# SUMMARY MINUTES
# NATIONAL TOXICOLOGY PROGRAM
# BOARD OF SCIENTIFIC COUNSELORS' MEETING

*April 6, 1994*

The National Toxicology Program (NTP) Board of Scientific Counselors (the Board) met on April 6, 1994, 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. Curtis Klaassen (Chairman), Paul Bailey, Arnold Brown, Elaine Faustman, Barbara Hansen, David Hoel, Claude Hughes, Lawrence Loeb, Fumio Matsumura, Franklin Mirer, Kenneth Reuhl, and Peter Working. All members were present except Drs. Faustman and Matsumura. Dr. Hoel and Dr. Mirer were new members.

I. Report of the Director: Dr. Kenneth Olden, Director. NTP and NIEHS, asked Board members for their comments on the NIEHS *Vision for the Future*, a document that lays out a new vision for the Institute which advocates strong roles in basic research, in prevention and intervention, and in communication with lay and scientific groups. He said the document had resulted in part from dialogues with our many constituents as well as the Society of Toxicology (SOT) and other professional organizations, and representatives of industry, labor, environmental, and economically disadvantaged groups. Dr. Olden noted the role of the Board's advisory review in the development of this document. These dialogues along with recommendations from the National Advisory Environmental Health Sciences Council-sponsored Task Force IV report were used to help structure this new vision which will help set priorities for NIEHS research both intramurally and extramurally. He said among input from the SOT was the point that we needed to put more emphasis on our accomplishments. Dr. Olden stressed that this was a living document and will be modified and changed.

Dr. Olden used charts derived by the NIH to point out that over the last 10 years (1983-1993) among the NIH institutes the NIEHS was last in increases in budget whether computed in current dollar percentages or in terms of constant dollars. He said that in FY 1995, all the Institutes would receive 3.9% increases with funding for special programs being added to that, e.g., AIDS and breast cancer research. Dr. Olden urged Board members to write to the Director, NIH, and others in the Government or Congress to help us make our case for increased funding to enable the NIEHS to address the public health issues for which it has the responsibility.

Dr. Olden noted that particularly since the reorganization of the NIEHS intramural program all of the NIEHS research with only a few exceptions impacts on the NTP. He said that recent discussions among the senior leadership had led to conclusions that lack of knowledge in certain areas were hindering our ability to address important public health issues. Specifically, he said: (1) we have inadequate models for extrapolation from animals to humans for high to low dose extrapolation and for other toxicological disease endpoints; (2) we need to develop a better understanding of genetic susceptibility; and (3) we need to exploit the tools we have in functional toxicology. All of the above will help us get at mechanisms of toxicology. He urged Board members to respond to future RFAs concerned with developing research in these areas.

Dr. Olden reported that the *Seventh Annual Report on Carcinogens* had been sent to the Secretary for final approval and we expected that it would be published in the near future. He said the Board would be asked to help look at the criteria for inclusion of substances in future volumes of the now *Biennial Report*.

During the meeting, Dr. Olden presented certificates and acknowledged the contributions of retiring members of the Board, Dr. Bailey, Dr. Hansen, and Dr. Loeb. Dr. Bailey also had served on the Technical Reports Review Subcommittee.

II. Update on Activities of the Technical Reports Review Subcommittee: Dr. Gary Boorman, ETP, NIEHS, reported on studies reviewed by the Subcommittee on November 16-17, 1993, noting that none were standard studies and they incorporated more mechanistic aspects in the design. He described a one-year mouse skin initiation/promotion study in mice that showed *o*-benzyl-*p*-chlorophenol to be a weak promoter but with activity as an initiator or complete promoter. There previously had been an NTP two-year gavage study. Studies with isoprene involved 26-week inhalation exposures followed by a 26-week recovery period, and as expected, based on a close structural similarity to 1,3-butadiene, the chemical was clearly carcinogenic to the liver, lung, forestomach and harderian gland of mice, while there were no carcinogenic effects in rats. Dr. Boorman then discussed 2-year dermal studies with diethylphthalate in rats and mice along with one-year initiation/promotion studies with diethylphthalate and dimethylphthalate which showed no evidence of initiating or promoting activity for either chemical. He said that studies in progress with dibutylphthalate were aimed at learning more the mechanisms of peroxisome proliferation in the liver. Dr. Boorman concluded by commenting on the results of two-year and lifetime studies with ozone in rats and mice as well as cocarcinogenesis studies in male rats. There was no evidence of carcinogenic activity or cocarcinogenicity in rats but there were increases in lung tumors in mice, especially in females. In response to a question from Dr. Hoel about pulmonary effects of ozone and implications for risk assessment, Dr. Boorman said there were minimal pulmonary effects seen in the collaborative studies sponsored through the Health Effects Institute except for increases in fibrotic lung disease which may be more of a concern than the lung tumors seen in mice, particularly since there were no differences in oncogene patterns between the lungs of control and ozone-exposed mice.

III. Proposed Workshop on Dose Selection: Dr. John Bucher, ETP, NIEHS, said there was a NTP commitment to hold a workshop but no specific date had been set. Among ongoing activities, the International Life Sciences Institute (ILSI) has sponsored meetings on determining optimal doses for quantitative risk assessment which may result in a monograph and there is involvement by NTP agency scientists. The topic of dose selection will be the subject of a half-day symposium at the summer Toxicology Forum with NTP involvement, and there have been proposals for a symposium at the 1995 Society of Toxicology meeting which also would have NTP involvement. Dr. Bucher reported that we are reviewing the basis for dose selection for more recent chronic studies, about 95-100, and will publish a paper or a monograph describing those lesions/organs that seem to be important in dose selection. He said that the NIEHS has convened small in-house working groups that are looking at toxicokinetics and the role of cell proliferation in toxicity and how this information can be used in dose selection. Dr. Bucher said there are tentative plans for a small workshop at NIEHS in 1995 on dose selection that would use data sets from NTP prechronic studies. These data sets would be made available to interested parties. Discussion: Dr. Mirer pointed out the importance for risk assessment of good negative studies performed at the Maximum Tolerated Dose (MTD).

IV. Mechanism-Based Toxicology and Risk Assessment: Dr. George Lucier, ETP, NIEHS, said he would combine discussion of the most recent NTP Executive Committee meeting into this presentation since many of the topics discussed at that meeting were related to mechanism-based toxicology and risk assessment. He said this subject was not new, noting the Advisory Review report of the NTP Board which emphasized the need for more mechanism-based studies, and that there has been increasing emphasis by program staff over the past few years in incorporating mechanistic considerations in studies. The problem supporting the need is that of the 70,000 substances presently in commerce, adequate toxicological data is available for only 10-20% while a conventional rodent bioassay costs $2 to 4 million and takes four to six years to complete. Thus, uses of mechanism based toxicology include: (1) to more rapidly screen chemicals and set priorities for further studies; (2) as a basis for reasonably assuming hazard (rebuttable presumption); (3) to determine quantitative dose-response relationships; (4) to understand species, strain, and individual differences in susceptibility; and (5) for species extrapolation. He stressed that increased use of mechanistic data in risk assessment does not mean that use of chronic bioassays should be diminished. Animal models are usually appropriate for estimating human risk but in some cases may over or underestimate human responses. Dr. Lucier commented on the NTP record in meeting risk assessment needs, noting that it was excellent in hazard identification, frequently inadequate in dose response, and not really addressed in exposure assessment.

