board of Scientific Counselors, U.S. Public Health Service, in the Conference Center, Building 101, South Campus, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina on March 14, 1989.

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

8:30 a.m. - 8:45 a.m. - Report of the Director, NTP

8:45 a.m. - 10:30 a.m. - Concept Reviews - NIEHS Division of Toxicology Research and Testing (DTRT):

I. Toxicology and Carcinogenesis Studies;

II. National Toxicology Program Chemical Repository and Safety Support;

III. Chemistry Support Services for the National Toxicology Program;

IV. Rodent-Disease Diagnostic Laboratories;

V. Genetic Monitoring of Inbred Rodents;

VI. Pathology Support for the National Toxicology Program;

VII. National Toxicology Program Pathology Repository and Archive; and

VIII. Statistical Analysis of Laboratory Studies.

10:45 a.m. - 12:15 p.m. and

1:00 p.m. - 2:00 p.m. - Concept Reviews - Testing/Methods Development/Validation Contracts - NIEHS, DTRT:

I. Expired Breath Analysis in Chemical Toxicity Assessment;

II. Immunotoxicity of Environmental Chemicals and Therapeutics;

III. Collaborative Study on Neurotoxicology Assessment,

IV. Mutagenicity Studies with Salmonella;

V. In Vivo Cytogenetics Testing;

VI. Mammalian Germ Cell Mutagenesis; and

VII. Identification of Tumor Suppressor Genes in Chemically-Induced Rodent Tumors.

2:00 p.m. - 2:15 p.m. - Update on Activities of the Technical Reports Review Subcommittee (Peer Review Panel);

2:15 p.m. - 3:00 p.m. - Update on Activities of the Reproductive and Developmental Toxicology Program Review Subcommittee and Report on Scientific Efforts in NTP Reproductive and Developmental Toxicology Programs.

3:00 p.m. - 4:00 p.m. - Review of Chemicals Nominated for NTP Studies. The nominations of five chemicals will be reviewed. The chemicals were evaluated by the NTP Chemical Evaluation Committee on December 1, 1988, and are (with CAS Nos. in parentheses): (1) Dimethylformamide (68-12-2); (2) Formamide (75-12-7); (3) Indium Phosphide (22398-80-7); (4) N-Methyl-pyrrolidone (872-50-4); and (5) Monomethylformamide (123-39-7). A Request for Comments on these chemicals was published in the Federal Register Vol. 54, No. 21, pp. 5279-5280, February 2, 1989.

The Executive Secretary, Dr. Larry G. Hart, National Toxicology Program, P.O. Box 12233, Research Triangle Park, North Carolina 27709, telephone (919) 541-3971; FTS 629-3971, will have available a roster of Board members and other program information prior to the meeting, and summary minutes subsequent to the meeting.

Dated:

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

AGENDA

NTP BOARD OF SCIENTIFIC COUNSELORS

NATIONAL TOXICOLOGY PROGRAM

March 14, 1989

CONFERENCE CENTER, BUILDING 101, SOUTH CAMPUS
NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES (NIEHS)
RESEARCH TRIANGLE PARK, NORTH CAROLINA

8:30 a.m. - 8:45 a.m. -	Report of the Director	Dr. D. Rall
8:45 a.m. - 10:30 a.m. -	Concept Reviews - NIEHS - Division of Toxicology Research and Testing (DTRT) - Research Support Contracts:	Dr. R. Griesemer
	I. Toxicity and Carcinogenicity Studies in Animals	Dr. J. Selkirk
	II. Chemical Repository and Safety Support	Dr. C. Jameson
	III. Chemistry Support Services	Dr. C. Jameson
	IV. Rodent Disease Diagnostic Laboratories	Dr. G. Rao
	V. Genetic Monitoring of Inbred Rodents	Dr. G. Rao
	VI. Pathology Support	Dr. G. Boorman
	VII. Pathology Archive	Dr. G. Boorman
	VIII Statistical Analysis of Laboratory Studies	Dr. J. Haseman
10:30 a.m. - 10:45 a.m. -	Break	
10:45 a.m. - 12:15 p.m. -	Concept Reviews - NIEHS DTRT-Testing/Methods Development/ Validation Contracts:	Dr. Griesemer
	I. Immunotoxicity of Environmental Chemicals and Therapeutics	Dr. M. Luster
	II. Expired Breath Analysis in Chemical Toxicity Assessment	Dr. J. Bucher

		III. Neurotoxicology Methods Validation	Dr. D. Dietz
		IV. Mutagenicity Studies with *Salmonella*	Dr. E. Zeiger
12:15 p.m. -	1:00 p.m.	- Lunch	
1:00 p.m. -	2:00 p.m.	- Concept Reviews - NIEHS	
		DTRT - (Continued)	
		V. *In Vivo* Cytogenetics	Dr. M. Shelby
		VI. Mammalian Germ Cell Mutagenesis	Dr. M. Shelby
		VII. Identification of Rodent Tumor Suppressor Genes	Dr. W. Caspary
2:00 p.m. -	2:20 p.m.	- Update on Activities of the Technical Reports Review Subcommittee (Peer Review Panel)	Dr. J. Huff Dr. R. Scala
2:20 p.m. -	2:30 p.m.	- Carcinogenicity of 1,3-Butadiene: An Update	Dr. R. Melnick
2:30 p.m. -	3:15 p.m.	- Update on Activities of the Reproductive and Developmental Toxicology Program Review Subcommittee and Report on Scientific Efforts in NTP Reproductive and Developmental Toxicology Programs	Dr. R. Miller Dr. B. Schwetz Dr. J. Haartz, NIOSH Dr. J. Heindel Dr. R. Chapin Dr. R. Morrissey
3:15 p.m. -	4:15 p.m.	- Review of Chemicals Nominated for NTP Studies	Board Dr. D. Canter

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS

March 14, 1989

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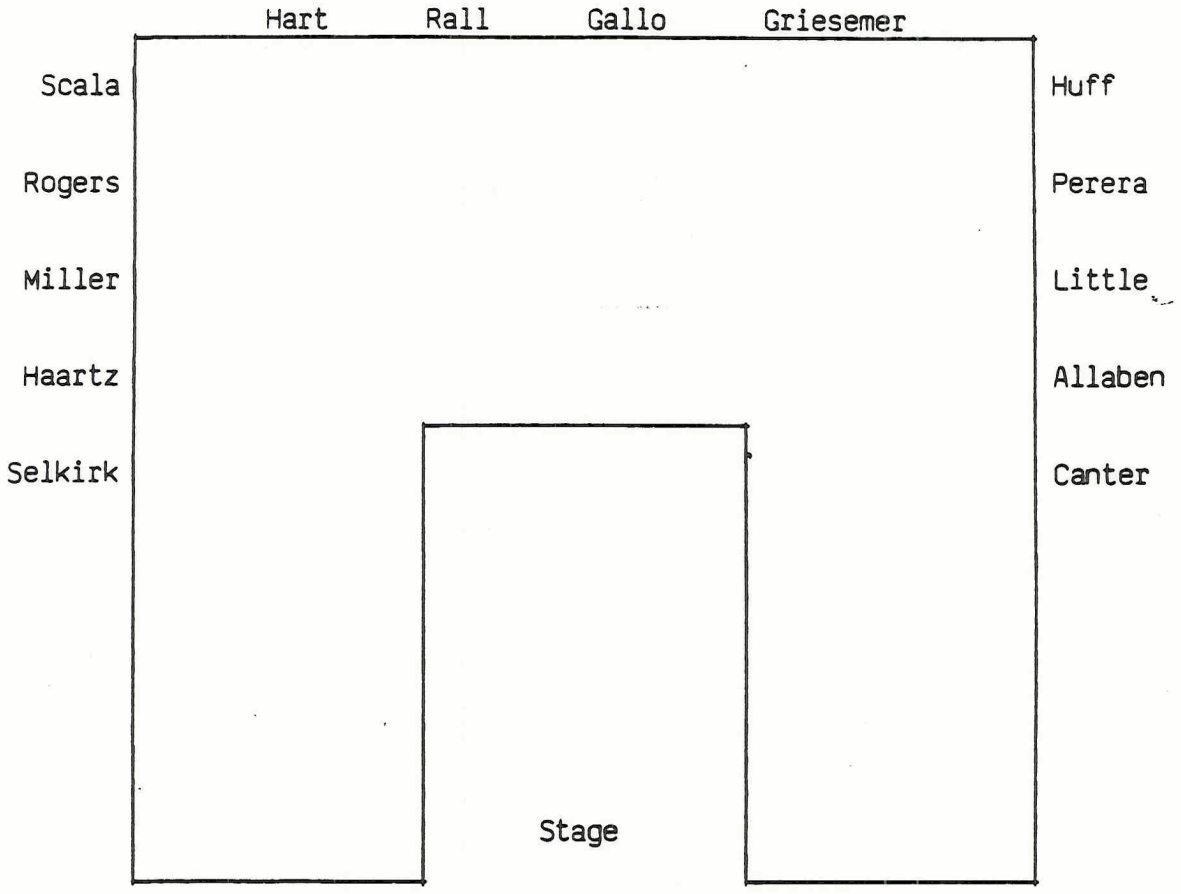
NTP BOARD OF SCIENTIFIC COUNSELORS MEETING

Conference Center, Building 101
National Institute of Environmental Health Sciences
Research Triangle Park, North Carolina

March 14, 1989



NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS

REVIEW OF PROJECT CONCEPTS

March 14, 1989

National Institute of Environmental Health Sciences

Research Triangle Park, North Carolina

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BACKGROUND
NATIONAL TOXICOLOGY PROGRAM

The National Toxicology Program was formed by the Secretary of Health and Human Services to coordinate and strengthen the Department's activities in characterizing the toxicity of chemicals. The Program is charged with (a) broadening the spectrum of toxicologic information obtained on selected chemicals, (b) increasing the number of chemicals studied, (c) developing and validating test methods, and (d) communicating the results to government agencies, the medical and scientific communities, and the public.

