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NATIONAL TOXICOLOGY PROGRAM

BOARD OF SCIENTIFIC COUNSELORS

November 2, 1993

Summary Minutes

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Contents — Page Numbers

SUMMARY MINUTES
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The National Toxicology Program (NTP) Board of Scientific Counselors (the Board) met on November 2, 1993, 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, Claude Hughes, Lawrence Loeb, Fumio Matsumura, Kenneth Reuhl, and Peter Working. All members were present except Drs. Brown and Loeb.

I. Report of the Director: Dr. Kenneth Olden, Director, NTP and NIEHS, announced that Dr. Bern Schwetz, Acting Director, Environmental Toxicology Program (ETP), NIEHS, had accepted the position of Director, National Center for Toxicologic Research (NCTR), Food and Drug Administration (FDA), effective November 1. He expected that our working relationship with the NCTR, which was already good, would be even better with Dr. Schwetz as Director. Dr. Olden said that a search committee would be formed in the near future. In the interim, Dr. George Lucier would serve as Acting Director, ETP, and Dr. John Bucher would serve as Acting Deputy Director.

Dr. Olden reported that the NIEHS budget for FY 1994 was $265.4 million, an increase of $13.8 million from FY 1993, and a larger increase was expected in FY 1995. Dr. Olden commented that the Senate Appropriations Committee had recommended that the Program start 15 chemicals on test yearly. He said we were committed to meeting that goal. Dr. Olden noted that the NIEHS had need of a clinical center which would enable the Institute to transfer laboratory studies ripe for clinical application into a clinical setting. This was being done through formal agreements with the excellent medical schools at the University of North Carolina in Chapel Hill and Duke University. The first area of study would be in pulmonary dysfunction with the program director at NIEHS to be Dr. Paul Nettesheim, Chief, Laboratory of Pulmonary Pathobiology, DIR. He reported that the first multicenter clinical trials had been announced recently and would involve evaluating the efficacy of a new chelating agent, succimer, for reducing blood lead levels in children. The trials would be conducted at four clinical centers under the overall coordination of Harvard and the Centers for Disease Control and Prevention.

Dr. Olden presented his thoughts on what some of the priorities for study by the NTP should be both in-house and through the extramural community: 1) there should be research on methods development and validation of new approaches and models for improving extrapolation of results from animals to humans and from high dose to low dose; 2) development of models for non-cancer endpoints; 3) development of models for predicting toxicity of chemicals for which little or no toxicity data exist; 4) there needs to be more toxicity testing both to provide chemical-specific data and to improve predictability; 5) cutting-edge technologies in cell and molecular biology need to be better incorporated into toxicology; 6) elucidation of carcinogenic and toxicologic mechanisms; 7) development of biomarkers of exposure, effect, and susceptibility; 8) need greater emphasis on cross species and human studies (rodent or other models do not exist for all human diseases) ; 9) more studies of mixtures; 10) relationship between poverty, malnutrition, and susceptibility to environmental exposures (environmental equity) with particular attention to the poor and ethnic minorities, children and senior citizens. He

noted a symposium cosponsored with EPA and ATSDR on environmental equity was planned for February; 11) molecular prevention/intervention; 12) better coordination/ collaboration among the various government agencies involved in risk assessment research; and 13) pursuing partnerships among government, industry and public interest groups.

II. Update on Activities of the Technical Reports Review Subcommittee: Dr. Larry Hart, NIEHS, reported that the Subcommittee had met on June 22, 1993, at which time they peer reviewed the draft Technical Reports for six long-term toxicology and carcinogenesis studies. He noted that the Subcommittee would meet again on November 16-17, with the entire first day being devoted to studies on ozone. This would include presentation of findings from extensive mechanistic studies performed either in-house or by investigators around the country under the auspices of the Health Effects Institute which examined effects of ozone on pulmonary function, structure and morphometry and examination of chemical markers. Peer review of the draft NTP Technical Report, which includes both 24 and 30 month as well as cocarcinogenicity studies, will conclude the day. Other long-term studies would be reviewed the second day along with a short-term toxicity study of isoprene. The Board was given a printout detailing the conclusions for the draft Reports peer reviewed in June, and summary information for the Reports to be reviewed November 16-17.

III. Report of Workshop on Predicting Chemical Carcinogenesis in Rodents: Dr. Michael Shelby, NIEHS, said the purposes of this international workshop held at the NIEHS on May 24-25, 1993, were to: assess the state-of-the-art of predicting chemical carcinogenesis; examine the strengths and weaknesses of different predictive methods; investigate the kinds of information that can be used to improve the prediction process; and stimulate efforts to improve prediction methods. The workshop derived from a paper published in *Mutagenesis* (5: 3-14, 1990) by Tennant, Spalding, Stasiewicz, and Ashby which predicted the outcome of rodent carcinogenicity studies on 44 chemicals under study by the NTP and for which the findings are now known. Other investigators or groups were invited to make predictions on these chemicals and discussion and comparison of results were a focus of the workshop. Dr. Shelby described the basis for the various methods used in predicting outcomes with the range of success being between 50 and 75%, leaving considerable room for improvement. The Tennant *et. al.* system gave the best prediction. Dr. Shelby summarized the conclusions of the workshop: 1) strong carcinogens and noncarcinogens are predicted with greater success than weak carcinogens or chemicals giving equivocal results; 2) use of extensive and varied information results in a more accurate prediction process; and 3) systems capable of learning, i.e., human or computer-based, have the greatest potential for improvement. He said the benefit of the workshop lies in the identification of data gaps and problems to be addressed in improving our ability to predict carcinogenicity, particularly for those chemicals that may act through non-genotoxic mechanisms. In response to questions about details of the workshop and recommendations made, the Board was provided with the original prediction paper by Tennant *et.al.*, the workshop workbook, a preprint of the meeting report, and a copy of a paper that illustrates a new approach to predicting chemical toxicity using machine learning to analyze NTP data sets.