Dr. Lucier listed some of the recent legislative and government acts and authorities calling for improvements in risk assessment and prevention strategies. He said that good epidemiology studies are desirable but tend to detect an effect while toxicology, mechanistic, structure activity and predictive toxicology studies can be part of prevention strategy. Dr. Lucier discussed mechanisms, biomarkers and dose response noting the importance of developing biomarkers for rate limiting molecular event(s) and being able to quantitate a biomarker over a wide dose range. He cited examples of dose response relationships for specific chemicals using biomarkers. Drawing on his extensive experience with the dioxins, Dr. Lucier delineated NIEHS objectives in dioxin research for strengthening the scientific foundation on which risk assessments are made. They range from investigating biochemical and toxic effects, mechanism of action, dose response, and biomarkers of exposure, through determining relevance of animal and cell models, to identifying sensitive groups, developing prevention/intervention strategies, and communicating with regulatory agencies and the public. Dr. Lucier commented on the need and development of genetic susceptibility markers.

Dr. Lucier reviewed what he saw as evolving and new directions for the NTP, these being: (1) chemical and issues nomination and selection — includes using bioassays to test hypotheses, and obtaining broader input from outside the Federal government; (2) prioritization for bioassay studies — includes using all prior data, perhaps to decide a chemical will be a carcinogen and we don't need to commit resources to test it while exploring ways to gain regulatory acceptance. An August workshop is tentatively planned to explore the scientific/regulatory interface; (3) dose selection/optimization; (4) risk assessment models; (5) NTP grants — will explore their use in developing mechanistic information where animals/tissues from the bioassay could be made available to extramural researchers with a good example here being the collaborative studies on ozone; (6) alternative/complementary tests — a problem/issue here is validation and development of regulatory acceptance; (7) the *Biennial Report on Carcinogens;* and (8) better communications to integrate the different types of information developed.

Discussion: Dr. Mirer inquired as to how the nomination/selection process had changed. Dr. Lucier said the major change in emphasis was in broadening the sources from which nominations are received, including more from the state regulatory agencies and industrial and labor communities. A new NTP liaison office was working on this. Dr. Mirer stated that the NTP shouldn't commit resources to testing agents that legislative authorities can compel industry to test. Dr. Hughes said that it looked as though the NTP was trying to do more with less or no increase in resources. Dr. Lucier commented that enhanced collaboration with intramural basic researchers in development of mechanistic data was a means to do more with limited resources. In public comment, Dr. James Bond, Chemical Industry Institute of Toxicology (CIIT), said that CIIT urged the NTP to expand the nomination process to include mechanistic considerations and this sort of information should be included in *Federal Register* announcements and other mailings to stimulate discussion and input from the scientific community and the public at large.

V. Report from NTP Advisory Group — Cell Proliferation/Apoptosis: Dr. Bucher said he had asked Dr. Robert Maronpot, Laboratory of Experimental Pathology (LEP), Environmental Carcinogenesis Program (ECP), DIR, NIEHS, to convene a group of NIEHS and non-NIEHS scientists to make recommendations on the use of cell proliferation data in prechronic assessment of chemicals. (The Board had received a written summary of the group's report prior to the meeting.) Dr. Maronpot said the report prepared several months ago summarized the current state of knowledge and made recommendations. The only addition or change in emphasis he would add at this point would be to accentuate the fact that we still lack a good technique to measure apoptosis. Discussion: Dr. Reuhl complimented the group for a well reasoned report and for the conservative cast in recommending selective application of the technology.

VI. *Biennial Report on Carcinogens*: Dr. J. Carl Barrett, ECP, DIR, NIEHS, said that Dr. Olden had asked him to chair an in-house committee to examine how the *Biennial Report* (formerly *Annual Report*) was prepared and then study and make recommendations as to whether and how we might improve the process. In looking to improve the listing of chemicals in the NTP *Biennial Report*, four issues were considered by this committee: (1) who should conduct the evaluation of chemicals for listing in the *Biennial Report on Carcinogens*?; (2) how can input from outside sources be ensured?; (3) is there a need for further review of the criteria for listing and who should conduct this review?; and (4) what should be the review process for the *Biennial Report*? He said that today he would focus primarily on the first issue of who should conduct the evaluation. After considering the options, Dr. Barrett said his committee made the following recommendation: "A new subcommittee of the NTP Board of Scientific Counselors should be created to handle the *Biennial Report on Carcinogens*. This subcommittee should include selected members of the NTP Board, *ad hoc* reviewers selected for each chemical, and liaison members from the NIEHS and other agencies. A support contract for bibliographic searches and draft preparations of nominations is needed. The new subcommittee should begin by convening a working group to review the criteria for listing in the *Biennial Report*. The NTP Board should approve the work of the subcommittee." He said this subcommittee would be analogous to the existing Technical Reports Review Subcommittee.

Public Comments: Dr. Klaassen announced that there had been requests by three individuals for time to make formal comments. Dr. Donald Hughes, Proctor and Gamble (retired), representing the American Industrial Health Council (AIHC), began by noting the societal impact of the *Biennial Report* through its use by Federal and state regulatory agencies. He said the AIHC had been very supportive of the recommendations of the NTP

Board's Advisory Review especially regarding incorporation and use of mechanistic studies to aid others in assessing human risk from findings in animals. Dr. Hughes concluded by stating that AIHC's recommendations to the NTP were: (1) to revise the listing criteria to incorporate weight-of-the-evidence evaluations and to consider all of the scientific evidence; and (2) to rely more heavily on independent experts to peer review the listing criteria and nominated chemicals. He said this was supportive of the proposals made by Dr. Barrett.

Dr. John Hadley, Owens-Corning Corporation, representing the North American Insulation Manufacturers Association (NIAMA), said NAIMA strongly supported the recommendations made by Dr. Barrett. He stated that the primary thrust of this review should focus on the classification category "reasonably anticipated to be a carcinogen." That review should be given the highest priority, be undertaken with a sense of urgency and employ the leading scientific experts in this area. Dr. Clay Frederick, Rohm and Haas Company, speaking for himself, commented that there was a question of how to use mechanistic data developed and one suggestion he had received was that it be encapsulated into the *Biennial Report* when appropriate. He said there were basically four options as to how to use data on adverse effects in animals. The only two options that made sense were - to do nothing because the data are not relevant to humans, or - to use a comprehensive biologically based risk assessment model. Guidance in use of the mechanistic data should be provided by the NTP in the *Report*. Dr. Klaassen announced that written comments from Ms. Betsy Shirley, The Society of the Plastics Industry, Inc., had been given to the Board. These comments were supportive of the other speakers and the NTP proposals.

Discussion: Dr. Mirer said he thought the current process for the *Biennial Report* worked well and didn't need to be changed. He said the proposals and comments if adopted would push the *Report* more toward a risk assessment document and that regulatory input should be sought. Dr. Brown and Dr. Bailey supported formation of a Board subcommittee. Dr. Hansen thought more than two classifications might be considered. Dr. Claude Hughes wondered whether even conducting this review would be impinging on regulatory agency purviews. Dr. Barrett responded that the NTP was only seeking the most inclusive scientific advice available, which might be to not change the process. Dr. William Allaben, FDA/NCTR, cautioned that the subcommittee's charge should not be to conduct risk assessments. Dr. Hoel supported formation of a subcommittee which should include an epidemiologist. Dr. Working said there should be no *a priori* assumption of change. Dr. Brown moved that the recommendation as presented by Dr. Barrett be approved, and Dr. Bailey seconded the motion.