To fulfill its mission, the NTP conducts a basic and applied research program through contracts with non-government laboratories and institutions, through agreements with other federal agencies, and in-house in its own laboratories. Approximately 80% of NIEHS/NTP's research activities are conducted through contracts or interagency agreements.

BACKGROUND CONCEPT REVIEWS

The Division of Toxicology Research and Testing currently has 160 research and resource contracts and interagency agreements. These contracts and agreements support a variety of activities — toxicologic characterization, testing, methods development, and program resources (i.e. chemistry, occupational health and safety, animal production, pathology, quality assurance, archives, etc).

Prior to issuance of a Request for Proposal (RFP), a project concept review is required by Public Health Service regulations. These project concepts in many instances consist of more than one contract or interagency agreement. Concept reviews are needed for new projects, for recompetitions with changes in statements of work, and for projects ongoing for 5 years or more since the last concept review. There are approximately 31 concepts projected to be reviewed by the NTP Board of Scientific Counselors over the next 5 years — 15 for this meeting in March 1989, 8 for the November 1989 meeting, and 8 over the following four years, 1990-1993 (see p. 28). As program plans evolve, others may be added.

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

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

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

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

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

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

National Toxicology Program Board of Scientific Counselors
Review of Concepts

March 14, 1989

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4	Toxicity and Carcinogenicity Studies in Animals	James Selkirk	Michael A. Gallo
5*	Chemical Repository and Safety Support	C. W. Jameson	Robert A. Scala
6*	Chemistry Support Services	C. W. Jameson	Robert A. Scala
7*	Rodent Disease Diagnostic Laboratories	Ghanta Rao	Robert A. Scala
8*	Genetic Monitoring of Inbred Rodents	Ghanta Rao	Adrianne E. Rogers
9*	Pathology Support	Gary Boorman	Adrianne E. Rogers
10*	Pathology Archive	Gary Boorman	Adrianne E. Rogers
11*	Statistical Analysis of Laboratory Studies	Joseph Haseman	Frederica Perera
13**	Expired Breath Analysis in Chemical Toxicity Assessment	John Bucher	Robert A. Scala
16	Immunotoxicity of Environmental Chemicals and Therapeutics	Michael Luster	Richard K. Miller
17**	Neurotoxicology Methods Validation	Dennis Dietz	Richard K. Miller
20	Mutagenicity Studies with Salmonella	Errol Zeiger	Frederica Perera
21	In Vivo Cytogenetics	Michael Shelby	John B. Little
23	Mammalian Germ Cell Mutagenesis	Michael Shelby	John B. Little
25**	Identification of Rodent Tumor Suppressor Genes	William Caspary	Frederica Perera

*Resource Contracts

**New Projects

NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW

CONTRACT TITLE: Toxicity & Carcinogenicity Studies in Animals
PROJECT OFFICER: James Selkirk, (919) 541-2548

OBJECTIVE: To continue to characterize the toxicologic effects of chemical and physical substances through studies in animals. These studies provide a rational basis for the protection of people from exposure to hazardous substances.

CONCEPT STATEMENT: The characterization of the toxicity of substances of public health concern is performed through studies in animals, usually laboratory rodents. The typical approach is the repeated administration of the substance to groups of animals for variable periods up to two years or more. The adverse health effects from short- or long-term exposures to different dose levels are evaluated clinically, toxicologically, and pathologically by comparison with groups of control animals not administered the substance.

Because of limited laboratory space and personnel within NIEHS, the toxicology studies are performed in non-government facilities through contracts or in other government facilities through interagency agreements. These activities are also supported by resource contracts in chemistry, animal production, health and safety, pathology, quality assurance, statistics, and report preparation, all of which have separate concept reviews.

PROPOSED CHANGES TO THE CURRENT WORK STATEMENT: The work to be performed during the next five years is expected to be essentially the same as in the preceeding period. Increasingly greater emphasis is being given, however, to non-cancer toxic effects on the various organs of the body.

NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW

CONTRACT TITLE: Chemical Repository and Safety Support
PROJECT OFFICER: Douglas Walters, (919) 541-3355

OBJECTIVE: The NTP evaluates a large variety of chemicals for toxicological effects. To meet these objectives, it is essential to obtain the exact chemical requested, to maintain the integrity of these chemicals and to ensure adequate safety measures are used in handling, storage and shipping operations. The program is responsible for providing repository and health and safety support services for the NTP. The NTP Chemical Repository is vital and necessary for preserving the integrity of the studies and for making accessible chemicals from previous studies. The proposed project will allow the program to continue to provide these resources.

CONCEPT STATEMENT: The chemical repository provides the source of test chemicals for NTP testing efforts and maintains the archival supply of surplus chemicals upon completion of NTP studies. Currently maintained in the archive are approxiamtely 1900 unique chemicals.

The chemical repository provides industrial hygiene support for the NTP laboratories. This support includes determination of a variety of chemical and physical properties when this information is unavailable in the literature such as solubility tests, permeability, flash point, vapor pressure, density, melting point and explosivity determinations, as well as other industrial hygiene support, necessary for the safe handling of test chemicals. This information also serves subsequently as an aid to investigators. In providing a central source of test chemicals and corresponding chemical-physical property information, this mechanism helps eliminate a possible source of interlaboratory variability. The chemical repository also serves as a central information resource for all analytical chemistry data as well as chemical-physical property data and safety handling and emergency information for all NTP chemicals.

In addition, the NTP has established procedures for disposing of older bulk test chemicals. The chemical repository categorizes for disposal those chemicals procured, studied and their data published at least 2 years ago. As approval is granted from the NTP, excess bulk chemicals are discarded. A smaller archive sample is always maintained to resolve potential questions about previous test results.

PROPOSED CHANGES TO THE CURRENT WORK STATEMENT: The work to be performed under the five (5) year recompetition is essentially the same except for the delineation of the waste disposal process for the older chemicals.

NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW

CONTRACT TITLE: Chemistry Support Services
PROJECT OFFICER: C. W. Jameson, (919) 541-4096

OBJECTIVE: To continue to provide analytical support services for the toxicity and carcinogenicity studies conducted by the NTP. To insure that the animals under study are exposed to the prescribed chemicals and at the specified dose levels, chemistry support is required, including full chemical characterization, dosage preparation, dose level verification and biological sample analysis. The NTP analytical chemistry support services are a necessary part of the toxicity and carcinogenicity studies and for the proper characterization and analysis of chemicals studied by the NTP.

CONCEPT STATEMENT: The chemicals used in the studies must be well characterized. The precise definition of the chemical nature of the substance is one of the cornerstones of toxicity testing. In addition, it is necessary that the in vivo toxicity studies be carried out with dosage formulations which are of known composition and stability. The chemicals selected for toxicity study can vary from highly volatile nonpolar organics to non-volatile polar organometalics and inorganics. Analysis of this broad range of materials requires personnel with a broad theoretical background as well as practical experience with analytical instrumentation. The chemistry support required includes chemical procurement and synthesis, bulk chemical characterization, and dosage formulation and analysis. Other special analytical services include analysis for the chemical or metabolites in tissues and biological fluids.

The contracts are used to support toxicity and carcinogenicity studies and other special studies, i.e. teratology, continuous breeding, immunotoxicology, chemical disposition, oncogene, cellular and genetic toxicology, and AIDS studies.

PROPOSED CHANGES TO THE CURRENT WORK STATEMENT: The work to be performed under the recompetetion is essentially the same with the addition of performing pilot studies in rodents to determine basic pharmacokinetic parameters and to determine the approximate concentration range of study chemicals (or metabolities) in biological samples over which the analytical methods must be validated.

NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW

CONTRACT TITLE: Rodent Disease Diagnostic Laboratories
PROJECT OFFICER: Ghanta N. Rao, (919) 541-7899

OBJECTIVE: To continue to provide rodent disease diagnostic services support to the rodent production colonies and the chemical toxicity and carcinogenicity studies. These are necessary to maintain the quality of animals supplied for the studies and to document the infection and disease status of the animals during the course of the studies.

CONCEPT STATEMENT: Centralized colonies of rodents with homogeneous genetic properties and defined health profiles ensure an adequate and continuous supply of defined quality animals for the NTP studies. Veterinary medical procedures are performed to characterize the health status of animals prior to and during the toxicity and carcinogenicity studies.