IV. Report of a Workshop on Trihalomethanes and Colorectal Cancer: Dr. June Dunnick, NIEHS, said the workshop on trihalomethanes and other environmental factors that contribute to colorectal cancer resulted from interest within the three programs under the NIEHS Division of Intramural Research and was planned in collaboration with

Dr. Dale Sandler and Dr. Ronald Melnick. The meeting was held to bring together scientists in the field to exchange ideas and review the current status of research on the biology, epidemiology and genetics of colorectal cancer. Speakers discussed effects of dietary factors, genetic changes occurring in the disease process, a mouse model for colorectal cancer, effect of growth factors, physical-chemical properties of brominated chemicals that relate to potency, and the association of trihalomethanes, especially those derived from chlorination of water, with tumor incidence. Dr. Dunnick reported on 14 NTP studies that gave evidence for colorectal tumors in the rat, with seven of these chemicals having incidences of greater than 10%. Among areas for future study were characterization of the spectrum of genetic changes in colorectal cancer, study of the metabolism of brominated chemicals including identification of the enzymes systems involved, and expansion of epidemiological investigations to areas of high human exposure to these chemicals.

V. Proposed Workshop on Criteria for Dose Selection: Dr. Bernard Schwetz, NCTR, said that Dr. Olden had asked for advice on whether it would be useful to convene a workshop on the use of the MTD (Maximum Tolerated Dose) in toxicology and carcinogenesis testing. Dr. Schwetz asked a group of NIEHS scientists to discuss this issue. After agreeing that criteria for dose selection should be the focus rather than the MTD, the group found several points of agreement, including that: 1) both scientific and political factors have to be considered; 2) over the past 15 years, the NTP criteria have changed as we've learned more about our animal models; 3) the primary emphasis on dose selection has been to minimize the toxicity and mortality rather than on prediction of the outcome of the study; 4) there needs to be better definition of the purpose or purposes of the study; and 5) in the past, study design has often been determined on the basis of default assumptions. Recommendations were that: 1) we need to document the current criteria for dose selection for the various endpoints of toxicity; 2) review these understandings with the other government agencies involved in the NTP; and 3) make refinements in the criteria and bring this to the Board for their input. Dr. Schwetz said we could then convene a workshop with a broader range of people to discuss not only the criteria but more importantly the types of data that need to be collected and how they should be used as the basis for dose selection. Discussion: Dr. George Lucier, NIEHS, commented that a major purpose should be to aid in selecting doses that would be relevant to those having to do risk assessment on a chemical. Dr. Hansen and Dr. Hughes both stressed the importance of considering nutrient background of animal diet in designing studies and how changes in macro and micro nutrients can influence outcomes.

VI. Proposed Symposium on Causes and Risks of Developmental Abnormalities: Dr. Jack Bishop, NIEHS, reported that a symposium on "New Approaches for Assessing the Causes and Risks of Developmental Abnormalities" sponsored by the National Academy of Sciences (NAS) was planned for spring 1994 at the NAS Auditorium, Washington, D.C. He commented that among the stimuli and background for this symposium was a series of six workshops bringing together developmental and molecular biologists with developmental toxicologists which were held at the NIEHS in the fall of 1992. Dr. Bishop said the primary objectives would be to: 1) stimulate additional interactions between developmental biologists and toxicologists, 2) present and discuss a spectrum of research ideas expanding more into areas of epidemiology, human health, and toxicological risk, and 3) identify data gaps and research needs. He reviewed the agenda for the three day meeting. Discussion: This centered on queries or suggestions of topics that might be included in the symposium. Among these were transplacental toxicology and carcinogenesis, epigenetic influences, and behavioral teratology.

VII. Plans for Contracts, Interagency Agreements and Grants — Developmental Biology/Toxicology: Dr. Jerrold Heindel, NIEHS, said the Request for Applications (RFA) he was going to discuss was the first NTP-sponsored RFA and derived from the series of six workshops held at the NIEHS in the fall of 1992. The RFA funded in the amount of $3 million to be used to fund 5 or 6 grants for a three year period on "Toxic Substance Effects on Developmental Gene Expression." He said the RFA is being issued to stimulate research at the interface between developmental biology and toxicology to further our knowledge of how environmental agents alter the basic processes of development and contribute to birth defects in human populations. A key component is the cross fertilization between molecular biologists/embryologists and developmental toxicologists. The RFA is not limited to any particular stage of development, organ, developmental abnormality or toxicant; the main criteria for consideration is the relevance of the environmental toxicant and the developmental abnormality to human health and the integration of biology with toxicology. The receipt date is January 21, 1994, and he expected awards to be made by July 1, 1994. Dr. Heindel said he would serve as a liaison between the NIEHS Extramural Program and the NTP including membership on the Chemical Nomination Committee which should aid in identifying future research needs that would be appropriate for the RFA process. Discussion: Dr. Klaassen thought this was a good way to get the academic community more involved in NTP projects. He stated that a major problem for himself and other academic investigators was to obtain NIH grant funding for investigator initiated projects that fall between basic and applied research. Dr. Hansen suggested that there needed to be an applied toxicology study section.

VIII. Discussion of FY 1994 NTP Research Plans and Chemical Studies: Dr. Schwetz introduced the topic by noting that under the NIEHS reorganization, parts of the NTP program represented by branches or laboratories would be reviewed by the Division of Intramural Research Board while the overall Program plans would be brought to the NTP Board for advice as originally intended. Dr. John Bucher, NIEHS, led the discussion of the FY 1994 *Annual Plan*, and prefaced his remarks by stating that the most important input would be for the Board to give us advice on whether the division of studies was appropriate both scientifically and in a public health sense, whether the balance between testing and mechanistic studies was right. Should we expend our resources on studies that attempt to prevent or reverse chemically-induced injury, e.g., the role of glycine deficiency in the toxicity of a variety of compounds? Are there important areas that we are not adequately addressing or not addressing at all?