In further discussion, Dr. Mirer said the phrase indicating the subcommittee would be reviewing nominated chemicals suggested to him that they would be moving from a hazard assessment process into risk assessment issues. Dr. Barrett explained that the intention of this part of the recommendation was to give the Board and other outside reviewers opportunity to provide review and make recommendations regarding inclusion of chemicals nominated for the *Biennial Report*. The Subcommittee will not be involved in performing risk assessments. Dr. Hughes stressed again that the regulatory agencies should have a say in the *Biennial Report* process. Dr. Olden said a subcommittee would be one additional step in the review process with the final step being the NTP Executive Committee, which includes all the Federal health regulatory agencies. He noted that the Institute of Medicine/National Academy of Sciences has made several recommendations that the criteria be reviewed and if the Board, which is the most appropriate body, is not

involved we would have to convene another committee to help us. Dr. Loeb endorsed formation of a subcommittee. Dr. Marilyn Wind, Consumer Product Safety Commission (CPSC), expressed concern that regulatory agencies such as CPSC have adequate representation in review of the criteria since they are charged with doing risk assessments. Dr. Olden agreed that this would be the case. Dr. Barrett emphasized that one stipulation in our recommendation was that the first charge of the subcommittee would be to convene a working group to review the criteria. Dr. Mirer requested several changes in wording of the motion, these being to replace 'handle' with 'review,' to delete '*ad hoc* reviewers selected for each chemical,' and to delete the sentence beginning 'A support contract...'. After some clarifying discussion that the subcommittee would be involved in initial review of nominated chemicals but not risk assessments, and the explanation that there was a need for logistical support for this process, Dr. Brown agreed to replace 'handle' and to delete 'selected for each chemical.' Dr. Bailey opined that *ad hoc* reviewers would be needed to provide expertise in evaluating mechanistic information. The Board then voted to approve the revised motion with seven yes votes. There were two abstentions (Dr. Mirer, Dr. Working).

The approved motion reads: "A new subcommittee of the NTP Board of Scientific Counselors should be created to review the *Biennial Report on Carcinogens*. This subcommittee should include selected members of the NTP Board, *ad hoc* reviewers, and liaison members from the NIEHS and other agencies. A support contract for bibliographic searches and draft preparations of nominations is needed. The new subcommittee should begin by convening a working group to review the criteria for listing in the *Biennial Report on Carcinogens*. The NTP Board should approve the work of the subcommittee."

VII. Environmental Science & Management Fellows (ESMF) Program: Dr. Olden introduced Ms. Linda Alexander, Director, ESMF Program, noting that the NIEHS currently has a fellow in residence. Ms. Alexander said the ESMF Program is a mid-career graduate fellowship program that was founded by the National Urban Fellows, Inc. in 1969 to address the need to increase the number of minority managers in public administration. The program seeks to take baccalaureate level persons in public policy or technical science areas and enroll them in a graduate program encompassing academic course work and field experience (mentorship) leading to a M.S. degree awarded by Tufts University Graduate School of Arts and Sciences. She said they are now recruiting their fourth class of fellows. Ms. Alexander introduced Mr. Niranjan Vescio, a chemist with the EPA, who had been in the first class of fellows. Mr. Vescio said he had done his mentorship at the EPA on the science, regulation and policy of environmental protection, and had gained an appreciation of the burdens placed on industry by regulations and the challenges to Federal agencies in formulating good regulations. He hoped to move into environmental management possibly in the international arena. Ms. Alexander introduced Ms. Marlene Richardson who is currently completing her mentorship with Dr. Olden. Ms. Richardson said she was completing her M.S. thesis on studies of prenatal care utilization in lead exposure in young children. She said her background was in public policy. She commented that the ESMF program was the only one she knew about that tried to bridge the gap between science and public policy. Ms. Alexander asked Board members to consider if they had staff members or students who might be eligible and appropriate for this program.

VIII. Report on Results, Accomplishments, New Contract Awards - Past Year: Dr. Bucher said he would cover several reports of contract-related activities for which tabular information had been provided to the Board, mention some specific new activities he

thought would be of interest, and conclude with the status of NTP grant activities in the developmental biology area. He began with the listing of chemicals for which studies were ongoing or completed over the last year in various genetic toxicology assays, noting the increasing emphasis on the *in vivo* micronucleus tests. Dr. Bucher pointed out the chemical studies planned, ongoing or completed during the past year in developmental and reproductive toxicology and immunotoxicity studies, noting there would be increasing use of 28-day reproductive toxicology studies in place of the more expensive and more time-consuming reproductive assessment by continuous breeding assay. He went over short-term studies including information on chemical disposition and toxicokinetics, and chronic studies began, ongoing or near starting.

Among other activities, Dr. Bucher commented on dietary restriction studies being done in-house comparing effects on carcinogenesis between restricted diets and *ad lib* feeding with a purpose being to try and stabilize long-term bioassays with regard to weight gain in rodents. He noted that the NCTR has had a large dietary restriction study ongoing for some time. A series of studies were being initiated on the perinatal effects of several classes of pesticides that should fill in data gaps identified in the recent NAS report. In research on electromagnetic fields (EMF), studies are underway on general toxicological characterization, developmental toxicity and effects on melatonin levels. Chronic studies will begin later in the year. In the area of alternative models, bioassays for carcinogenicity are underway in two small fish species, the Medaka and the guppy. Studies are underway to evaluate the adverse effects of retroviral vectors being used for gene therapy. A series of multigenerational studies in rodents are looking at the effects of low levels of estrogenic chemicals, alone or in combination, on hormonal status, reproductive function, and induction of preneoplasia. Recent studies have shown retinoic acid to be teratogenic during the sensitive pregastrulation phase of embryogenesis. Dr. Bucher concluded by saying that most of the projects he had described were in the FY 1994 NTP *Annual Plan* that had been reviewed by the Board in November.

Dr. Jerrold Heindel, Division of Extramural Research and Training, NIEHS, said he wanted to update the Board on the status of the Request for Applications (RFA) titled "Toxic Substance Effects on Developmental Gene Expression." He noted that this first NTP-sponsored RFA was issued to stimulate research at the interface between developmental biology and toxicology to further our knowledge of how environmental agents alter basic processes of development and contribute to birth defects in human populations. Of 33 applications, only eight had previous NIEHS funding, thus representing a lot of 'new blood.' Twenty had previous funding from the National Institute of Child Health and Human Development, 10 had no toxicology background, and eight had no current funding. Three were rated outstanding, three rated high excellent, and three low excellent. Dr. Heindel said a program plan had been developed to fund five or six grants for three years. This plan will be presented to the National Advisory Environmental Health Sciences Council in late May for their concurrence. The plan would be to have grantees come to the Institute once a year to interact with developmental scientists here and perhaps have a symposium once during the three-year period. He said there would be a program announcement indicating continuing interest in this area.

IX. Toxicology Review Team Activities: Dr. Allaben updated the Board on activities at NCTR under the Interagency Agreement (IAG) with NIEHS. He reminded them that the purpose was to conduct comprehensive toxicological assessments on FDA priority chemicals/agents nominated to the NTP. An important point is that the studies be

designed to have regulatory utility that can be used in decisions about risk assessment/risk management. He discussed the Toxicology Study Selection and Review Committee (TSSRC) for each chemical, which had responsibility for planning, oversight and review of applied and mechanistic studies, including studies of noncancer endpoints, and considering development and use of alternative assays. Dr. Allaben reported on initial studies with chloral hydrate, a hypnotic used extensively in pediatric medicine and dentistry. He noted that the TSSRC for chloral hydrate included scientists from NICHD, NIEHS, NCTR, and the American Academy of Pediatrics as well as the principal scientist from the FDA's Center for Drug Evaluation and Research (CFSAN) who will have responsibilities with regard to conducting risk-benefit assessments. Dr. Allaben said range finding studies had been completed in rats and mice and a mouse chronic study has been designed. Pharmacokinetic and metabolism studies were conducted in both species and also being investigated was the modulation of toxicity as a consequence of dietary restriction. Also described were *in vitro* metabolism, mutagenesis, and macromolecular binding studies with chloral hydrate and metabolites in an alternative system - the new born mouse assay. Dr. Allaben also briefed the Board on the status of studies with Fumonisin $B_1$ (FB1), the second chemical to be studied under the IAG. The TSSRC for the FB1 studies includes scientists from the USDA, Agriculture Canada, CFSAN, academe, and from the NIEHS and the NCTR. There have been three TSSRC meetings on FB1 and six critical studies have been selected. They anticipate two-year studies in rats and mice will begin in the fall. Since little is known about FB1 metabolism, extensive metabolism/pharmacokinetic studies are planned in rodents and nonhuman primates. Among other mechanistic studies are those in cellular/molecular biology, mutagenesis and macromolecular binding, and initiation/promotion (pending outcome of IARC studies). Also planned are developmental studies in rabbits and rats, which will begin in mid-summer, and neurobehavioral studies in rats, which are ongoing. CFSAN will supply the purified (98% pure) FB1.