These contracts provide rodent disease diagnostic laboratory support for monitoring the microbial status and comprehensive health status of the animals and for investigating any disease conditions that might appear. Animals from the production colonies are examined for pathogenic microorganisms and parasites. These evaluations include pathologic examination of selected tissues for microbial and parasitic lesions. Serum samples from sentinel animals in the toxicology studies are evaluated for viral antibody profiles. In addition, sentinel animals and tissues from animals on studies are evaluated for microbial or parasitic disease conditions. These programs are necessary to insure the production of disease-and-infection-free rats and mice for the study of environmental chemicals.

PROPOSED CHANGES TO THE CURRENT WORK STATEMENT: None

NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW

CONTRACT TITLE: Genetic Monitoring of Inbred Rodents
PROJECT OFFICER: Ghanta N. Rao, (919) 541-7899

OBJECTIVE: To continue to provide genetic monitoring of inbred stocks at the rodent production centers. This contract is necessary to maintain the genetic integrity of the rodent production colonies as well as the animals supplied for toxicity and carcinogenicity studies.

CONCEPT STATEMENT: This project provides genetic monitoring to assure the genetic integrity of inbred stocks at all production facilities producing F344/N rats, B6C3F1 hybrid mice and other strains of rodents for the NTP. Genetic loci are being monitored by electrophoresis of erythrocyte lysates, kidney homogenates and serum proteins. The genetic integrity of inbred rodents is also being evaluated by skin grafting. In addition kidneys from B6C3F1 hybrid mice received at the study laboratories are subjected to isoenzyme analysis by electrophoresis. Constant monitoring for biochemical genetic variants of foundation and production stock and test animals will ensure that data from NTP animal studies are collected from genetically homogenous rats and mice.

PROPOSED CHANGES TO THE CURRENT WORK STATEMENT: None

NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW

CONTRACT TITLE: Pathology Support
PROJECT OFFICER: Michael Elwell, (919) 541-5066

OBJECTIVE: To continue to provide pathology support including necropsy assistance and tissue section preparation for studies conducted in-house as well as for supplemental studies on pathology specimens generated through contracted studies; reviewing and assessing the pathology of toxicology and carcinogenicity studies performed by contractors; serving on NTP Pathology Working Group reviews; and technical support as needed for quality assessment of pathology evaluations.

CONCEPT STATEMENT: Among the objectives of the NTP are the improvement of the understanding of the nature of chemically-induced lesions in rodents and the development of criteria and standardized terminology for the diagnosis, interpretation and documentation of these lesions. In addition to in-house research, a major responsibility is the review of pathology data from the toxicity and carcinogenicity studies performed by private laboratories. Large amounts of data are generated from these studies, including anatomic and clinical pathology evaluations of animals in all phases of the experiments. Studies include pathology data from 90-day exposures as well as from interim sacrifice and two-year studies. In a two-year study there are approximately 10,000 slides with multiple tissues on each slide for evaluation. During the 90-day studies there are clinical pathology studies which include hematology slides for evaluation as well as clinical chemistry results for interpretation. A program of the magnitude and diversification of the NTP requires cooperation and collaboration of numerous testing facilities. For these studies, there is a need for assurance of uniformity and the consistent, accurate application of diagnostic criteria and procedures for pathology. This is accomplished through a variety of pathology tasks which are performed prior to, during, and after study completion.

PROPOSED CHANGES TO THE CURRENT WORKSTATEMENT: The work to be performed under the proposed five-year recompetition is essentially the same as described above. Estimated workload for histology laboratory support is decreased but additional technical effort is required for the computerized entry of pathology data into the Toxicology Data Management System (TDMS) and for the evaluation of clinical pathology data from prechronic studies.

NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW

CONTRACT TITLE: Pathology Archive
PROJECT OFFICER: Gary Boorman, (919) 541-3440

OBJECTIVE: To continue to provide storage and retrieval for all of the histological slides, paraffin blocks, formalin-fixed wet tissues, paper data, and microfiche/microfilm for the toxicity and carcinogenicity studies that have been conducted by the Program.

CONCEPT STATEMENT: The NTP Archive files and preserves the data and specimens from over 350 rodent toxicology and carcinogenicity studies. Materials from reproductive and teratology studies are also saved. The Archive coordinates and tracks data flow from study laboratories, inventories and files materials, reviews pathology data and specimens, and supports NTP and outside auditors in their review of these studies. The Archive periodically identifies older studies for disposal of selected materials.

Since carcinogenicity studies are time consuming and expensive, access to pathology specimens from these studies is invaluable. The pathology specimens provide a unique resource for the analysis of chemical-related lesions using new techniques as they become available.

PROPOSED CHANGES TO THE CURRENT WORK STATEMENT: The work to be performed under the recompetition is changed very little. Greater emphasis will be given to microfiching data and to collecting frozen tissues for oncogene analysis.

NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW

CONTRACT TITLE: Statistical Analysis of Laboratory Studies
PROPOSED PROJECT OFFICER: Joseph K. Haseman, (919) 541-4996

OBJECTIVE: To continue to provide statistical and computational expertise and resources to summarize, analyze, and aid in the interpretation of data from various laboratory animal experiments conducted by the NTP. These investigations consist of long-term carcinogenesis experiments, 14 and 90 day studies, reproduction and fertility studies, and various other toxicological experiments.

CONCEPT STATEMENT: One of the primary research efforts of the NTP is to carry out long-term laboratory animal carcinogenicity studies to evaluate the carcinogenic potential of chemicals to which humans may be exposed. Data from these experiments (e.g., survival, body weight, food consumption, histopathology) are stored in the Toxicology Data Management System (TDMS). Statistical software used to analyze these data are developed and maintained by the current contractor, with modifications and additions provided to the NTP as needed. TDMS provides both descriptive statistics (growth and survival curves) and detailed statistical analyses (e.g., survival-adjusted tests for increased tumor incidence). Specialized analyses of data stored in TDMS are also provided, and new computational tools are developed as required by changes in experimental design. A database for sentinel animals is maintained and a historical control data base of tumor incidences is under development.

NTP research efforts are being increasingly directed toward the assessment of non-cancer toxicity endpoints (data which at present are not captured by TDMS). For example, in addition to evaluating organ weights, clinical chemistry and hematology, the NTP has recently increased the scope of its studies to include parameters such as neurobehavioral changes. Further, the NTP has begun to prepare a series of Technical Reports (TRs) devoted to pre-chronic study results and interpretation. The current contractor provides detailed reports that summarize the results of statistical analyses of pre-chronic data, and these evaluations form an integral part of the new TRs. Further, the contractor is responsible for maintaining a computerized database for these parameters and supplies a report that summarizes these data on an annual basis. The current volume of pre-chronic studies is approximately 20-25 per year.

Reproduction and fertility studies form a significant number of the other toxicological experiments carried out by the NTP. The contractor is responsible for analyzing these data and preparing written reports summarizing the results.

PROPOSED CHANGES TO THE CURRENT STATEMENT OF WORK: The work to be performed under the recompetition is essentially the same as that described above. Minor proposed changes include the following:

(1) The contractors will be required to analyze data from NTP long term studies with experimental designs that can not be directly analyzed using TDMS programs. This will require the modification of existing TDMS

programs or the development of new statistical software.

(2) The contractors will be expected to maintain a historical control database for reproduction and fertility parameters.

(3) The data processing involving TDMS will be performed on the VAX computer cluster at the NIEHS rather than at the computer facility at the National Center for Toxicological Research (NCTR).

(4) The contractors may be asked to review the presentation of statistical methodology and/or the interpretation of data analyses in manuscripts that report the results of statistical evaluations carried out by the contractors.

(5) Certain additional responsibilities related to the new NTP prechronic TRs may also be needed. This will become clearer as the new TRs evolve.

NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW

CONTRACT TITLE: Expired Breath Analysis in Chemical Toxicity Assessment
PROJECT OFFICER: Dr. John R. Bucher, (919) 541-4532

OBJECTIVE: To evaluate possible applications of expired breath analysis in laboratory animals as part of an overall toxicologic assessment following chemical administration. The goal of this research is to develop a noninvasive method of toxicologic characterization that would be applicable to humans in workplace situations, and which could provide a basis for extrapolation of potential risk from rodents studies to the human situation.

CONCEPT STATEMENT: The effort described in this concept is directly related to the NTP charge to develop new and improved methods for toxicological assessment of environmental and other chemicals. Expired breath analysis is a noninvasive technique that can be performed repeatedly with animals or humans. In some ways it is analogous to clinical pathology assessments, but may be more sensitive for identifying early stages of toxic responses and can provide mechanistic information in cases where the underlying processes responsible for the exhaled substances have been established.

Expired breath analyses have been used extensively in clinical gastroenterology to study malabsorption syndromes based on detection of fermentation products (usually hydrogen or methane) generated within the gut. A second familiar application, trapping of expired radioactive compounds, is an essential part of most disposition and metabolism studies. However, the breath of healthy people and animals contains numerous low molecular weight volatile components such as isobutane, ethane, ethylene, butane, pentane, hexane, isohexane, acetone, and acetic and propionic acids. These are produced by endogenous metabolic and catabolic reactions, many of which are as yet not well characterized. Certain volatiles appear primarily under conditions of tissue necrosis and the possibility that these might be biomarkers for increased cell turnover is a promising area that has not yet been investigated.