Dr. Bucher reviewed changes in scientific direction that have occurred over the last one to three years: **genetic toxicology** — moving away from large-scale efforts using *in vitro* and *in vivo* tests to correlate with rodent carcinogenicity findings while retaining the most useful of these assays for screening purposes. We are expanding the use of transgenic animals and biomarkers for detecting mutagenic events in both somatic and germ cells with renewed emphasis on development of assays for aneuploidy; **carcinogenesis** — while retaining use of the two-year bioassay, we have expanded the use of toxicokinetics, cell proliferation measures, and the evaluation of molecular changes in rodent tumors. In 1994, we plan to continue development of immunohistochemistry for assay of growth factor related gene products and cell cycle-related factors for the further evaluation of pathologic specimens from short-term and long-term studies; **toxicology and chemical disposition** — studies will be designed on the new chemicals approved for study as well as continuation of those on which studies have already begun. New directions will be charted in new initiatives on the safety of retroviral vectors used in gene therapy, and the

use of glycine supplementation in prevention or reversal of chemically-induced toxicity; **alternative methods** — development and evaluation of alternative methods are being pursued in all program areas; and **NIOSH and NCTR projects** — Dr. Bucher commented that studies included by our sister agencies are peer reviewed by their own scientific review boards. Comments would be most useful on how well their efforts integrate into the overall *Plan*.

Discussion: **genetic toxicology** — Dr. Working said he liked the emphasis on germ cell mutagenesis, especially in females, an area that has been understudied, while continuing work in more traditional areas such as the specific locus test and in development of a multiple endpoint assay. He also liked the inclusion of zygote and early embryo studies. Dr. Faustman commented on the implications of early zygote exposure, especially the regulatory implications; **carcinogenesis** — With regard to studies on peroxisome proliferators, Dr. Faustman inquired as whether these were collaborative efforts. Dr. Bucher noted they were primarily done on contract but tissues were earmarked for collaborative efforts with university and other laboratories. Dr. Reuhl wondered how 'issue specific investigations' were chosen. Dr. Bucher responded that this approach is evolving as the Program traditionally has been chemically driven with some focus on chemical class studies. More mechanistic investigations have resulted as add-ons proposed by staff scientists to chemical studies, e.g., dibutyl phthalate and the issue of peroxisome proliferation. He said we were still working on how best to incorporate issues into the nomination process. Dr. Hansen stated that we should stress the complementarity of alternative models in the sense that we can't replace some whole animal systems. Dr. Lucier agreed noting that we have to use the experimental system that gives us the best data, which ultimately will enable us to reduce the use of whole animals. Dr. Matsumura commented on mechanistic studies on the biology of mouse liver tumors especially with regard to activation of H-*ras* protooncogene. Dr. Robert Maronpot, NIEHS, said these studies were still in progress while stressing the importance of careful harvesting of tissues for use with a host of evolving molecular biological techniques. In response to a query about magnetic resonance imaging, Dr. John McLachlan said the collaboration with The Center for In Vivo Microscopy at Duke was a happy marriage between NIEHS scientific problems and Duke technology; **toxicology** — Dr. Reuhl inquired as to the status of neurotoxicology in the NTP. Dr. McLachlan commented that with the recent departure of the chief of the one intramural basic neurosciences laboratory, the NIEHS was currently evaluating the directions that neurotoxicology should take. Dr. Jean Harry, NIEHS, said we were trying to evaluate the neurobehavioral studies needed on a chemical by chemical basis, e.g., carbon disulfide and glycine supplementation studies. Dr. Klaassen stated that the effects of carbon disulfide on cholesterol metabolism should be studied. Under the respiratory toxicology area, Dr. Klaassen asked for a status update on the expired breath analysis in chemical toxicity assessment project. Dr. Bucher reported that we can quantify most of the low molecular components of rat breath and changes in these components could be used as a measure of clinical pathology. Administration of cytochrome P 450 inhibitors results in large increases or changes in breath components and these effects are being further characterized, while there don't seem to be changes in animals treated with chemicals that induce cell proliferation or peroxisomal proliferation.

There was considerable discussion about the efforts in the area of reproductive and developmental toxicology. Dr. Working noted two areas being under-served — female reproductive toxicology, especially non-developmental and non-endocrine, and transplacental toxicology. Dr. Hughes inquired as to the pace of short-term testing for

reproductive toxicity testing. Dr. Robert Chapin, NIEHS, reported that reproductive assessment by continuous breeding remains the primary test system coupled with 28-day studies as a preliminary screen, noting that we are currently evaluating chemical products of water chlorination at the request of the EPA. Dr. Schwetz added that Dr. Chapin was active in trying to anticipate chemical hazards associated with new industries, e.g., photovoltaic chemicals. In response to a question from Dr. Faustman, Dr. Schwetz commented on NTP efforts including FETAX and , with NIOSH, use of *Drosophila* as a screen; **chemical disposition** — Dr. Klaassen observed that there were a plethora of chemicals to be studied, and remarked favorably on the use of microdialysis/mass spectrometry for repetitive *in vivo* analysis of blood levels of chemicals; **Superfund project** — Dr. Faustman noted the possibilities for collaboration between the NTP and outside laboratories. Dr. Schwetz agreed and added that these possibilities also existed for development, validation and use of alternative species.

IX. Formation of a New Toxicology Review Team — Fumonisin B_1: Dr. William Allaben, NCTR, said that Fumonisin B_1 (FB1) was the second chemical to begin study under the Interagency Agreement (IAG) between NIEHS and FDA. The Toxicology Study Selection and Review Committee (TSSRC) serves as the review team with *ad hoc* members chosen specifically for each chemical. For FB1, in addition to FDA and NIEHS scientists, scientists from the USDA, Agriculture Canada, Emory University, and Columbia University are members. He said the purposes of the committee for FB1 (and other chemicals) were to conduct a comprehensive literature review, to identify and recommend studies which will enhance the regulatory utility of toxicity and carcinogenicity studies including appropriate mechanistic studies, to consider use and development of alternative assay systems, and to monitor progress of the studies. Dr. Allaben reported that FB1 is a metabolite of *Fusarium* species of fungus, primarily *F. moniliforme*, that contaminates corn crops and is found in processed human and animal food where it is known to cause severe health problems in horses, swine and poultry. Limited laboratory animal studies report hepatotoxicity and hepatocarcinogenicity of FB1 and there are epidemiology studies from South Africa and China of potential human health problems. He described planned Phase I studies (range finding, developmental toxicity, pharmacokinetics and metabolism) scheduled for early 1994, and Phase II studies (two-year chronic studies in rats and mice and specific mechanistic studies). Key to carrying out the planned studies is isolation of sufficient quantities of purified FB1 which is a responsibility of the FDA's Center for Food Safety and Applied Nutrition.