X. Concept Reviews, ETP, DIR, NIEHS:

(1) **Toxicity and Carcinogenicity Studies in Animals** — (Attachment 3, p. 4) Dr. Bucher introduced the concept, and Dr. Arnold Brown, Board member, served as principal reviewer. Dr. Bucher said the continuing objectives were to characterize the toxicologic and carcinogenic effects of chemical, biological and physical substances through studies in animals. The only changes are to increasingly give greater emphasis to noncancer toxicity and to investigations into mechanisms of toxicity and carcinogenicity. Dr. Brown stated that the Board should support continuation of this program and lauded the addition of new emphases mentioned. He moved that the concept be approved. Dr. Hoel seconded the motion. Dr. Loeb said that he could not support the concept without more specific definitions of mechanism studies and an idea of costs. Dr. Brown noted that bioassays were still the primary component and essential to the NTP's mission and he didn't need to have the detail requested to approve the concept. Dr. Hoel inquired as to what kinds of mechanism studies were involved. Dr. Bucher said that in many cases this would involve a different level of investigation beyond histopathology, such as at the biochemical and molecular levels. Dr. Lucier commented that it was difficult to give a generic answer since the types of mechanistic studies often would be chemical-specific. The Board voted to approve the concept with eight yes votes to one no vote (Loeb).

(2) **Potential for Environmental and Therapeutic Agents to Induce Immunotoxicity** — (Attachment 3, pp. 5-7) Dr. Michael Luster, NIEHS, introduced the concept, and Dr. Paul Bailey, Board member, served as principal reviewer. Dr. Luster

said there were two types of collaborative efforts being supported under this continuing project area, a research and development (R&D) contract for immunotoxicity testing in rodents that is due to be recompeted, and an IAG with NIOSH for development and application of biological monitoring protocols for immunotoxicity studies in humans which can be used to assess human exposures and to evaluate the utility and predictiveness of immunotoxicity data from animal studies. Agents are examined for their potential to produce hypersensitivity, exacerbate autoimmune disease, and/or induce immunosuppression. He listed chemicals studied and the components of the two-tiered immune panel used for a number of years. Dr. Luster described a proposed battery for assessing immunotoxic potential. He said that up to about 30% of the R&D effort might be apportioned to mechanistic studies, methods development, and modifications of existing methods, all of this depending in part on capabilities of the contractor. Dr. Bailey said this was an extension of an existing contract which has provided important information and served to improve the science of immunotoxicology, and as such, he strongly supported the concept. Dr. Loeb asked what the yearly costs have been for the existing contract and what has been accomplished. Dr. Luster replied that the cost was about $600,000 per year and that was for seven tests of immunosuppression and an equal number of tests for hypersensitivity as well as the 30% effort noted for mechanistic studies and methods development or modifications. Among accomplishments, he cited valuable data provided to the National Institute of Allergy and Infectious Diseases on the toxicity of combinations of anti-AIDS therapeutics, and data provided to the FDA on the immunotoxic effects of silicone breast implants. Dr. Bailey moved that the concept be approved. Dr. Hughes seconded the motion which was approved unanimously by the Board.

(3) **DNA/RNA Isolation and Molecular Analysis** — (Attachment 3, pp. 8-9) Dr. Claudia Thompson, Laboratory of Biochemical Risk Analysis (LBRA), DIR, NIEHS, introduced the concept. She stated that the objective of this proposed contract was to provide technical support for the extraction of DNA and RNA from biological samples and the performance of polymerase chain reaction (PCR)-restriction fragment length polymorphisms (RFLP) analysis for laboratory programs at the NIEHS, particularly in the LBRA, Epidemiology Branch (EB) and the Environmental Toxicology Program (ETP). Dr. Thompson said DNA and/or RNA would be derived from frozen human blood, blood cells, paraffin embedded tissues and a wide variety of rodent tissues. The primary use of the DNA would be for PCR based gene polymorphism or mutation studies and the primary use of the RNA would be for PCR based quantitation of gene expression. She gave examples of ongoing or planned studies where these analyses could be used in detecting exposure-gene interactions and their effects on disease outcomes as well as providing a mechanism for archiving DNA or RNA derived from rodent tissues obtained through NTP toxicology and carcinogenesis studies.

Dr. Loeb said that he was supportive of some of the studies to be done but could not support the concept without some estimates of cost. Dr. William Johnston, NIEHS, responded that cost estimates could not be provided without closing the meeting. Dr. Hansen asked whether all candidate genes and PCRs would undergo an internal project review process. Dr. Thompson reported that genes chosen will have been well characterized in the laboratory, e.g., some that hold promise as cancer susceptibility genes, including glutathione-s-transferase and N-acetyl transferase. Dr. Hoel wondered whether it was appropriate for the NTP Board to approve concepts for intramural research or epidemiologic studies. Dr. George Lucier, NIEHS, stated that NTP scientific activities encompassed much more than bioassays and applied animal studies. Especially with increased emphasis on mechanistic studies, a growing number of intramural

research projects were considered to be at least in part NTP-related, and as well, more emphasis was being given to exploring links to human studies. Dr. Bailey moved that the concept be approved. Dr. Brown seconded the motion which was approved by four yes votes to two no votes with two abstentions. Dr. Klaassen asked if the two abstainers would comment on their vote. Dr. Reuhl said he was concerned by the vagaries of how the technology would be applied and how much would be for NTP projects. Dr. Lucier estimated that at least one-third of the applications would be directly NTP-related in mechanistic research in bioassays and in improving dose-response in the low dose region. Dr. Hughes, who also abstained, said he thought that most of the application would be to human studies such that the concept should have been reviewed more appropriately by a different Board.

XI. Environmental Toxicology and Alternative Methods - Overview of NIEHS Activities: Dr. William Stokes, NIEHS, said the 1993 NIH Revitalization Act reinforced the NTP mandate to develop improved test methodology and had specific language directing the NIEHS to develop and validate alternative methods. He said he would give a brief overview of current NIEHS activities both extramurally and intramurally. Under the extramural program, in the research grants program in FY 1993, there were 29 R01 grants, $3.62 million, with emphasis on *in vitro* systems, transgenics, nonmammalian systems, and computer modeling. Five NIEHS Marine and Freshwater Biomedical Core Centers receive core support, $1.2 million in FY 1993, to support the development of aquatic organisms as models for environmental health research. Dr. Stokes discussed the role of Small Business Innovative Research Grants, which supported the development of the Big Blue® transgenic mouse, and the NIEHS Superfund Basic Research Program in developing alternative test methodologies. Within the intramural program, there are a number of projects in development and evaluation: transgenic models, including fish models, the Frog Embryo Teratogenesis Assay: Xenopus (FETAX); transgenic cells/tissue cultures; and computer-based prediction models. He briefly described each. With regard to future directions in alternative methods in the NTP, Dr. Stokes pointed to: (1) use of alternative systems as models for studying mechanisms of toxicity; (2) their use as improved test methods and screening tests; (3) their use as biological assay systems, especially field applications and in study of mixtures; and (4) use as environmental sentinels. Discussion: There were questions about the advantages or utility of transgenic animals for detecting carcinogens. A primary advantage given was the shorter onset time for tumor induction in some transgenic animals such as the p53 knockout and the TG.AC mouse model and with a concomitant reduction in costs.