The National Institute of Occupational Safety and Health and the Environmental Protection Agency have supported research that has developed analysis methodology for expired breath of humans. This has been directed primarily toward determining exposures to volatile industrial chemicals, but these analyses have revealed the presence of many of the compounds listed above even though the methodologies have not been optimized for their detection (Wallace et al., 1984).

Individual breath components have been studied under a variety of experimental conditions. Analysis of expired ethane and pentane is now an accepted method for the assessment of lipid peroxidation in vivo (Wendel and Dumelin, 1981). These hydrocarbons are derived from omega 3 and omega 6 polyunsaturated fatty acids during the propagation reactions of peroxidation. Administration of carbon tetrachloride to rats causes increases in expired ethane and pentane within minutes (Gee et al., 1981). Exposure to high concentrations of oxygen does not elevate pentane and

ethane levels (Roberts et al., 1983). Interestingly, expired pentane is increased under conditions of intravenous infusions of nutrient lipid emulsions (Wispe et al., 1985), but normal fatty acid metabolism does not release ethane or pentane. Patients with alcoholic cirrhosis exhale increased pentane (Moscarella et al., 1984). Patients with fatty liver do not. Patients with liver cirrhosis also exhale increased acetic and propionic acids, the origins of which are unclear, and liver injury has also been studied quantitatively by measuring exhaled mercaptans such as dimethyl sulfide or methanethiol after feeding methionine (Chen et al., 1969a, 1969b). Exposure to halothane anesthesia results in increases in isobutane exhalation in humans, but not in rats (Hemple et al., 1980). It has long been recognized that acetone and other ketone bodies are exhaled during starvation or in diabetes.

These selected examples for applying breath analyses have been established the usefulness of the approach. What has been lacking is an overall effort to identify breath components more completely, both volatile hydrocarbons and the more polar compounds which are frequently lost through adsorption to trapping materials, and to evaluate the utility of these compounds to act as biomarkers for specific metabolic or toxic processes. We should establish baseline information on the consistency of breath components throughout the day, as animals age, under conditions of fasting (ketosis) or administration of corn oil, and perhaps throughout the estrus cycle. We should evaluate possibility that chemically included increased cell turnover in a particular organ may provoke consistent changes in the pattern of expired breath components, and we need to understand how agents which inhibit cytochrome P450 activity affect the exhalation of substances such as pentane which is known to undergo metabolism by the mixed function oxidase system (Frank et al., 1980). All of this information is essential to understand the full potential of this technique for use in screening individuals who are exposed to potentially hazardous chemicals in the workplace, and to evaluate the utility of this tool for extrapolating potential identified risks from animal bioassays to humans.

The initial efforts of this new research project will center on a complete characterization of volatile breath components of the Fischer 344 rat. Technical questions that will be addressed concern the generation of breath samples via pass-through or rebreathing systems and the optimal methodologies for collection of polar compounds (methods for collection of nonpolars are available). Once these initial studies are completed, efforts will move to evaluating the consistency of breath components in the F344 rat over time, under conditions of fasting, corn oil administration, and other conditions as outlined above. If the results of these studies justify continued work with this methodology, efforts would shift towards the characterization of changes in breath components with chemical administration, focusing on chemicals which have produced pathologic changes in prechronic studies, and for which data are available from 2-year exposure studies.

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NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW

CONTRACT TITLE: Immunotoxicity of Environmental Chemicals and Therapeutics
PROJECT OFFICER: Dr. Michael I. Luster, (919) 541-4188

OBJECTIVE: To continue to examine the potential immunotoxicity of environmental chemicals, drugs or biologics (e.g. cytokines) on the immune response in laboratory animals. The immune effects that are to be monitored include immunomodulation (i.e., the ability of a xenobiotic to either suppress or enhance various immune responses) as well as alter resistance to challenge with infectious agents or transplantable tumor cells. In addition, other xenobiotics will be studied for their ability to act as sensitizers and produce hypersensitivity disease. The immuntoxicology effort is important since the data derived serves as important adjunct data to more traditional toxicological endpoints monitored in the toxicity studies.

CONCEPT STATEMENT: The immunotoxicology data serves as an additional endpoint in the other toxicology studies which can be used in risk assesment. The extent to which exposures to chemical xenobiotics alter the immunocompetence of the general population remains a critical issue. Several lines of evidence indicate that the continued concern for the immunotoxic potential of xenobiotics is warranted. Firstly, a substantial data base exists demonstrating that a broad spectrum of chemical xenobiotics are immunosuppressive in laboratory animals. These immunological effects are often accompanied by increased susceptibility to challenge with infectious agents or tumor cells. Secondly, although extrapolation from rodents to humans is difficult, in several instances parallel effects on the immune system have been found between experimental animals and humans who have been therapeutically, inadvertently or occupationally exposed to selected chemical xenobiotics including PCBs, dibenzofurans, TCDD, benzene and PBBs. In addition, a large number of xenobiotics are known or presumed sensitizers (i.e., they produce allergy) in humans including isocyanetes, anhydrides, and many pesticides.

PROPOSED CHANGES TO THE CURRENT WORK STATEMENT: The work to be performed under the recompetition is essentially the same as described above. However, currently the contractor(s) performs a Tier I testing configuration as the initial test to examine for immunomodulation. It is proposed that a pre-screen be established where the contractor(s) determines dose levels and examines the antibody plaque forming cell assay before examination in the standard testing scheme.

In addition, since strain difference appear to play a role for certain xenobiotics in their ability to manifest immunotoxicity, it is proposed that for source xenobiotics the testing of a second mouse strain or rats, in addition to the $B6C3F_1$ strain be included.

Lastly, there will be some minor modifications in the presently used testing panel. In Tier I, the cytotoxic T lymphocyte assay (CTL) will replace the DHR test while lymphocyte enumeration will be moved from Tier II to Tier I. The existing macrophage test will be replaced by a macrophage anti-tumor assay.

NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW

CONTRACT TITLE: Neurotoxicology Methods Validation
PROJECT OFFICER: Dennis Dietz, (919) 541-2272

OBJECTIVE: Based upon recommendations from an international panel of experts in the area of neurotoxicology, the World Health Organization (WHO), within the framework of the International Programme on Chemical Safety (IPCS), is coordinating an international collaborative study on methods for the identification of chemicals having potential neurotoxicity in humans.

This collaborative study will utilize a functional observational battery and automated measures of motor activity to study a group of coded chemicals with known neurotoxic and non-neurotoxic potential to: (1) evaluate the reliability, sensitivity, and specificity of a set of neurobehavioral methods designed to identify chemicals that are potential neurotoxic agents in humans, (2) begin to develop a data base for the neurobehavioral effects of substances with known neurotoxicity which can serve as a reference for evaluating compounds with unknown nervous system toxicity, and (3) analyse data from the 10-12 participating laboratories for dose- and time-dependent effects of chemical treatments and for the influence of interlaboratory variability on these results.

The goal of the present project is the participation of the National Toxicology Program (NTP) as one of the 10-12 laboratories in the International Collaborative Study on Neurotoxicology Assessment. This is important since NTP is the principal nonregulatory U.S. federal agency responsible for the identification and characterization of potential neurotoxic agents.

CONCEPT STATEMENT: The effort described in this concept conforms to the NTP's goal to develop new and improved methods for toxicological assessment of chemicals and is responsive to an increased public interest concerning the actions of chemical or physical agents on the nervous system. Ultimately, the experience and data base generated in this study may be used to establish an internationally recognized protocol for the initial screening of chemicals for potential neurotoxicity.

The need to develop a strategy to assess chemicals for potential neurotoxicity has been recognized by many expert panels and groups (USNAS, 1975, 1977, 1984; Leukroth, 1987; WHO, 1986). Accordingly, the NTP has adopted a sequential or tiered approach to evaluate chemicals for neurotoxicity. The first tier or level is to determine if a chemical might produce neurotoxicity following exposure, i.e., hazard detection, and usually consists of some type of functional observational battery.

The decision to advance to the second tier is based upon the data suggesting that a chemical produces neurotoxicity in first tier studies. Much of the neurotoxicity testing done at the NTP is at the secondary level. The information used to decide to evaluate a chemical in the NTP battery comes from a variety of sources, including neurotoxicological data already existing in the literature, structure activity relationships, data

from a first tier functional observational battery or following reports of neurotoxicity from humans exposed to the chemical. This second level helps ascertain whether or not the nervous system is a primary target for the chemical and determines dose- and time-effect relationships using relatively sensitive endpoints.

The NTP recognizes a third level when previous data have suggested a specific neurotoxic effect (i.e., sensory deficit, cognitive loss). Experiments done at the third level are intended to characterize or study the mechanism of action associated with a neurotoxic agent.

An NTP contract laboratory will be selected to participate in the WHO validation study of neurotoxicity assessment using the Functional Observational Battery (FOB) and automated Motor Activity (MA) in rats. The FOB consists of a sequence of observations designed to detect changes in arousal (hyperactivity, hyperreactivity, and lethargy), emotionality, other signs of central nervous stimulation (convulsions and tremors), limb weakness or paralysis, autonomic nervous system toxicity, and sensory disturbances. This study will be conducted under GLP compliance and NTP requirements regarding animal care, chemistry, and health and safety will be met. All participating laboratories will study the effects of aliquots of the same seven substances. Five substances have been selected by the IPCS expert committee for their known patterns of neurotoxicity and two nonneurotoxic substances will serve as controls in the initial phase of interlaboratory validation.