X. Chemicals Nominated for FY 1994: Dr. Errol Zeiger, NIEHS, said 15 chemicals had been nominated to be considered for toxicological testing in FY 1994. At its meeting on September 1, 1993, the NTP Interagency Committee for Chemical Evaluation and Coordination (ICCEC) had recommended 12 of these for toxicological studies while the other three chemicals were deferred for additional information or because they were under consideration by other government agencies. Dr. Zeiger discussed the chemicals, ICCEC recommendations, and supporting information (Attachment 3). Discussion: Dr. Hughes suggested that mechanistic studies of receptor mechanisms need to be incorporated into studies on ecdysterone. Dr. Hansen wanted to know if 'Low to Moderate' priority meant a chemical would not be tested. Dr. Zeiger said this was correct but factors may arise that lead to a change in priority. Dr. George Lucier, NIEHS, commented that the NIEHS is trying to broaden input on chemicals nominated and as part of this, a chemical nominations committee has been formed within the Institute to solicit and review nominations of chemicals as well as issues/concepts for NTP study from NIEHS scientists. Dr. Zeiger said we would welcome nominations from Board members. He

noted that the NTP is trying to broaden both the base of nominations and the sources from which they are received.

XI. F344 Rat Studies - Report of Workshop and Recommendations for Changes: Dr. G. N. Rao, NIEHS, said the NIH-07 open formula nonpurified diet had been the selected diet for NTP studies since 1980 and contains ~24% protein, ~5% fat, and ~3.5% fiber. The purpose of the workshop was: a) to review the effects of changes in protein, fat and fiber concentrations of diet on modifying chronic disease (e.g., nephropathy), tumor incidences (e.g., leukemia, mammary, and pituitary tumors), and survival of rats, especially Fischer-344 (F344) rats, in two-year studies; and b) to discuss modifications in diet composition and ingredients, as appropriate. The workshop was held at the NIEHS on June 23, 1993. Dr. Rao said data from NIEHS studies comparing NIH-07 with a diet containing ~15% protein were presented which indicated that a 15% nonpurified diet was adequate for growth and maintenance of male and female rats and the ~30% decrease in protein consumption markedly decreased the severity of nephropathy. There was broad support by workshop participants for lowering protein concentration of nonpurified diet for rats to 15%. Dr. Rao discussed NIEHS studies with diet of varying fat content and comparisons with fat (corn oil) as vehicle for gavage studies on body weight gain and decreases or increases in incidences of several tumors. Discussion by participants indicated that the effect of corn oil (fat) in diet may not be the same when given by gavage and it was difficult to judge the beneficial effect of higher fat content from data available. Thus, the NIEHS recommendation to increase fat content of diet was delayed until further review and identification of a beneficial effect of higher fat content (>5%) in diet on decreasing incidence or delaying onset of leukemia is completed. Dr. Rao reported that fiber due to its low caloric value appears to lower body weight gain. However, fiber: a) may decrease intestinal transit time and absorption of chemicals; b) may change gut flora and associated metabolism of chemicals; and c) may bind and decrease bioavailability, so a decision to increase fiber content of rat diet was delayed until further review and identification of beneficial effects of fiber on delaying development of mammary tumors was completed. Dr. Rao concluded by stating that the purposes of his proposal to increase fat was to decrease incidence of leukemias and to increase fiber was to balance calories with increased fat as well as to decrease the development of mammary tumors while producing a diet more akin to human diet.
Discussion: Dr. Reuhl expressed concern with how changes in diet may affect the NTP historical control database. Dr. Rao acknowledged his concern and said we would have to establish new databases from the early studies with a new diet. However, he noted that we don't often go back very far in time with the current historical database as the incidences of a number of tumors have been changing with time. Dr. Klaassen asked what the time table was for changing the diet. Dr. Rao replied that it would take about six months to finalize our plans and he would come back to the Board and Technical Reports Review Subcommittee to inform them.

XII. Selected Ongoing Research Programs - Environmental Toxicology and Alternative Systems: Dr. Schwetz introduced the topic by commenting that this initiative represents a move from usual laboratory animal species to evaluation and use of those found in the natural environment. In addition to possible uses as monitors of environmental pollution and new models for studying environmentally induced disease, the use of these alternative species is responsive to the recent advisory review report of the Board and the mandates of the recently enacted NIH Revitalization Act. Thus, an established workgroup within the DIR headed by Dr. Heinrich Malling has been moved into the ETP. Dr. Schwetz reported that another component of this effort was still under negotiation and

would involve transfer from the Department of Defense to the NIEHS of an ecotoxicology program at Fort Dietrick, Maryland.

1) **Developmental Toxicity - FETAX** — Dr. Jean Harry, NIEHS, noted that the NTP has been actively involved for a number of years in development of *in vitro* screens in the area of teratology and developmental toxicology. A key problem in implementing new alternative methods is insufficient validation of models for effectiveness in large-scale, multilaboratory studies. The purpose of an interagency agreement with the Army laboratories at Fort Dietrick was to address this critical need. One system chosen for study was the FETAX (Frog Embryo Teratogenesis Assay: *Xenopus)*. This is a whole embryo system which in 96 hours undergoes cleavage, gastrulation, and organogenesis in a manner similar to mammals. The assay allows measurement of a number of endpoints, including malformations, motor activity, pigmentation, as well as survival and growth endpoints. Dr. Harry displayed and discussed data obtained in a multilaboratory validation study with FETAX. She stated that a goal of this project was to identify a screening tool to aid in prioritizing the vast numbers of chemicals for further assessment of developmental toxicity *in vivo*. The wealth of basic biological information known about this species allows us to examine patterns of perturbations for underlying mechanisms of developmental toxicity which may apply across species.

2) **Carcinogenicity - Medaka/Guppy** — Dr. William Stokes, NIEHS, reported on studies with two small freshwater fish being conducted through a contract by Dr. Gary Boorman's group. The Medaka or Japanese rice fish has a low spontaneous tumor incidence, is easy to breed in large numbers, has a short time-to-tumor duration compared with rodents, and about two dozen rodent carcinogens have been tested to some extent in this species. The guppy also can be bred in large numbers, is somewhat less sensitive to carcinogens, and has a slightly lower spontaneous tumor incidence than Medaka. The initiative started with a NIEHS-sponsored workshop in 1991 on carcinogenesis testing with fish. The first project, using both species, will use three chemicals, all of which are proven carcinogens or preliminary data indicate a carcinogenic effect in rats and mice, to be tested in a long-term study. The first phase will include a lifespan control study to assess spontaneous tumor incidence, the second phase will include subchronic exposure studies, and the third phase will be a 16-month continuous exposure which includes a special 9-month exposure followed by a 7-month grow-out period. The second project is a large scale low dose carcinogenicity study of N-nitrosodiethylamine (DEN) in Medaka in collaboration with the EPA, NCI, and DOD to be conducted by the same contractor. The NIEHS is providing pathology working group support for the study.