XII. New Initiatives in Alternative/Environmental Sentinel Test Methodology at Fort Detrick: Dr. Lucier introduced Mr. Hank Gardner, U.S. Army Biomedical Research and Development Laboratory (USABRDL), Fort Detrick, Maryland. Dr. Lucier said that as a result of the Base Closing Act, there were ongoing negotiations with the Department of Defense (DOD) to enact a transfer of Mr. Gardner's lab to the NIEHS. He noted that there was already a collaboration with studies on FETAX were being performed at Fort Detrick under an IAG. Mr. Gardner said he appreciated the opportunity to give the Board an overview of studies in his laboratory. Since 1983, the USABRDL has conducted a research program focused on the development of new methods for assessing the carcinogenic hazard resulting from exposure to chemicals in the workplace or the environment. Early on, emphasis was on single chemicals while more recently emphasis has been more on mixtures that would be found in hazardous waste sites and effluents. He said the very expensive remediation efforts at Army sites must be based on sound, risk-based characterizations of the potential hazards posed by these sites to both human

and ecological receptors. In addition to the usual contaminants, one has military unique materials and breakdown products to deal with. Mr. Gardner said they have both fixed and mobile labs. Most of his interest has been with carcinogenesis assays but studies are also being pursued in developmental toxicology, immunotoxicology, genetic toxicology and acute toxicity. Their successes are due in part to collaborations with investigators around the country.

Mr. Gardner said the use of small fish species in carcinogenesis studies is not new; the NCI has been conducting studies in this area since the 1960s. His laboratory has used predominantly the Japanese Medaka as an experimental animal. With extramural scientists, they have looked at oncogene and tumor suppressor gene activation and cell proliferation in livers from Medaka exposed to carcinogens. He commented on other collaborative studies developing a technique for analyzing tissue for the presence of free radical induced damage to the bases of DNA, a technique proven valuable in assessing animals obtained from contaminated sites. Mr. Gardner commented on their studies with FETAX which is a 96-hr whole embryo assay used for assessing developmental toxicity of chemicals or mixtures. With exogenous metabolic activation, the system has been shown to be 90% accurate in predicting mammalian teratogens. The assay is currently undergoing an interlaboratory validation with partial funding by the NIEHS. Mr. Gardner described their 48 foot mobile laboratories which enable them to go to sites of contamination, collect and analyze samples and run experiments, such as Medaka or FETAX assays. Analytical instrumentation includes HPLC and GC/MS. Through collaborations with other Federal agencies and universities, they are expanding the number of sites available for use and evaluation of the mobile laboratories, and also providing the opportunity for scientists from these organizations to do research in the mobile facilities.

XIII. Reports of Research Programs on Alternative Methods:

(1) **Ecological Toxicology and Genetics** — Dr. Frank Johnson, NIEHS, said he had recently come to the ETP from the Laboratory of Genetics, DIR, where his research had involved analysis of genotype/environment relationships in various types of insects with studies based on the thesis that genetic polymorphism is adaptive. The results generally showed a high degree of correlation between genetic and environmental variability. He said he is currently studying this in the laboratory looking at heavy metal contamination with yeast populations. He described experiments with lead and nickel suggesting that eventually mutation and selection will result in increased tolerance to the toxicant and perhaps even dependence in the very long term. In the balance between mutation and selection, he thought the importance of selection had been underemphasized. Dr. Johnson then listed the large number of Federal agencies that are funding and/or conducting ecotoxicology testing and research, noting the large involvement of the private sector especially in schools, colleges and universities, as well as religious organizations. He concluded by stating that the health of the environment is inextricably intertwined with human health, and it is not possible to harm the environment, and the creatures in it, without also harming humans, either directly or indirectly.

(2) **A Transgenic Model for Comparing Environmental Mutagenesis in Rodents and Alternative Species** — Dr. James Burkhart, NIEHS, said an objective of his research was to measure mutations directly at the DNA level independent of any requirement for phenotypic expression so that responses could be correlated between a

variety of organisms using the same target gene. The approach, developed by Dr. Heinrich Malling, was to make the host organism transgenic for a phage vector. The *am*3 mutation of bacteriophage øX174 was used to demonstrate that spontaneous mutation frequencies are similar in transgenic cells in culture, various tissues of mice, and in transgenic fish. Two species of fish were made transgenic for this gene marker, the Medaka and the Mummichog, an estuarine species found all up and down the U. S. east coast. Dr. Burkhart described experiments with mice made deficient for glucuronyl transferase. He discussed collaborations with Dr. Daniel Casciano at NCTR, and with Dr. Barrett's laboratory at NIEHS where hamster cells and human transformed cell lines are being made transgenic for their gene marker. Dr. Burkhart said that from the work to date, the following conclusions can be drawn: (1) an identical gene can be used to investigate environmental mutagenesis in aquatic species, laboratory mammals, and cultured cells; (2) øX174 as a transgenic mutation target can be recovered independent of variations in CpG methylation between tissues and species; (3) efficiency of recovery is such that the numbers of animals required for investigation of somatic and germinal mutagenesis can be significantly reduced; and (4) this type of approach can begin to be a model for combining research in basic mechanisms with the applied need for hazard assessment.

Dr. Lucier concluded the discussion by noting that NIEHS efforts in the area of alternative methodologies are evolving and the Board's continuing input and guidance will be appreciated as we develop our plans in response to the mandates of the FY 1993 NIH Revitalization Act. He emphasized the commonality between ecological distress and human health effects.

ESTIMATED STATE MEDIAN INCOME FOR 4-PERSON FAMILIES, BY STATE—Continued

[FY 1995 [1]]

| States | Estimated state median income, 4-person families [2] | 60% of estimated state median income, 4-person families |
|---|---|---|
| Wyoming ..... | 43,126 | 25,876 |

[1] In accordance with 45 CFR 96.85, each state's estimated median income for a 4-person family is multiplied by the following percentages to adjust for family size: 52% for one person, 68% for two persons, 84% for three persons, 100% for four persons, 116% for five persons, and 132% for six persons. For family sizes greater than six persons, add 3% to 132% for each additional family member and multiply the new percentage by the state's estimated median income for a 4-person family.

[2] Prepared by the Bureau of the Census from the March 1993 Current Population Survey, 1990 Decennial Census of Population and Housing, and 1992 per capita personal income estimates, by state, from the Bureau of Economic Analysis.

Note—FY 1995 covers the period of October 1, 1994 through September 30, 1995. The estimated median income for 4-person families living in the United States is $44,615 for FY 1995.

[FR Doc. 94-5538 Filed 3-9-94; 8:45 am]

BILLING CODE 4184-01-P

## Public Health Service

### National Toxicology Program; Board of Scientific Counselors, Meeting

Pursuant to Public Law 92-463, notice is hereby given of a meeting of the National Toxicology Program (NTP) Board of Scientific Counselors, U.S. Public Health Service, in the Conference Center, Building 101, South Campus, National Institute of Environmental Health Sciences (NIEHS), 111 Alexander Drive, Research Triangle Park, North Carolina, on April 6, 1994.

The meeting will open to the public from 8:30 a.m. to adjournment with attendance limited only by space available. The preliminary agenda topics with approximate times are as follows:

8:30 a.m.–9 a.m.—Report of the Director, NTP.