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NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW

CONTRACT TITLE: Mutagenicity Studies With Salmonella
PROJECT OFFICER: Errol Zeiger, (919) 541-4482

OBJECTIVE: To evaluate the mutagenicity of pure chemicals and complex mixtures using Salmonella typhimurium.

CONCEPT STATEMENT: To support the above objective, two types of contracts are proposed: (1) the use of standard protocols to test for coded chemicals; and (2) the use of specially designed or selected protocols for substances that cannot be adequately studied using standard protocols. The chemicals to be tested will be those nominated to the NTP for mutagenicity testing, structural analogues of these chemicals, chemicals nominated for carcinogenicity or reproductive toxicity testing, and other chemicals of interest to the NIEHS or other NTP agencies. A number of chemicals will be tested in more than one laboratory, or at different times in the same laboratory, as a mechanism for monitoring laboratory performance and to resolve weak or equivocal responses.

Standard Protocols: These contracts will permit the routine testing of coded chemicals for mutagenicity in more than one laboratory. Testing will be performed using the preincubation procedure, with oxidative and reductive metabolism from rat and hamster liver (S-9) preparations. Gases and highly volatile liquids will be tested in sealed chambers.

Special Protocols: This contract will allow the selection or design of protocols for specific substances that cannot be adequately tested using the standard protocols employed by the other Salmonella testing laboratories. Examples include a determination as to the extent to which intra- and extracellular glutathione are required for the mutagenicity of dichloromethane; the mutagenicity of ozone; whether wood dusts, or extractable materials from the dusts, are mutagenic; and the ability of liver homogenates from various animal species to metabolize chemicals to mutagens. Chemicals and complex mixtures for mutagenicity evaluation will use protocols developed from a variety of tester strains and metabolic activation procedures, and the use of different chemical extraction or sample preparation techniques for complex mixtures.

PROPOSED CHANGES TO THE CURRENT WORK STATEMENT: None

NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW

CONTRACT TITLE: In Vivo Cytogenetics
PROJECT OFFICER: Michael Shelby, (919) 541-3455

OBJECTIVE: To provide methods development and testing capacity that will permit the improvement, assessment and application of short term, in vivo studies for genetic toxicity. The awards will include projects involving tests for micronuclei, chromosomal aberrations and sister-chromatid exchanges in mice and the development of testing capabilities in rats. Studies are generally conducted using bone marrow cells as the target. Study results from these studies are used in the process of selecting chemicals for study in long term toxicity and germ cell mutagenicity studies, and in the overall evaluation of long term toxicity study results.

CONCEPT STATEMENT: Numerous short-term assays are available to determine the genetic toxicity of chemicals. These assays use a variety of organisms, ranging from viruses and bacteria to intact mammals, and are capable of detecting the induction of a broad spectrum of mutational events. A major activity of the NTP has been the evaluation and application of tests for induced cytogenetic effects in laboratory rodents, and has centered on tests for chromosomal aberrations (ABS), sister-chromatid exchanges (SCE), and more recently, micronuclei (MN) in the bone marrow cells of B6C3F1 mice.

Results of tests on approximately 75 chemicals provide evidence that the bone marrow tests for ABS and SCE are effective and reproducible means of detecting the genetic toxicity of chemicals in whole animals. Evaluation of this data base indicates that the performance of these studies in detecting carcinogens and in discriminating between carcinogens and noncarcinogens is equal to or exceeds the performance of most in vitro short-term studies. Bone marrow ABS and SCE studies on the 73 chemicals previously used by the CGTB to assess the performance of four in vitro short-term studies are nearly complete. Upon completion of these studies, an assessment, similar to that done on the four in vitro studies, of the performances of the in vivo tests will be conducted. We have also begun studies on approximately 50 of those 73 chemicals in the mouse bone marrow micronucleus study in an effort to characterize the performance of this study. Preliminary results indicate that the micronucleus study will be an effective and economical means to detect in vivo cytogenetic effects and may replace the chromosome aberration study as a primary screen for in vivo genetic toxicity.

Test results support a continued effort in evaluating the performance of in vivo cytogenetic tests, refining protocols and improving the economy in terms of dollars and animals, and in defining their role in the evaluation of chemical hazards. Our immediate plans are to complete testing of the 73 chemicals in the ABS and SCE tests and to publish those results. Meanwhile, the MN test is being evaluated through use of a modified protocol that was developed in this program. Based upon the micronucleus test results, our plans for next year are to reduce ABS and SCE testing capacity and to increase MN testing while maintaining the capacity to conduct in-depth ABS studies on chemicals of interest.

PROPOSED CHANGES TO THE CURRENT STATEMENTS OF WORK: The work to be performed over the next 5 years will be essentially the same as that being conducted at the present. The only significant changes anticipated in the statements of work will involve the number of chemicals to be tested in any given period of time. As mentioned above, we anticipate an increase the number of micronucleus tests, a decrease the numbers of ABS and SCE tests and to continue scoring micronuclei in blood smears from mice on 90-day studies. The only other changes that can be anticipated involve possible improvements in protocols or data analysis and reporting.

NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW

CONTRACT TITLE: Mammalian Germ Cell Mutagenesis
PROJECT OFFICERS: Jack Bishop, (919) 541-1876
Michael Shelby, (919) 541-3455

OBJECTIVE: To continue to provide in vivo data relating to the effects of mutagenic agents upon mammalian germ cells and to increase the number of chemicals tested per year as well as broaden the spectrum of endpoints measured with respect to various classes of mutational events. This data base is the primary source of chemical germ cell mutagenicity data in the U.S. and serves as a major resource for assessing risks for increased incidence of inherited diseases and birth defects associated with exposure to environmental mutagens.

CONCEPT STATEMENT: Among the naturally occurring and synthetic chemicals to which humans are exposed are those with the capacity to interact with DNA and give rise to mutations. If humans are exposed to mutagenic chemicals and that exposure leads to mutations in the germ cells, the risk of genetically based disease is increased in subsequent generations. If chemically induced genetic damage in human germ cells is to be minimized or avoided, it is important that potentially mutagenic chemicals be evaluated using assays in which heritable germ cell mutations are detected in a mammalian system. Several assays have been developed to detect the induction of mutations in germ cells. One of these is the mouse germ cell electrophoretic mutation assay, a method that utilizes tissue preparations from the offspring of chemically exposed mice. These tissues are analyzed electrophoretically for the presence of variant proteins and the mutational origin of detected variants are then confirmed by breeding tests.

The mouse electrophoretic germinal mutation assay offers two major advantages in evaluating chemicals for mammalian germ cell mutagenicity. The first is that their assay detects changes in specific enzymes and thereby offers the opportunity to relate genetic changes to changes in enzyme structure and function and ultimately to effects on health or fitness. The second is the potential availability of a large number of loci for analysis which ultimately means that fewer mice are needed to observe an effect.

During the 6 year course of NTP sponsored studies using this assay, a number of important tasks have been accomplished. The mutagenicity of N-ethyl- N-nitrosourea has been studied extensively; a dose response curve has been developed for treated spermatogonia and the sensitivity of female germ cells has been determined. The effectiveness of x-rays at inducing electrophoretic mutants has been demonstrated in males and females using both acute and split-dose exposures. Ethylene oxide was detected as a mouse germ cell mutagen in male postmeiotic germ cell stages and ethylene dibromide was shown to be nonmutagenic under the conditions of the tests conducted.

Of substantial importance was the detection of two mouse models for human genetic diseases: beta-thalassemia and carbonic anhydrase deficiency. Stocks of animals were established for both conditions and have been shared

with other investigators for purpose of collaborative research on molecular characterization and gene therapy.

This assay has now been used successfully in demonstrating the germ cell mutagenicity of both chemicals and ionizing radiation. Based on the success of these studies, the U.S.E.P.A. is in the process of establishing the assay as an option when a test for heritable mutations in mammals is required. However, it is advantageous, particularly with regard to efficiency, to combine the endpoints used in various germ cell mutagenicity assays into a single assay. Such an assay would permit the detection of mutations at a greater number of loci and should provide the opportunity to recover a wider spectrum of genetic damage than is provided by any single endpoint. The feasibility of such a system has been demonstrated by Ehling and his co-workers using what they call the "multiple endpoint approach". In their studies, the progeny of treated parents are screened for evidence of mutations in genes controlling morphological characteristics (7 loci), cataract development (ca. 30 loci), electrophoretic patterns (23 loci), and enzyme activity (12 loci).

To partially overcome the limitations of the individual assays, it is proposed to develop and evaluate the utility of an assay that combines some of these endpoints into a single system. Because the mouse electrophoretic assay offers advantages such as availability of a large number of loci for analysis and an endpoint which is a direct measure of a molecular change in an enzyme, the proposed contracts will involve the continued use of this assay to test chemicals for germ cell mutagenicity, but the assay will be expanded to include other endpoints/loci. In so doing, the efficiency and the information obtained from the assay will be improved. The combined endpoint approach would be supportive of the EPA's "Guidelines for Mutagenicity Risk Assessment" which stresses that the entire spectrum of heritable mutational lesions be considered in the assessment of genetic risk (Federal Register, Vol. 51, No. 185, pages 34006-34012, 1986).