3) **Genotoxicity - Transgenic Fish** — Dr. James Burkhart, NIEHS, said this study was in collaboration with Dr. Malling and investigators at Duke University with the objective of the research being to study somatic and germinal mutations directly at the DNA level independent of any requirement for phenotypic expression so that responses can be correlated between a variety of organisms using the same target gene. He said a major problem in making correlations of mutagenesis among tissues and species is that the level and specificity of responses are very different. In the initial phase of this study, the *am*3 mutation of bacteriophage ϕX174 was used to demonstrate that spontaneous mutation frequencies are similar in transgenic cells in culture, various tissues of mice *in vivo*, 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 east coast, for which there is considerable carcinogenic and toxicological data. Dr. Burkhart said they hoped to collaborate with the group at Fort Dietrick. He concluded

that this type of approach can combine comparative research in basic mechanisms and functional modeling with the applied need for hazard assessment.

Discussion: Much of the discussion had to do with concerns about trying to make comparisons across such a wide range of species in view of the qualitative and quantitative biological differences. Dr. Schwetz said that while we define the objectives/work plan/directions for the group, we are trying to learn what others are doing across and outside the United States.

XIII. NIH Revitalization Act: Dr. Stokes said the 1993 NIH Revitalization Act was signed by the President in June as a bill to amend the Public Health Service Act to revise and extend the programs of the NIH, and for other purposes. Among its specific mandates for the NIEHS is a charge to establish within the Institute a program for conducting applied research and testing regarding toxicology to be known as the Applied Toxicological Research and Testing Program. The six objectives of this Program are: (1) to expand knowledge of the health effects of environmental agents; (2) to broaden the spectrum of toxicology information that is obtained on selected chemicals; (3) to develop and validate assays and protocols, including alternative methods that can reduce or eliminate the use of animals in acute or chronic safety testing; (4) to establish criteria for the validation and regulatory acceptance of alternative testing and to recommend a process through which scientifically validated alternative methods can be accepted for regulatory use; (5) to communicate the results of research to government agencies, to medical, scientific, and regulatory communities, and to the public; and (6) to integrate related activities of the Department of Health and Human Services. Dr. Stokes noted that objectives (1), (2), part of (3), (5), and (6) have been ongoing goals of the NTP. He remarked on the Board's recommendations concerning alternative methods in their recent Advisory Review report and the Program's response. Dr. Stokes then discussed five aspects of how the Institute proposed to respond to these mandates: (1) establish an NIEHS coordinating committee of division and program directors to assure attainment of Act objectives and integrate research; (2) organize workshops and conferences to identify the most promising methods for additional research, development, and validation studies; (3) continue to expand test method development and validation activities; (4) establish a joint *ad hoc* Subcommittee on Alternative Test Methods and Validation of the DIR and NTP Boards of Scientific Counselors; and (5) establish an Interagency Coordinating Committee on the Validation of Alternative Methods. Dr. Stokes concluded by describing the NIH Plan for the Use of Animals in Research. The first Plan had been developed over the summer by the Interagency Coordinating Committee for the Use of Animals in Research that also included representatives from the FDA, the EPA, the CPSC, and the National Science Foundation (NSF). The Plan was submitted by the Director, NIH, to House and Senate committees in late 1993. Dr. Richard Griesemer was the NIEHS representative and Dr. Stokes the alternate.

XIV. Functional Toxicology: Dr. McLachlan said functional toxicology was a new approach to detecting biologically acting xenobiotics based on the principle that a biologically active exogenous chemical may act by binding to a hormone receptor and mimicking the action of an endogenous hormone or by binding and blocking the action of an endogenous hormone. He commented that if we have some knowledge of the overall physiology or toxicology of a chemical or hormone we can screen for this action. Using estrogens as prototype hormones, he illustrated the diversity of structures of exogenous chemicals that exert an estrogenic response. Dr. McLachlan said we propose to set up a laboratory where chemicals can be screened *in vitro* using a panel of human or animal

cells that have been transfected with a specific receptor and reporter gene, e.g., the estrogen receptor. By using a variety of different receptors, the screening of xenobiotics for biological functions can be quite broad, including the estrogen receptor, progesterone receptor, androgen receptor, glucocorticoid receptor, retinoid receptor, etc. Dr. McLachlan reported that a scientist with experience in studying ligands for orphan receptors had recently come to the NIEHS, where he has a joint appointment between the Laboratory of Reproductive and Developmental Toxicology and the Environmental Toxicology Program to establish a functional toxicology effort. A collaborative arrangement will be made with the Agency for Toxic Substances and Disease Registry. He concluded that chemicals could then be classified by their function *in vitro* which, in some cases may be a useful guide for toxicological studies.

XV. Concept Reviews, ETP, DIR, NIEHS:

(1) **Tumor Incidence in the Offspring of Mutagen-Treated Mice** — (Attachment 4, pp. 2-3) Dr. Michael Shelby, NIEHS, introduced the concept, and Dr. Peter Working, Board member, served as principal reviewer. Dr. Shelby said this new project proposes to investigate the issue of germ cell mutation and increased risk of cancer in subsequent generations by determining tumor frequency in the offspring of mice exposed to known germ cell mutagens. Its overall objective is to improve our ability to predict the long-term impact of exposure to germ cell mutagens on the health of the generations following exposure. Dr. Shelby stated that the case for conducting this study using cancer as the genetic endpoint is supported by several lines of evidence, including the fact that more than 300 genetic traits in humans are associated with an increased risk of cancer. He listed experimental and epidemiologic factors and data supporting induced germ cell mutations leading to an increased incidence of cancer in progeny, and outlined the approach to be taken.