9 a.m.–9.25 a.m.—Meeting Reports/Upcoming Meetings:

Update on Activities of the Technical Reports Review Subcommittee

Planned Workshop on Dose Selection

Report on NTP Executive Committee

9:25 a.m.–10:05 a.m.—Mechanism Based Toxicology and Risk Assessment.

10:05 a.m.–10:25 a.m.—Report from Advisory Group on Cell Proliferation/Apoptosis.

10:45 a.m.–12 noon—Biennial Report on Carcinogens—Discussion of Possible Role of the Board in Review Process.

1:15 p.m.–1:35 p.m.—Report on Results/Accomplishments and New Contract Awards in Past Year.

1:35 p.m.–1:50 p.m.—Toxicology Review Team Activities.

1:50 p.m.–2:50 p.m.—Concept Reviews.

3:10 p.m.–3:20 p.m.—Environmental Toxicology and Alternative Methods—NIEHS Role.

3:20 p.m.–4 p.m.—New Initiatives in Alternative/Environmental Sentinel Test Methodology at Fort Detrick.

4 p.m.–4:45 p.m.—Reports of Research Programs on Alternative Methods at NIEHS.

4:45 p.m.–5 p.m.—Discussion.

Adjournment

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

Dated: March 3, 1994.

**Richard A. Griesemer,**

*Deputy Director, National Toxicology Program.*

[FR Doc. 94-5494 Filed 3-9-94; 8:45 am]

BILLING CODE 4140-01-M

### National Toxicology Program (NTP) Board of Scientific Counselors' Meetings; Announcement of NTP Draft Technical Reports Projected for Public Review From June 1994 Through Fall 1995

To earlier inform the public and allow interested parties to comment or obtain information on long-term toxicology and carcinogenesis studies prior to public peer review, the National Toxicology Program (NTP) again publishes in the **Federal Register** a current listing of draft Technical Reports projected for evaluation by the NTP Board of Scientific Counselors' Technical Reports Review Subcommittee during their next four meetings from June 1994 through the fall of 1995. We plan to continue updating the listing with announcements in the **Federal Register** once or twice a year. The next meeting dates are June 21–22, 1994 and November 29–30, 1994. Specific dates for 1995 meetings will be established at a later time.

The attached table 1 lists draft Technical Reports for long-term studies on chemicals within known or approximate dates of reviews and includes Chemical Abstracts Service (CAS) registry numbers, primary use, route of administration, species, exposure levels, and NTP report numbers (if assigned).

Technical Reports of short-time toxicity studies are currently reviewed by mail; however, when necessary may be reviewed in open meetings. The attached Table 2 lists the draft Technical Reports of short-term toxicity studies tentatively projected for review by mail during 1994 and also includes Chemical Abstracts Service (CAS) registry numbers, primary use, route of administration, species, exposure levels, and NTP report numbers (if assigned).

Those interested in having more information about any of the studies listed in this announcement, should contact Central Data Management as early as possible by telephone or by mail to: MD-A0-01, NIEHS, P.O. Box 12233, Research Triangle Park (RTP), North Carolina 27709 (919-541-3419). The program would welcome receiving toxicology and carcinogenesis data from completed, ongoing or planned studies by others as well as current production data, human exposure information, and use and use patterns.

The Executive Secretary, Dr. Larry G. Hart, P.O. Box 12233, Research Triangle Park, North Carolina 27709, telephone 919/541-3971, will furnish final agendas and other program information prior to a meeting, and summary minutes subsequent to a meeting.

Attachments.

Dated: March 3, 1994.

**Richard Griesemer,**

*Deputy Director, National Toxicology Program.*

# AGENDA
# BOARD OF SCIENTIFIC COUNSELORS
# NATIONAL TOXICOLOGY PROGRAM

*April 6, 1994*

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, NTP | Dr. K. Olden, NIEHS |
| 8:45 a.m.-9:10 a.m. | Meeting Reports/Upcoming Meetings<br>— Technical Reports Review Subcommittee | Dr. G. Boorman, NIEHS |
| | — Upcoming Workshop on Dose Selection | Dr. J. Bucher, NIEHS |
| | — NTP Executive Committee<br>- Nomination Process<br>- Priority for 2-Year Study Selection<br>Congressional Mandate for Regulatory Acceptance of Alternate Test Data | Dr. G. Lucier, NIEHS |
| 9:10 a.m.-9:50 a.m. | Mechanism-Based Toxicology and Risk Assessment | Dr. Lucier |
| 9:50 a.m.-10:10 a.m. | Report from NTP Advisory Group<br>— Cell Proliferation/Apoptosis | Dr. Bucher<br>Dr. R. Maronpot, NIEHS |
| 10:10 a.m.-10:30 a.m. | *Coffee Break* | |
| 10:30 a.m.-10:45 a.m. | Environmental Science Management Fellows Program | Ms. L. Alexander, Environmental Science and Management Fellows Program |
| 10:45 a.m.-12 noon | Biennial Report on Carcinogens<br>— Review of criteria for inclusion<br>— Proposed review by Board Subcommittee<br>Public Comments | Dr. C. Barrett, NIEHS |
| 12 noon-1:15 p.m. | *Lunch* | |
| 1:15 p.m.-1:35 p.m. | Report on Results/Accomplishments<br>New Contract Awards-Past Year | Dr. Bucher |
| 1:35 p.m.-1:50 p.m. | Toxicology Review Team Activities<br>— Chloral hydrate, fumonisin $B_1$ (IAG) | Dr. W. Allaben, NCTR |

***April 6, 1994***

| | | |
|---|---|---|
| 1:50 p.m.-2:50 p.m. | Concept Reviews:<br>— Toxicity and Carcinogenicity Studies in Animals<br>— Potential for Environmental and Therapeutic Agents to Induce Immunotoxicity<br>— DNA/RNA Isolation and Molecular Analysis | <br>Dr. Bucher<br>Dr. M. Luster, NIEHS<br>Dr. C. Thompson, NIEHS |
| 2:50 p.m.-3:10 p.m. | *Break* | |
| 3:10 p.m.-3:20 p.m. | Environmental Toxicology and Alternative Methods - Overview of NIEHS Activities | Dr. W. Stokes, NIEHS |
| 3:20 p.m.-4:00 p.m. | New Initiatives in Alternative/Environmental Sentinel Test Methodology at Fort Detrick | Mr. H. Gardner, U.S. Army Biomedical Research and Development Laboratory |
| 4:00 p.m.-4:45 p.m. | Reports of Research Programs on Alternative Methods at NIEHS:<br>— Ecological Toxicology and Genetics<br>— A Transgenic Model for Comparing Environmental Mutagenesis in Rodents and Alternative Species | <br>Dr. F. Johnson, NIEHS<br>Dr. J. Burkhart, NIEHS |
| 4:45 p.m.-5:00 p.m. | Discussion | |
| | Adjourn | |

# National Toxicology Program
# Board of Scientific Counselors

*April 6, 1994*

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Dr. Paul T. Bailey (6/94)
Toxicology Program Manager
Stonybrook Laboratories Inc.
P. O. Box 1029
Princeton, NJ 08543-1029

(Toxicology)

Dr. Arnold L. Brown (3/96)
University of Wisconsin Medical School
1300 University Avenue
Room 1217
Madison, WI 53706

(Carcinogenesis, Pathology)

Dr. Elaine F. Faustman (3/95)*
Professor and Associate Chair
Department of Environmental Health
University of Washington SC-34
F561, 1705 N. E. Pacific
Seattle, WA 98105

(Developmental Toxicology)

Dr. Barbara C. Hansen (6/94)
Professor of Physiology
Director, Obesity & Diabetes Research Center
University of Maryland
10 South Pine Street
MSTF 600
Baltimore, MD 21201