PROPOSED CHANGES TO THE CURRENT WORKSTATEMENT: The work to be performed under the recompetition involve the continued use of this assay to test chemicals for germ cell mutagenicity. However, since the electrophoretic assay is now established as a valid method for detecting germ cell mutagens, the addition of other endpoints to the system offers the opportunity to improve and expand the capacity of the assay to yield data on the genetic effects induced by chemicals in mammalian germ cells. Therefore, the proposed contracts will place primary emphasis upon expanding the assay to include other endpoints/loci. In so doing, the efficiency and the information obtained from the assay will be improved. The proposed contract work is necessary for the NTP to continue its central role in the evaluation of environmental agents for germ cell mutagenicity and in doing so, support the public, industrial and governmental efforts to assess human genetic risks.

NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW

CONTRACT TITLE: Identification of Rodent Tumor Suppressor Genes
PROJECT OFFICER: William Caspary, (919) 541-2150

OBJECTIVE: To identify tumor suppressor genes in rodents. An approach to be taken is to develop DNA probes for the hybrid B6C3F1 mouse that can be used to study spontaneous and carcinogen-induced loss of tumor suppressor genes. There is evidence for the involvement of two distinct classes of genes in carcinogenesis - proto-oncogenes and tumor suppressor genes. While evidence is mounting for the role of tumor suppressor genes in human cancers, their role in chemically induced tumors in animals has not been ascertained as yet. We propose to initiate a study of the loss of tumor suppressor genes in chemically induced tumors of rodents.

CONCEPT STATEMENT: Two classes of genes have been identified and implicated in the etiology of cancer: proto-oncogenes and tumor suppressor genes. Proto-oncogenes appear to play a role in normal cellular growth and differentiation, and when activated to oncogenes, the processes controlling these functions are disrupted leading to neoplastic growth (Bishop, 1987). Tumor suppressor genes are also normal genes and provide signals for control of cell proliferation. Their activity must be lost or inactivated for cells to become neoplastic (Barrett and Wiseman, 1987).

Activated oncogenes have been found in human and rodent tumors (Varmus, 1984; Barbacid, 1986; Reynolds et al, 1986; Stowers et al, 1987; Reynolds et al, 1987; Reynolds et al, 1988). The transforming ability of oncogenes has been associated with overexpression of the proto-oncogene product, point mutations, partial deletions or translocations. In humans, the large majority of transforming genes detected by this approach are members of a highly conserved ras family of genes (Parade, et al, 1982), a finding that has been supported by examination of spontaneous and chemically induced tumors in rodents. In contrast to the requirement that proto-oncogenes become activated in the carcinogenic process, the normal function of tumor suppressor genes must be inhibited during the development of certain tumors (Cavenee et al, 1986; Ponder, 1978). In humans, there is considerable evidence that the retinoblastoma locus (Rb) at 13q14 is the site of such a gene. Anomalies in this region have been implicated in the development of not only retinoblastoma, but also osteosarcoma, ductal breast cancer and small cell lung cancer. Cytogenetically visible deletions have been observed in other human tumors including Wilm's tumor (chromosome 11), acoustic neuroma (chromosome 22), carcinoma of the lung (chromosome 3) and colon (chromosome 5) implying that tumor suppressor genes may also occupy these sites.

Historically, the concept of tumor suppressor genes can be traced to a proposal by DeMars (1969), whereby in certain familial cancers recessive heterozygous genes may become homozygous leading to expression the cancer phenotype. This was refined in the two hit models of retinoblastoma developed by Knudson (1971, 1985). Another line of evidence for tumor suppressor genes comes from studies on the genetic regulation of the tumor phenotype in somatic cell hybrid experiments. The recognition that tumor suppressor genes play a role in the etiology of human cancer caused a surge

of activity to determine whether loss of Rb1 gene function is pleiotropic and whether other genes play a similar role for other tumors. Confirmation of this has been provided in studies by Cavenee and coworkers (1983) using RFLPs to search for heterozygous DNA segments which become homozygous in various tumors.

The loss of tumor suppressor genes is often associated with the concomitant loss of other genes on the same chromosome which can be shown by loss of heterozygosity of chromosomal markers. The approach of this proposal will take advantage of the heterozygous nature of the hybrid B6C3F1 mouse. DNA probes which detect restriction fragment length polymorphisms (RFLPs) in B6C3F1 mice will be identified and mapped to specific chromosomes. Tumor DNA will then be digested with a variety or restriction endonucleases and examined by Southern analyses with these RFLP probes in order to detect chromosomal regions which have lost heterozygosity. If non-random losses of heterozygosity on a specific chromosome are found for a given tumor type, this would suggest that tumor suppressor genes may be linked to these RFLP markers.

DNA probes have already been identified that detect RFLPs on twelve different chromosomes of the B6C3F1 mouse. A contract laboratory will use these initially to screen tumor DNAs for regions of the mouse genome that may harbor tumor suppressor genes. In addition, several hundred DNA probes have been mapped in the mouse by RFLP analysis. A contract laboratory will digest C57/B16 and C3H/He DNA with a variety of restriction enzymes and perform Southern analyses with these and additional anonymous DNA probes to identify new RFLP markers for the B6C3F1 mouse. If the chromosomes of the suspected suppressor genes in the mouse can be predicted based on homology with man (as it is for retinoblastoma, Wilm's tumor, etc.), then DNA probes in that chromosomal region can be examined. If the location is unknown, then markers from throughout the genome can be utilized to search for losses of heterozygosity in tumor DNA.

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SCHEDULE OF CONCEPT REVIEWS

	MARCH 1989	NOVEMBER 1989	1990-1993
TESTING:			
Toxicity and Carcinogenicity	X		
Salmonella	X		
In Vitro Cytogenetics			X
In Vivo Cytogenetics	X		
Chemical Disposition			X
Immunotoxicity	X		
Inhalation Toxicology On-site Facility		X	
Developmental Toxicity Testing			X
Reproductive Toxicity Testing		X	
RESOURCE SUPPORT:			
Health and Safety Support			X
Chemical Repository and Safety Support	X		
Chemistry Support Services	X		
Rodent Disease Diagnostic	X		
Genetic Monitoring on Inbred Rodents	X		
Pathology Support	X		
Pathology Archive	X		
Pathology Quality Assurance			X
Statistical Analysis of Laboratory Studies	X		
DEVELOPMENT/VALIDATION/OTHER RESEARCH:			
Genetic Tox. Test System Dev. & Valid:			
Mammalian Germ Cell Mutagenesis	X		
Mouse Germ Cell Specific Locus and Chromosomal Mutations		X	
Development of Mutagenesis Assays Using Transgenic Mice			X
Transfection of Human P450s into Human Cells		X	
Aneuploidy Test Systems			X
Development of Tests for Nongenotoxic Carcinogens:			
Mammalian Cell Assay Systems		X	
In Vitro Transformation of Oncogene Primed Cells by Genotoxic Chemicals		X	
Molecular Mechanisms of Genotoxicity:			
Identification of Rodent Tumor Suppressor Genes	X		
Mouse Endogenous Retroviral LTR Elements for Studying Gene Transposition in Environmental Carcinogenesis		X	
Response of Centromeres to DNA Damaging Agents			X
Expired Breath Analysis in Chemical Toxicity Assessment	X		
Neurotoxicology Methods Development	X		
Methods Development to Assess Human Metabolism of Chemicals		X	

CARCINOGENICITY OF 1,3-BUTADIENE: AN UPDATE

NTP Board of Scientific Counselors

March 14, 1989

R. Melnick, CTEB

INCIDENCE OF PRIMARY TUMORS IN MALE B6C3F1 MICE EXPOSED TO 1,3-BUTADIENE FOR 60 WEEKS

TARGET: NEOPLASM	EXPOSURE CONCENTRATION (ppm)		
	0	625	1250
Malignant Lymphoma+	0/50	23/50*	29/50*
Heart: Hemangiosarcoma+	0/50	16/49*	7/49*
Lung: Alveolar-bronchiolar neoplasm+	2/50	14/49*	15/49*
Forestomach: Squamous cell neoplasm	0/49	7/40*	1/44

+ Increasing trend, $P < 0.05$.
* Increased compared to control (0 ppm), $P < 0.05$.

INCIDENCE OF PRIMARY TUMORS IN FEMALE B6C3F1 MICE EXPOSED TO 1,3-BUTADIENE FOR 61 WEEKS

TARGET: NEOPLASM	EXPOSURE CONCENTRATION (ppm)		
	0	625	1250
Malignant Lymphoma+	1/50	10/49*	10/49*
Heart: Hemangiosarcoma+	0/50	11/48*	18/49*
Lung: Alveolar-bronchiolar neoplasm+	3/49	12/48*	23/49*
Forestomach: Squamous cell neoplasm+	0/49	5/42*	10/49*
Mammary gland: Acinar cell carcinoma+	0/50	2/49	6/49*
Ovary: Granulosa cell neoplasm+	0/49	6/45*	12/48*
Liver: Hepatocellular neoplasm+	0/50	2/47	5/49*

+ Increasing trend, $P < 0.05$.
* Increased compared to control (0 ppm), $P < 0.05$.