Dr. Working remarked that there are at least three possible outcomes of exposure to germ cell mutagens believed to exist, and the outcomes for the first two have been well studied while the third category of risk which is proposed in this concept has yet to be evaluated in a controlled system, with ample resources and knowledge of the genetics of meiosis and physiology of the reproductive system.. He said this proposal promises to rectify that situation. He cautioned against oversimplifying but to learn from more recent experiences with the carcinogenesis bioassay in incorporating add-on experiments to maximize the use of the animals. He also said possible sex-related differences need to be examined. Dr. Working summarized by saying that the proposal merges two important strengths, the experience of the project officer in germ cell mutation and general reproductive studies and the long experience of the NTP in conducting and evaluating rodent carcinogenesis studies. Dr. Working moved that the concept be approved. The motion was seconded and approved unanimously by the Board.

(2) **Rodent Disease Diagnostic Laboratories** — (Attachment 4, p. 4) Dr. G. N. Rao, NIEHS, introduced the concept, and Dr. Paul Bailey, Board member, served as principal reviewer. Dr. Rao said the objective was to continue to provide, through contract rodent disease diagnostic services, support to the rodent production colonies and the chemical toxicity and carcinogenicity studies. To emphasize the importance of these services, he noted that in 1984 when this program was started, nearly all long-term studies had one or more viral infections while by 1989, nearly all studies were free of viral infections. Dr. Rao concluded that the rodent disease diagnostic support is necessary to produce and maintain animals free of viral infections and maintain animals free of

diseases and viral infections during the long-term studies. Dr. Bailey stated that the Board should support continuation of these essential services through approval of the concept. The motion was seconded and approved unanimously by the Board.

(3) **Genetic Monitoring of Inbred Rodents** — (Attachment 4, p. 5) Dr. G. N. Rao, NIEHS, introduced the concept, and Dr. Paul Bailey, Board member, served as principal reviewer. Dr. Rao said the objective is to procure services for genetic monitoring through a contract mechanism. The contract is necessary to maintain the genetic integrity of the rodent production colonies as well as animals supplied for toxicity and carcinogenicity studies. Dr. Rao pointed out that prior to instituting this service, genetic variants were a problem, whereas now constant monitoring for biochemical genetic variants of foundation and production stock and test animals ensures that data are collected from genetically homogeneous rats and mice. Dr. Bailey stated that the Board should support continuation of these essential services through approval of the concept. Dr. Hughes seconded the motion which was approved unanimously by the Board.

The Chair, Dr. Klaassen, concluded the meeting by thanking Dr. Schwetz for his important contributions to the NTP and to toxicology internationally, and wished him well in his new position as Director, NCTR.

Attachment 1

disclosure of which would constitute a clearly unwarranted invasion of personal privacy.

Name of Panel: NHLBI SEP on Respiratory Afferents and the Control of Breathing
Dates of Meeting: October 13–15, 1993
Time of Meeting: 7:30 p.m.
Place of Meeting: The Clarion Hotel, Rockville, Maryland
Agenda: To evaluate and review grant applications.
Contact Person: Dr. Jon Ranhand, 5333 Westbard Avenue, room 554, Bethesda, Maryland 20892. (301) 594–7439

Name of Panel: NHLBI SEP on Vasoactive Hormones: Receptor and Signaling Mechanisms
Dates of Meeting: October 31—November 2, 1993
Time of Meeting: 7:30 p.m.
Place of Meeting: Marriott Suites Bethesda, Bethesda, Maryland
Agenda: To evaluate and review grant applications.
Contact Person: Dr. Jon Ranhand, 5333 Westbard Avenue, room 554, Bethesda, Maryland 20892. (301) 594–7439

Name of Panel: NHLBI SEP for the Review of Minority Training Applications
Dates of Meeting: November 18–19, 1993
Time of Meeting: 8:30 a.m.
Place of Meeting: Holiday Inn Chevy Chase, Chevy Chase, Maryland
Agenda: To evaluate and review grant applications.
Contact Person: Dr. Kathryn Ballard, 5333 Westbard Avenue, Room 550, Bethesda, Maryland 20892. (301) 594–7450.

(Catalog of Federal Domestic Assistance Programs Nos. 93.837, Heart and Vascular Diseases Research; 93.838, Lung Diseases Research; and 93.839, Blood Diseases and Resources Research, National Institutes of Health)

Dated: September 27, 1993.

Susan K. Feldman,

Committee Management Officer, NIH.

[FR Doc. 93–24222 Filed 10–1–93; 8:45 am]

BILLING CODE 4140–01–M

Warren Grant Magnuson Clinical Center Board of Scientific Counselors; Meeting

Pursuant to Public Law 92–463, notice is hereby given of the meeting of the Board of Scientific Counselors, Warren Grant Magnuson Clinical Center (CC), October 21–22 1993, in Building 10, room 2C–124.

The meeting will be open to the public from 9 a.m. to 5 p.m. on October 21 for review of the activities of the Clinical Center Diagnostic Radiology, Nuclear Medicine, and Positron Emission Tomography Departments. Attendance by the public will be limited to space available.

In accordance with the provisions set forth in sec. 552b(c)(6), title 5, U.S.C. and sec. 10(d) of Public Law 92–463, the meeting will be closed to the public on October 22 from 1 p.m. to adjournment for the review, discussion, and evaluation of individual programs and projects, including consideration of personnel qualifications and performance, the competence of individual investigators, and similar items, the disclosure of which would constitute an unwarranted invasion of personal privacy.

Dr. Martin I. Goldenberg, Executive Secretary of the Board of Scientific Counselors, CC, Building 10, room 1C–121, National Institutes of Health, Bethesda, Maryland 20892 (Telephone: (301) 496–5939) will provide a summary of the meeting, a Roster of Board Members, and substantive program information upon request. Individuals who plan to attend and need special assistance, such as sign language interpretation or other reasonable accommodations, should contact Dr. Goldenberg in advance of the meeting.

Dated: September 27, 1993.

Susan K. Feldman,

Committee Management Officer, NIH.

[FR Doc. 93–24218 Filed 10–1–93; 8:45 am]

BILLING CODE 4140–01–M

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 Science (NIEHS), 111 Alexander Drive, Research Triangle Park, North Carolina, on November 2, 1993.