(Physiology)

Dr. David G. Hoel (3/96)
Professor and Chairman
Department of Biometry and Epidemiology
Medical University of South Carolina
Charleston, SC 29426-2503

(Biostatistics, Risk Assessment)

Dr. Claude L. Hughes Jr. (3/95)
Dept. of Obstetrics & Gynecology
Room 210 Baker House
Duke Hospital South Division
Trent Drive
Durham, NC 27710

(Reproductive Physiology)

Dr. Curtis D. Klaassen (3/95)
Professor
Department of Pharmacology and Toxicology
University of Kansas Medical Center
39th and Rainbow Boulevard
Kansas City, KS 66160

(Toxicology)

Dr. Lawrence A. Loeb (6/94)
Professor and Director
Gottstein Memorial Laboratory and
Department of Pathology SM-30
University of Washington D-525 HSB
Seattle, WA 98195

(Carcinogenesis)

* Not present

Dr. Fumio Matsumura (3/95)*
Professor
Institute of Toxicology & Env. Health
University of California
Old Davis Road
Davis, CA 95616-8615

(Toxicology)

Dr. Franklin E. Mirer (3/96)
Director
Health and Safety Department
International Union, UAW
8000 East Jefferson Avenue
Detroit, MI 48214

(Toxicology, Occupational Health)

Dr. Kenneth R. Reuhl (3/96)
Professor
Department of Pharmacology and Toxicology
School of Pharmacy
Rutgers University
Piscataway, NJ 08855-0789

(Neurotoxicology)

Dr. Peter K. Working (3/95)
Director, Pharmacology/Toxicology
Liposome Technology, Inc.
1050 Hamilton Court
Menlo Park, CA 94025

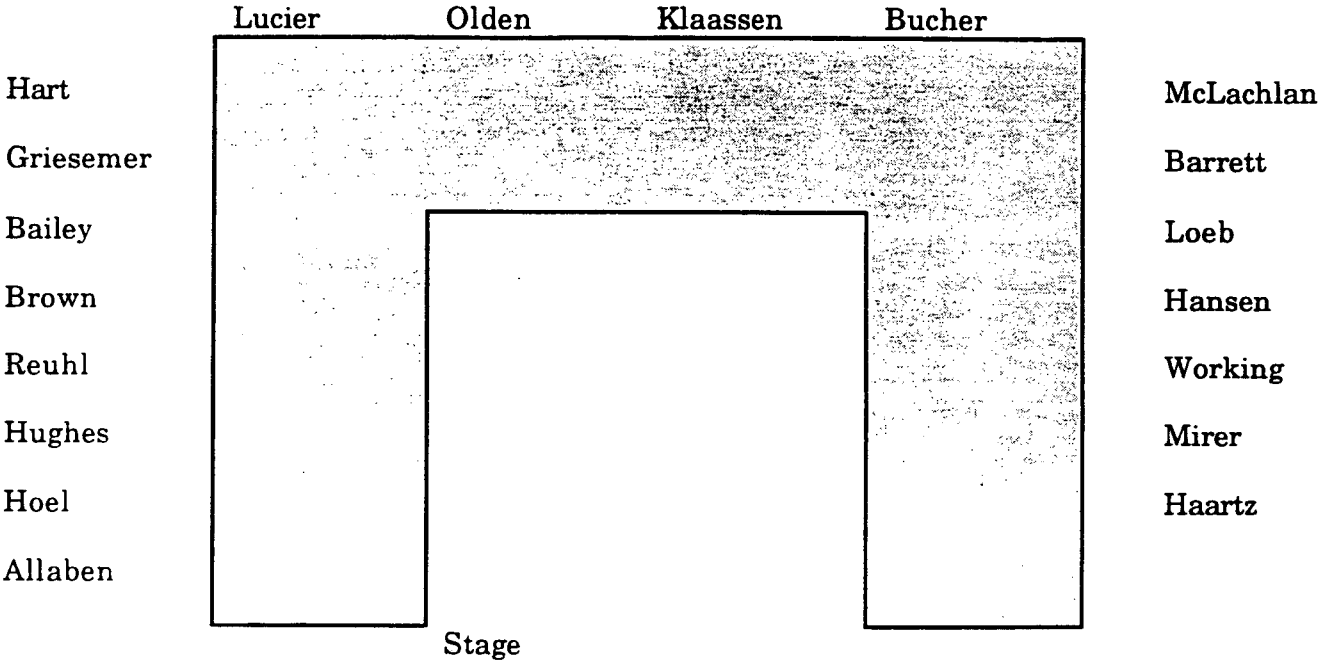
(Reproductive Toxicology, Genetics)

* Not present

National Toxicology Program
Board of Scientific Counselors Meeting

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

*April 6, 1994*



Environmental Toxicology Program

Division of Intramural Research

National Institute of Environmental Health Sciences

# CONCEPT REVIEWS

*Prepared for:*

National Toxicology Program
Board of Scientific Counselors

**April 6, 1994**

## CONCEPT REVIEWS

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Potential for Environmental and Therapeutic Agents to Induce Immunotoxicity
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# BACKGROUND CONCEPT REVIEWS

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

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

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

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

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

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

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

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

## NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW

**CONCEPT TITLE:** Toxicity and Carcinogenicity Studies in Animals

**PRESENTER:** Dr. John Bucher, Toxicology Branch, ETP

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

**BACKGROUND:** 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, 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 preceding period. Increasingly greater emphasis is being given, however, to non-cancer toxic effects on the various organs of the body and investigations into mechanisms of toxicity and carcinogenicity.

## NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW

**CONCEPT TITLE:** Potential for Environmental and Therapeutic Agents to Induce Immunotoxicity

**PRESENTER:** Michael I. Luster, Environmental Immunology and Neurobiology Section, LBRA

**OBJECTIVES:** The objective of the proposed project is to determine the potential of various environmental and therapeutic agents to produce immunotoxicity. Immunotoxicity will be assessed using well established experimental models. Agents will be examined for their potential to produce hypersensitivity (allergic contact dermatitis), exacerbate autoimmune disease and/or induce immunosuppression. These studies will: (1) improve our ability to predict the types of environmental agents that will affect the human system as well as their potential impact; (2) provide relevant and quantitative data that can be used in risk assessment; and (3) increase our basic understanding of immune-mediated diseases.

**BACKGROUND:** The value of incorporating immunological experimental data for the toxicological assessment of drugs, chemicals, and biologicals for human risk assessment has been increasingly accepted. For example, in addition to previously established test guidelines or proposals by the EPA and FDA for hypersensitivity and immunosuppression testing (e.g., Sjoblad, 1988; Hoyle and Cooper, 1990), EPA and FDA have recently prepared documents providing guidelines for immunotoxicity testing of pest control agents and drugs, respectively. Furthermore, EPA has established reference doses (Rf or NOAEL/SF) using immunotoxicity data for several compounds including 1,1,2-trichloroethane, 2,4-dichlorophenol and dibutyltinoxide, while the Agency for Toxic Substances and Disease Registry has derived "minimum risk levels" for arsenic, dieldrin, nickel, 1,2-dichloroethane and 2,4-dichlorophenol from immune endpoints.

The sensitivity of the immune system by some environmental agents observed in experimental studies is due as much to the general properties of a chemical (e.g., reactivity to macromolecules) as to the complex nature of the immune system which encompasses antigen recognition and processing; cellular interactions involving cooperation, regulation and amplification; cell activation, proliferation and differentiation; and mediator production by various cell types and their products. Because of this complexity, the initial strategies among immunologists working in toxicology and safety assessment have been to select and apply a tiered panel of assays to identify immunosuppression or, in rare instances, immunostimulatory agents in laboratory animals (Luster et al., 1988; NRC, 1992). Data collected from these tests have been utilized to examine a variety of com-pounds and the database generated from these studies, which consists of over 50 compounds, has been collected and analyzed in an attempt to improve the accuracy and efficiency of screening chemicals for immunosuppression and to better identify those tests that predict immune-mediated diseases (Luster et al., 1992; 1993).