EXPERIMENTAL DESIGN FOR THE SECOND LONG-TERM INHALATION STUDY OF 1,3-BUTADIENE IN MICE

Species, sex:	B6C3F1 mice, males and females
Animals/group:	70; 50/group for 2 years, plus 10/group for sacrifices at 40 and 65 weeks
Exposure concentrations:	0, 6.25, 20, 62.5, 200, 625 ppm
Duration of exposure:	6 hr/day, 5 day/wk for 40, 65, or 103 wk

SURVIVAL AND FREQUENCY OF MALIGNANT TUMORS IN B6C3F1 MICE EXPOSED TO 1,3-BUTADIENE FOR 2 YEARS

	EXPOSURE CONCENTRATION (ppm)					
	0	6.25	20	62.5	200	625
MALES						
Survival[a] (%)	70	78	48*	44*	8*	0*
Malignant tumors (%)	33	55	62+	68+	73+	86+
FEMALES						
Survival[a] (%)	74	66	48*	22*	0*	0*
Malignant tumors (%)	24	63+	68+	63+	80+	88+

[a] Percentage of animals surviving 2 years of exposure, excluding those deaths due to interim sacrifices at 40 and 65 weeks.

* Decreased compared to control (0 ppm), $P < 0.05$.

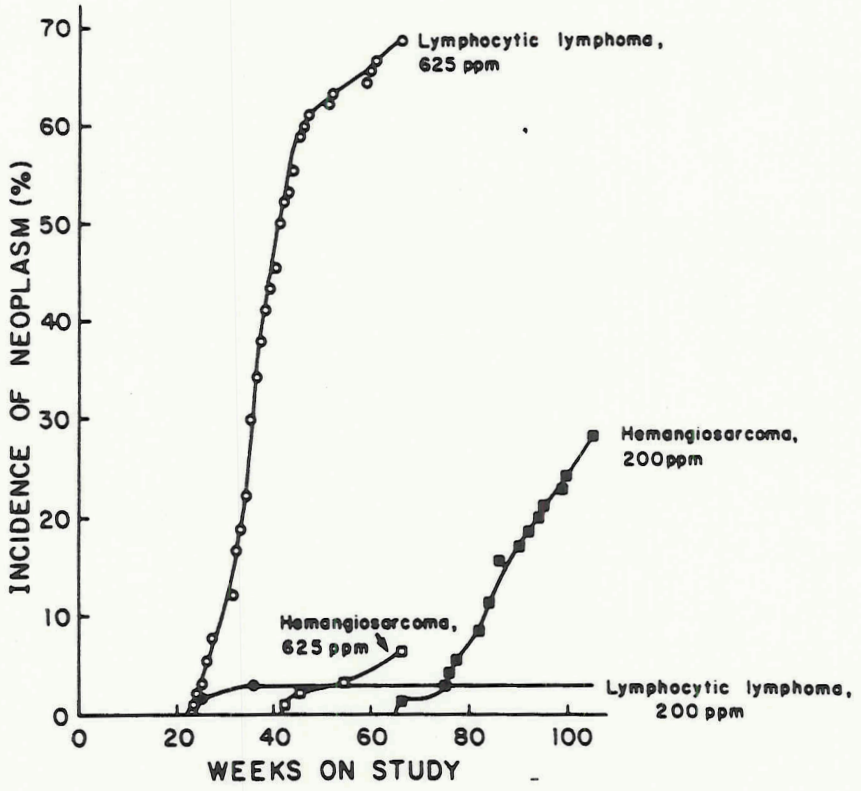
+ Increased compared to control (0 ppm), $P < 0.05$, based on adjustment for intercurrent mortality by logistic regression analyses.

INCIDENCE OF PRIMARY TUMORS IN MALE B6C3F1 MICE EXPOSED TO 1,3-BUTADIENE FOR 2 YEARS

TARGET: NEOPLASM	EXPOSURE CONCENTRATION (ppm)					
	0	6.25	20	62.5	200	625
Lymphocytic lymphoma	2/70 (3%)	1/60 (2%)	2/60 (3%)	4/69 (6%)	2/70 (3%)	62/90* (69%)
All malignant lymphomas	4/70 (6%)	3/60 (5%)	8/60 (13%)	11/69* (16%)	9/70* (13%)	69/90* (77%)
Heart: hemangiosarcoma	0/70 (0%)	0/60 (0%)	1/60 (2%)	5/58* (9%)	20/70* (29%)	6/90* (7%)
Lung: alveolar-bronchiolar neoplasm	22/70 (31%)	23/60 (38%)	20/60 (33%)	33/69* (48%)	42/70* (60%)	12/88* (14%)
Forestomach: squamous cell neoplasm	1/70 (1%)	0/60 (0%)	1/60 (2%)	5/60 (8%)	12/70* (17%)	13/89* (15%)
Harderian gland: neoplasm	6/70 (9%)	7/60 (12%)	11/60 (18%)	24/69* (35%)	33/70* (47%)	7/90* (8%)
Liver: hepatocellular neoplasm	31/70 (44%)	27/60 (45%)	35/59 (59%)	32/59 (54%)	39/70* (56%)	12/89 (13%)
Preputial gland: neoplasm	0/70 (0%)	0/60 (0%)	0/60 (0%)	0/69 (0%)	5/70* (7%)	0/90 (0%)

* Increased compared to chamber control (0 ppm), $P < 0.05$, based on adjustment for intercurrent mortality by logistic regression analyses.

Cumulative incidence of lymphocytic lymphomas and hemangiosarcomas of the heart versus weeks on study for male B6C3F1 mice exposed to 200 or 625 ppm 1,3-butadiene

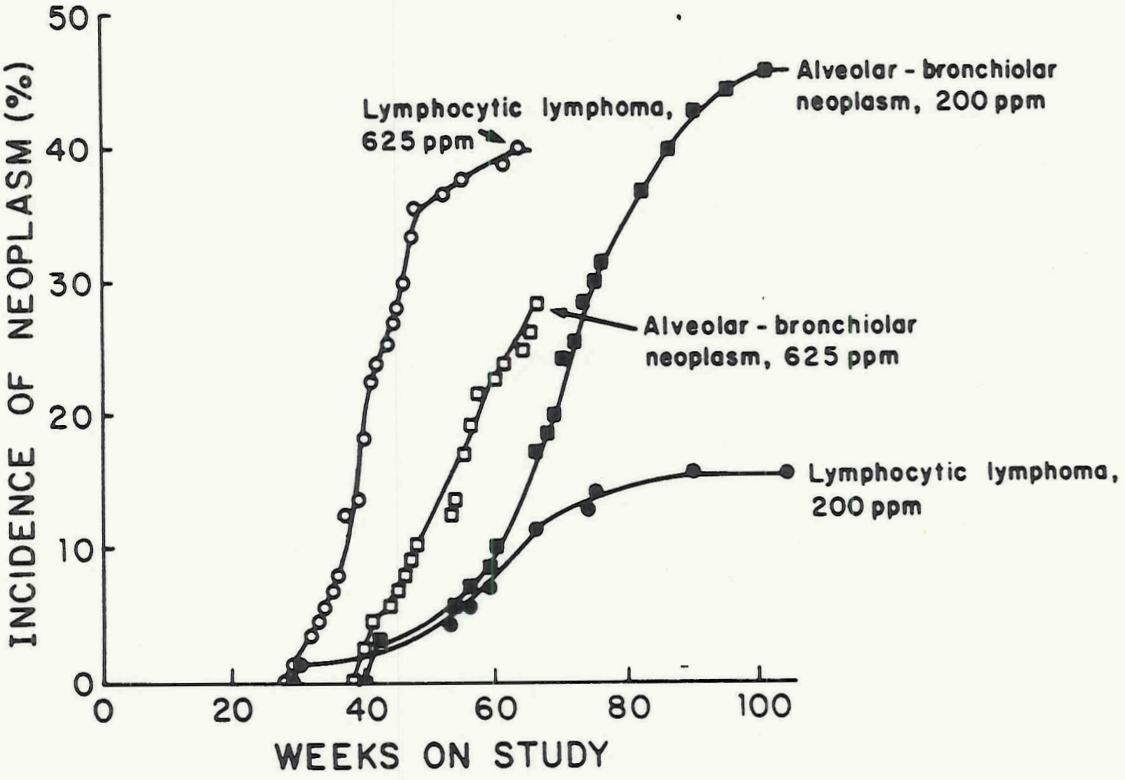


INCIDENCE OF PRIMARY TUMORS IN FEMALE B6C3F1 MICE EXPOSED TO 1,3-BUTADIENE FOR 2 YEARS

TARGET: NEOPLASM	EXPOSURE CONCENTRATION (ppm)					
	0	6.25	20	62.5	200	625
Lymphocytic lymphoma	2/70 (3%)	4/60 (7%)	6/60 (10%)	3/70 (4%)	11/70* (16%)	36/90* (40%)
All malignant lymphomas	10/70 (14%)	14/60 (23%)	18/60* (30%)	10/70 (14%)	19/70* (27%)	43/90* (48%)
Heart: hemangiosarcoma	0/70 (0%)	0/60 (0%)	0/60 (0%)	1/59 (2%)	20/70* (29%)	26/90* (29%)
Lung: alveolar-bronchiolar neoplasm	4/70 (6%)	15/60* (25%)	19/60* (32%)	27/70* (39%)	32/70* (46%)	25/88* (28%)
Forestomach: squamous cell neoplasm	2/70 (3%)	2/60 (3%)	3/57 (5%)	4/68 (6%)	7/70* (10%)	28/89* (31%)
Harderian gland: neoplasm	9/70 (13%)	10/60 (17%)	7/60 (12%)	16/69* (23%)	22/70* (31%)	7/90 (8%)
Liver: hepatocellular neoplasm	17/69 (25%)	20/60 (33%)	23/60* (38%)	24/60* (40%)	20/60* (33%)	3/90 (3%)
Mammary gland: adenocarcinoma	0/70 (0%)	2/60 (3%)	2/60 (3%)	6/70* (9%)	13/70* (19%)	13/90* (14%)
Ovary: granulosa cell neoplasm	0/69 (0%)	0/59 (0%)	0/59 (0%)	9/70* (13%)	11/70* (16%)	6/89 (7%)

* Increased compared to chamber control (0 ppm), $P < 0.05$, based on adjustment for intercurrent mortality by logistic regression analyses.