The meeting will be 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.–8:45 a.m.—Report of the Director, NTP
8:45 a.m.–9:15 a.m.—Meeting Reports:
—Update on Activities of the Technical Reports Review Subcommittee
—Workshops on Predicting Chemical Carcinogenesis in Rodents, and Trihalomethanes and Other Environmental Factors that Contribute to Colorectal Cancer
9:15 a.m.–9:45 a.m.—Upcoming or Proposed Meetings

NTP FY 1994 Program Plans

9:45 a.m.–10:05 a.m.—Plans for Contracts, Interagency Agreements, and Grants
10:20 a.m.–11:20 a.m.—Chemical Studies (By Toxicity Endpoint):
Completed or Ongoing
Planned
Nominated—Twelve chemicals approved for study by the Interagency Committee for Chemical Evaluation and Coordination (formerly Chemical Evaluation Committee) on September 1, 1993, will be presented for discussion, and are (with CAS Nos. in parentheses): (1) Allyl Acetate (591–87–7); (2) Allyl Alcohol (107–18–6); (3) p-tert-Butyl-catechol (98–29–3); (4) Cyclohexene Oxide (286–20–4); (5) 2, 4-Decadienal (25152–84–5); (6) Decalin (91–17–8); (7) Dicyclohexylcarbodiimide (538–75–0); (8) Diisopropyl-carbodiimide (693–13–0); (9) Ecdysterone (5298–74–7); (10) 2,4-Hexadienal (142–83–6); (11) Malachite Green (Chloride: 569–64–2) (Oxalate: 18015–76–4); and (12) Tetralin (119–64–2). Brief time will be allowed for public comment on these nominations.
11:20 a.m.–12 Noon—Formation of New Toxicology Review Teams:
—Fumonisin B1
—Riddelliine
Update on Ongoing Review Teams
12:50 p.m.–1:50 p.m.—Selected Ongoing Research Programs:
Ecotoxicology Initiative—Aquatic Models
1:50 p.m.–2:20 p.m.—Workshop on Diet for F344 Rats in Long-Term Studies: Meeting report and recommendations
2:35 p.m.–3:05 p.m.—NIH Revitalization Act of 1993, Establishment of the Applied Toxicological Research and Testing Program (ATRTP) at NIEHS and activities to be conducted under the ATRTP
3:05 p.m.–4:05 p.m.—Concept Reviews:
A. Role of Glycine in Toxicity of Chemicals
B. Germ Cell Mutations and Cancer
C. Rodent Disease Diagnostic Laboratories
D. Genetic Monitoring of Inbred Rodents

Adjournment

The Executive Secretary, Dr. Larry G. Hart, National Toxicology Program, P.O.

Box 12233, Research Triangle Park, North Caroline 27709, telephone (919) 541–3971, will have available a roster of Board members and other program information prior to the meeting and summary minutes subsequent to the meeting.

Dated: September 27, 1993.

Kenneth Olden,

Director, National Toxicology Program.

[FR Doc. 93–24223 Filed 10–1–93; 8:45 am]

BILLING CODE 4140–01–M

National Toxicology Program; Chemicals (12) Nominated for Toxicological Studies; Request for Comments

SUMMARY: The National Toxicology Program (NTP) is soliciting public comments on 12 chemicals nominated for toxicological studies. These comments will assist the NTP in making informed decisions about whether to perform toxicological studies on these chemicals.

FOR FURTHER INFORMATION CONTACT: Dr. Errol Zeiger, A0–01, National Toxicology Program, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709, (919) 541–4482.

SUPPLEMENTARY INFORMATION: The NTP was established in 1978 as a cooperative effort within the Public Health Service of the Department of Health and Human Services to coordinate toxicology research and testing activities within the Department, to provide information about potentially toxic chemicals to regulatory and research agencies and the public, and to strengthen the science base in toxicology. The chemical nomination and selection process remains integral to the effective operation and success of the NTP with respect to the testing of chemicals using current methodologies, the validation of new testing methodologies, and the evaluation of mechanisms of toxicity.

As part of the nomination and selection process, the NTP Interagency Committee for Chemical Evaluation and Coordination (ICCEC) (formerly the Chemical Evaluation Committee (CEC)), composed of representatives from Federal agencies participating in the NTP, evaluates chemicals nominated to the Program and makes recommendations for study. Nominated chemicals which have been reviewed by the ICCEC are published in the **Federal Register** with request for comment. The purpose is to encourage active participation in the NTP chemical evaluation process, thereby helping the NTP to make more informed decisions as to whether to select, defer or reject chemicals for toxicology study. Comments and data submitted in response to this announcement will be reviewed by NTP technical staff for use in the further evaluation of the nominated chemicals. The NTP chemical nomination and selection process is summarized in the NTP FY 1991 Annual Plan, pages 17–19.

On September 1, 1993, the ICCEC met to evaluate chemicals nominated to the NTP for toxicological studies. The attached table lists the chemicals, their Chemical Abstract Service (CAS) registry numbers, and the types of toxicological studies recommended by the ICCEC.

Interested parties are requested to submit pertinent information on all of the nominated chemicals. The following types of data are of particular relevance:

(1) Modes of production, present production levels, and occupational exposure potential;

(2) Uses and resulting exposure levels, where known;

(3) Completed, ongoing and/or planned toxicologic studies in the public or private sector including detailed experimental protocols and results; and

(4) Results of toxicological studies of structurally related compounds.

Please submit all information in written form by November 1, 1993, to Dr. Zeiger by mail or by FAX, (919) 541–4704. Any submissions received after the above date will be accepted and utilized if possible.

Dated: September 27, 1993.

Kenneth Olden,

Director, National Toxicology Program.