Studies in humans exposed occupationally, inadvertently or thera-peutically have helped confirm the parallelism of immunotoxicity observed in experimental animals. For example, as observed in rodents, individuals exposed to certain pesticides, halogenated aromatic hydrocarbons, UV-B radiation and heavy metals manifest subclinical immune changes (i.e., function and/or surface lymphocyte subpopulations). Similarly, the ability

of many chemicals (e.g., isocyanates, anhydrides) and therapeutics (penicillin) to produce allergic hypersensitivity is well established. Less studied, but of potential concern, are agents that may exacerbate autoimmune disease. In this respect, elevated levels of serum autoantibodies have been observed in individuals exposed to organic solvents in a number of studies.

Selection of the most appropriate animal model for immuno-toxicology studies has been a matter of concern. Ideally, toxicity testing should be performed with a species that will elicit chemical-related pharmacology and toxicities similar to those anticipated in humans (i.e., the test animals and humans will metabolize the chemical similarly and will have identical target organ responses and toxicity). For most immunotoxic therapeutics, rodent data on target organ toxicities and comparability of doses have been generally predictive of what was later to be observed in the clinic. Exceptions to the predictive value of rodent toxicological data are infrequently seen but have occurred such as in studies of glucocorticoids, which are lympholytic in rodents, but not in primates. Although certain compounds may exhibit different pharmacokinetic properties in rodents than in humans, rodents still appear to be the most appropriate animal model for examining the immunotoxicity of non-species-specific compounds, based on established similarities of toxicological profiles, ease of generating host susceptibility challenge and immune function data, availability of genetically altered mice and development of mouse hypersensitivity tests.

We propose to employ rodent models for evaluating the potential of various environmental agents to produce immunosuppression, allergic contact dermatitis or exacerbate autoimmune disease. A tiered panel (Luster et al., 1988) will be used to identify agents that may produce immunosuppression and include measures for immunopathology, hematology, immune function assays, immune cell phenotyping and host resistance tests. Hypersensitivity testing will be conducted in mice using the ear swelling test (Gad et al., 1986) and local lymph node assay (Kimber et al., 1989). If positive, antigen-specific IgE levels will be determined. All proposed tests for immunosuppression and hypersensitivity have successfully completed interlaboratory validations. We also propose to examine selected environmental agents for their ability to exacerbate autoimmune disease in autoimmune-prone mice. Work is our laboratory has shown that diethylstilbestrol accelerates disease in non-obese diabetic mice. These mice develop insulin-dependent diabetes mellitus, an autoimmune process against islet cells of the pancreas. This has not been validated as a screening test and will be considered accordingly, such as inclusion of positive controls.

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# NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW

**CONCEPT TITLE:** DNA/RNA Isolation and Molecular Analysis

**PRESENTER:** Claudia L. Thompson, Laboratory of Biochemical Risk Analysis, EBMP

**OBJECTIVE:** The objective of this contract is to provide technical support for the isolation of DNA and RNA from biological samples and to perform PCR-RFLP analysis for laboratory programs at NIEHS. In particular this contract will support studies in LBRA, EB, and NTP that are related to environmental causes of diseases in man, but will also be available for use by other laboratories at NIEHS. The work to be performed under this contract includes extracting DNA and/or RNA that are derived from human frozen whole blood, human lymphocytes or white blood cells, paraffin embedded tissues and a wide variety of rodent tissues including but not limited to liver, lung, spleen, kidney and reproductive tract tissues. The primary use of the DNA will be for PCR based gene polymorphism or mutation studies. The primary use of the RNA will be for PCR based quantitation of gene expression. In addition to DNA and RNA extractions, other tasks to be included in this contract include but are not limited to PCR-RFLP analysis.

**BACKGROUND:** NIEHS has a strong research interest in issues concerning risk assessment. These include the field of genetic susceptibility and disease, molecular dosimetry, the development of biomarkers for exposure analysis and mechanistically based modeling. The Laboratory of Biochemical Risk Analysis (LBRA) and the Epidemiology Branch (EB), Environmental Biology and Medicine Program, Division of Intramural Research, National Institute of Environmental Health Sciences have had a strong interest in uncovering exposure-gene interactions and its effect on disease outcome. It has been known that the incidence rates for cancer and many other environmentally related diseases vary markedly within a population. Contributing to the differences in disease rates are variability in exposure and genetic factors that affect the conversion of an exposure to deleterious molecular events ultimately leading to disease. Epidemiologic studies have identified a number of carcinogen-metabolism genes that are polymorphic in the population and hold promise as cancer susceptibility genes (glutathione-s-transferase, N-acetyl transferase, aryl hydrocarbon hydroxylase, dimethyl-nitrosamine demethylase and debrisoquine dehydroxylase genes). From animal and in vitro studies we know that for many chemicals, bioactivation is necessary to elicit genotoxic effects. Many carcinogens act by damaging DNA by forming DNA carcinogen adducts which can lead to mutations and ultimately cancer. For example, mutations in proto-oncogenes or tumor suppressor genes can affect the growth control homeostasis of the cell. Numerous animal and human studies have identified mutations in both oncogenes and tumor suppressor genes for many tumor types.

Population based studies provide the opportunity to identify and understand gene-environment interactions for selected diseases. The ability to discover new examples of how gene-environment interactions can differentially modulate disease risk in the population depends upon careful analysis of exposure and genetic risk factors. It also requires development of new methodologies for more sensitive and accurate assessment of exposure and assays for new candidate susceptibility genes.

In studies carried out in LBRA, it has been determined that individuals with the "at-risk" form of glutathione-s-transferase gene (GSTM1) suffer a 70% increased risk of bladder cancer in smokers and that the frequency of the "at risk" genotype differs greatly between

African-Americans and European-American ancestry (Bell, JNCI (1993) 85: 1159-1164). This finding demonstrates how an interaction between exposure (smoking) and genetics (GSTM1) differentially modulates risk for cancer in different populations. Studies are ongoing to analyze the frequency of "at risk" genotypes for a number of carcinogen metabolizing genes in different ethnic populations and case/control molecular epidemiological studies for different cancers and moreover to assess gene-environment interactions in these studies.

Better methods are needed for assessing exposure to potential carcinogens. The role of exposure has been difficult to define in human populations because traditional methods, primarily questionnaire based, have not been accurate. Biomarkers that detect exposure at the molecular level may significantly improve sensitivity as well as specificity. We have recently developed a reverse transcriptase based PCR method to quantify changes in gene expression that are caused by exposure to carcinogens like TCDD and benzo(a)pyrene (Vanden Heuvel, Carcinogenesis (1993) 14: 2003-2006). Ongoing projects are examining how the presence of increased levels of DNA damage, protein adducts, altered gene expression and mutations are related to increase risk for disease development.

In addition to the human based studies ongoing in LBRA and EB, the NTP has had a long history in performing rodent bioassays on candidate human carcinogens. The availability of tissues from bioassays for molecular analysis provides a unique opportunity to develop mechanistic models to be used in risk assessment. Moreover, studies that complement ongoing human molecular epidemiologic studies can provide useful across species comparisons at the molecular level.

This contract will provide needed support in processing the large number of samples for either DNA/RNA or for performing PCR analyses that molecular epidemiologic studies require to gain useful information in assessing gene-environment interactions in human populations as well as providing a mechanism for archiving DNA or RNA derived from rodent tissues obtained through the NTP bioassay.