Cumulative incidence of lymphocytic lymphomas or alveolar-bronchiolar neoplasms versus weeks on study for female B6C3F1 mice exposed to 200 or 625 ppm 1,3-butadiene



STOP-EXPOSURE STUDY OF 1,3-BUTADIENE IN MALE B6C3F1 MICE

BUTADIENE CONCN. (ppm)	EXPOSURE DURATION (weeks)	TOTAL EXPOSURE* (ppm-weeks)
625	13	8,125
200	40	8,000
625	26	16,250
312	52	16,224

* After the exposure period, mice (N=50/group) were placed in control chambers for the remainder of the 104 week study.

INCIDENCE OF PRIMARY TUMORS IN THE STOP-EXPOSURE (SE) GROUPS OF MALE B6C3F1 MICE EXPOSED TO 1,3-BUTADIENE

TARGET: NEOPLASM	EXPOSURE CONCENTRATION (ppm)				
	0 Control	200 SE 40 wk (8,000)[a]	625 SE 13 wk (8,125)	312 SE 52 wk (16,224)	625 SE 26 wk (16,250)
Lymphocytic lymphoma	2/70 (3%)	6/50 (12%)	17/50* (34%)	3/50 (6%)	30/50* (60%)
All Malignant lymphomas	4/70 (6%)	12/50* (24%)	24/50* (48%)	15/50* (30%)	37/50* (74%)
Heart: hemangiosarcoma	0/70 (0%)	15/50* (30%)	7/50* (14%)	33/50* (66%)	13/50* (26%)
Lung: alveolar-bronchiolar neoplasm	22/70 (31%)	35/50* (70%)	27/50* (54%)	32/50* (64%)	18/50* (36%)
Forestomach: squamous cell neoplasm	1/70 (1%)	6/50* (12%)	8/50* (16%)	13/50* (26%)	11/50* (22%)
Harderian gland: neoplasm	6/70 (9%)	27/50* (54%)	23/50* (46%)	28/50* (56%)	11/50* (22%)
Preputial gland: neoplasm	0/70 (0%)	1/50 (2%)	5/50* (10%)	4/50* (8%)	3/50* (6%)

[a] Total exposure expressed as ppm-weeks.

* Increased compared to control, $P < 0.05$.

Summary Data on Chemicals for Review by the Board of Scientific Counselors
on March 14, 1989

(CAS) Number	Nomination Source	Domestic Production (lbs.)	Estimated Worker Exposure	NTP Testing Status	Other	Chemical Evaluation Committee Recommendation (Priority)	Chemical Selection Principles	Rational/Remarks
1. Formamide (75-12-7)	1) EDF[a] 2) NCI	$1.0\text{-}10.0\times10^6$ (1977)[b]	2,724[c]	-Negative in Salmonella -Negative in Drosophila for sex-linked recessive lethal mutations	--	-Carcinogenicity (Moderate) -Reproductive effects (High)	3,4,8	-Widespread use -Potential for exposure -Lack of carcinogenicity data -Adequate teratogenicity data available -Structural interest--parent compound of formamides chemical class
2. N-Methylformamide (123-39-7)	EDF	$<1.0\times10^3$ (1977)[b]	--	--	--	-Salmonella assay	3,4	-Low production -No longer used in cancer chemotherapy clinical trials -Adequate teratogenicity data available -Other members of formamides chemical class to be tested; studies on formamide and dimethylformamide expected to provide information on N-methylformamide

ATTACHMENT 5

(CAS) Number	Nomination Source	Domestic Production (lbs.)	Estimated Worker Exposure	NTP Testing Status	Other	Chemical Evaluation Committee Recommendation (Priority)	Chemical Selection Principles	Rational/Remarks
3. Dimethylformamide (68-12-1)	EDF	5.0×10^7-1.0×10^8 (1977)[b] 7.5×10^7 (1982)[d]	93,648[c]	-Negative in Salmonella -Positive in mouse lymphoma in one study; negative in two other studies -Negative for chromosomal aberrations and sister chromatid exchanges in CHO cells in culture -Selected for carcinogenicity	--	-Reproductive effects (High)	3,4,5,8	-Widely used solvent -Potential for exposure -OSHA interested in testing -Adequately tested for teratogenicity -Structural interest -Selected previously for NTP carcinogenicity studies -Anecdotal data on carcinogenicity in certain types of workers
4. Indium phosphide (22398-80-7)	NIEHS	$<1.0 \times 10^3$ (1977)[b]	--	--	--	-Stability studies -Toxicity including immunotoxicity -Carcinogenicity (Moderate)	2	-Used in electronics industry -Potential for increased use as proposed replacement for silicon -Potential for acute toxicity -Exposure to inorganic compounds often causes pulmonary asthma -Carcinogenicity testing pending results of stability studies

(CAS) Number	Nomination Source	Domestic Production (lbs.)	Estimated Worker Exposure	NTP Testing Status	Other	Chemical Evaluation Committee Recommendation (Priority)	Chemical Selection Principles	Rational/Remarks
5. N-Methylpyrrolidone (872-50-4)	1) Private individual 2) NIEHS 3) CPSC	$1.0x10^3$-$1.0x10^9$ (1977)	58,418[c]	-Negative in Salmonella	--	-Quantitative dermal absorption studies -Reproductive effects (High) -Carcinogenicity in mice (Moderate)	4,8	-High production volume -Widespread use -Used as a replacement for methylene chloride -Potential for exposure -Previous carcinogenicity study performed only in rats -Adequately tested for teratogenicity

a) Environmental Defense Fund

b) U.S. Environmental Protection Agency. 1977 Production Statistics for Chemicals in the Nonconfidential Initial TSCA Chemical Substance Inventory

c) National Occupational Exposure Survey (1981-1983). National Institute for Occupational Safety and Health

d) Chemical Economics Handbook. 1984. SRI International, Menlo Park, California

Testing Recommendations for Chemicals Reviewed by Board of Scientific Counselors on March 14, 1989

Chemical (CAS Number)	Nomination Source	Testing Recommendations (Priority)	Rationale/Remarks
1. Formamide (75-12-7)	1) EDF[a] 2) NCI	-Carcinogenicity (Moderate) -Reproductive effects (High)	-Extensive use -Potential for exposure -Lack of carcinogenicity and reproductive effects data -Interest in formamides chemical class
2. N-Methylformamide (123-39-7)	EDF	-Salmonella assay	-Low production -Chemical may no longer be used -Perform reproductive effects studies if structure-activity data are needed for chemical class
3. Dimethylformamide (68-12-1)	EDF	-Reproductive effects (High)	-Widely used solvent -Potential for exposure -Concern as to toxicity based on clusters of testicular cancers reported in exposed male workers -Previously selected for NTP carcinogenicity studies -Interest in formamides chemical class -Reproductive studies pending review of industry data by Dr. R. Miller and NTP DART[b] staff
4. Indium phosphide (22398-80-7)	NIEHS	-Stability studies -Toxicity including immunotoxicity (High)	-Used in electronics industry -Potential for increased use as replacement for silicon -Lack of toxicity data -Toxicity studies pending results of stability studies -Review chemicals used in semi-conductor industry for new NTP nominations

ATTACHMENT 6

Chemical (CAS Number)	Nomination Source	Testing Recommendations (Priority)	Rationale/Remarks
5. N-Methylpyrrolidone (872-50-4)	1) Private individual 2) NIEHS 3) CPSC	-Quantitative dermal absorption studies -Reproductive effects (High) -Carcinogenicity in mice (Moderate to high)	-High production -Widely used solvent -Potential for exposure as replacement for methylene chloride, and other solvents -Previous carcinogenicity study in rats judged to be adequate -Recommend that NTP ask EPA to consider requiring industry to perform testing under Toxic Substances Control Act

a) Environmental Defense Fund (EDF)
b) Developmental and Reproductive Toxicology Program (DART)