TABLE

Chemical name, CAS No.	Nominating agency	Recommended testing
Allyl acetate (591–87–7).	NCI	Short-term toxicity; chemical disposition.
Allyl alcohol (107–18–6).	NCI	Carcinogenicity.
p-tert-Butylcatechol (98–29–3).	NCI	Metabolism; short-term toxicity; skin tumor promotion; class study.
Cyclohexene oxide (286–20–4).	NCI	Mechanistic studies; limited carcinogenicity studies.
2,4-Decadienal (25152–84–5).	NCI	Full toxicological evaluation; possible carcinogenicity studies.
Decalin (91–17–8).	NCI	Carcinogenicity; chemical disposition.
Dicyclohexylcarbodiimide (538–75–0).	NCI	General toxicity studies.
Diisopropylcarbodiimide (693–13–0).	NCI	General toxicity studies.
Ecdysterone (5298–74–7).	NCI	Full toxicological evaluation; carcinogenicity.
2,4-Hexadienal (142–83–6).	NCI	Full toxicological evaluation; possible carcinogenicity studies.
Malachite green (569–64–2/18015–76–4).	FDA	Carcinogenicity; mechanistic studies.
Tetralin (119–64–2).	NCI	Carcinogenicity; chemical disposition.

[FR Doc. 93–24224 Filed 10–1–93; 8:45 am]

BILLING CODE 4140–01–M

Substance Abuse and Mental Health Services Administration

Current List of Laboratories Which Meet Minimum Standards To Engage in Urine Drug Testing for Federal Agencies and Laboratories That Have Withdrawn From the Program

AGENCY: Substance Abuse and Mental Health Services Administration, HHS (Formerly: National Institute on Drug Abuse, ADAMHA, HHS).

ACTION: Notice.

SUMMARY: The Department of Health and Human Services notifies Federal agencies of the laboratories currently certified to meet standards of Subpart C of Mandatory Guidelines for Federal Workplace Drug Testing Programs (53 FR 11979, 11986). A similar notice listing all currently certified laboratories will be published during the first week of each month, and updated to include laboratories which subsequently apply for and complete the certification process. If any listed laboratory's certification is totally suspended or

Attachment 2

AGENDA
BOARD OF SCIENTIFIC COUNSELORS
NATIONAL TOXICOLOGY PROGRAM

November 2, 1993

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.-9:15 a.m.	Meeting Reports	
	— Technical Reports Review Subcommittee	Dr. L. Hart, NIEHS
	— Predicting Chemical Carcinogenesis in Rodents Workshop	Dr. M. Shelby, NIEHS
	— Trihalomethanes and Colorectal Cancer Workshop	Dr. J. Dunnick, NIEHS
9:15 a.m.-9:30 a.m.	Upcoming/Proposed Meetings	
	— Workshop on Criteria Proposed for Dose Selection	Dr. B. Schwetz, NIEHS
	— Symposium on Causes and Risks of Developmental Abnormalities	Dr. J. Bishop, NIEHS

NTP FY 1994 Program Plans

9:30 a.m-9:50 a.m.	Plans for Contracts, Interagency Agreements and Grants	
	— Developmental Biology/Toxicology	Dr. J. Heindel, NIEHS
9:50 a.m.-10:05 a.m.	*Break*	
10:05 a.m.-11:20 a.m.	FY 1994 Research Plans and Chemical Studies	
	Introduction and Charge to the Board	Dr. J. Bucher, NIEHS
	With Emphasis on Toxicology, Carcinogenesis, Genetic Toxicology, Chemical Disposition, Special Programs and Alternative Methods	Dr. B. Schwetz, NIEHS
	Comments by Board and Interactive Discussion Between Board and Staff of NTP Agencies	
11:20 a.m.-11:35 a.m.	Formation of New Toxicology Review Team	
	— Fumonisin B_1	Dr. W. Allaben, NCTR

11:35 a.m.-12:05 p.m.	Chemicals Nominated for FY 1994	
	— Chemicals approved for study by Interagency Committee for Chemical Evaluation and Coordination (ICCEC)	Dr. E. Zeiger, NIEHS
	— Public Comment	
12:05 p.m.-12:50 p.m.	*Lunch*	
12:50 p.m.-1:20 p.m.	F344 Rat Diet Studies - Report of Workshop and Recommendations for Changes	Dr. G. Rao, NIEHS
1:20 p.m.-2:20 p.m.	Selected Ongoing Research Programs - **Environmental Toxicology and Alternative Systems**	Dr. B. Schwetz, NIEHS
	— Developmental Toxicity — FETAX	Dr. J. Harry, NIEHS
	— Carcinogenicity — Medaka/Guppy	Dr. W. Stokes, NIEHS
	— Genotoxicity — Transgenic Fish	Dr. J. Burkhart, NIEHS
	— Discussion	
2:20 p.m.-2:35 p.m.	*Break*	
2:35 p.m.-3:20 p.m.	NIH Revitalization Act	Dr. W. Stokes, NIEHS
	— Creation of Applied Toxicological Research and Testing Program at NIEHS	
	— NIH Plan for the Use of Animals in Research	
	— Proposed subcommittee on alternative test methods and validation from the NTP and Division of Intramural Research Boards	
	Functional Toxicology	Dr. J. McLachlan, NIEHS
3:20 p.m.-4:05 p.m.	Concept Reviews	
	— Tumor Incidence in the Offspring of Mutagen-treated Mice	Dr. M. Shelby, NIEHS
	— Rodent Disease Diagnostic Laboratories	Dr. G. Rao, NIEHS
	— Genetic Monitoring of Inbred Rodents	Dr. G. Rao, NIEHS

NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS

November 2, 1993

Dr. Paul T. Bailey (3/94)
Mobil Oil Corporation
Environmental & Health Sciences Laboratory
P. O. Box 1029
Princeton, NJ 08543-1029

(Toxicology)

Dr. Elaine F. Faustman (3/95)
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Department of Environmental Health
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Seattle, WA 98105

(Developmental Toxicology)

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

(Reproductive Physiology)

Dr. Lawrence A. Loeb (3/94)*
Professor and Director
Gottstein Memorial Laboratory and Department of Pathology SM-30
University of Washington D-525 HSB
Seattle, WA 98195

(Carcinogenesis)

Dr. Kenneth R. Reuhl (3/94)
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Department of Pharmacology and Toxicology
School of Pharmacy
Rutgers University
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(Neurotoxicology)

Dr. Arnold L. Brown (3/96)*
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Madison, WI 53706

(Carcinogenesis, Pathology)

Dr. Barbara C. Hansen (3/94)
Professor of Physiology
Director, Obesity & Diabetes Research Center
University of Maryland
10 South Pine Street
MSTF 600
Baltimore, MD 21201

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

(Toxicology)

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

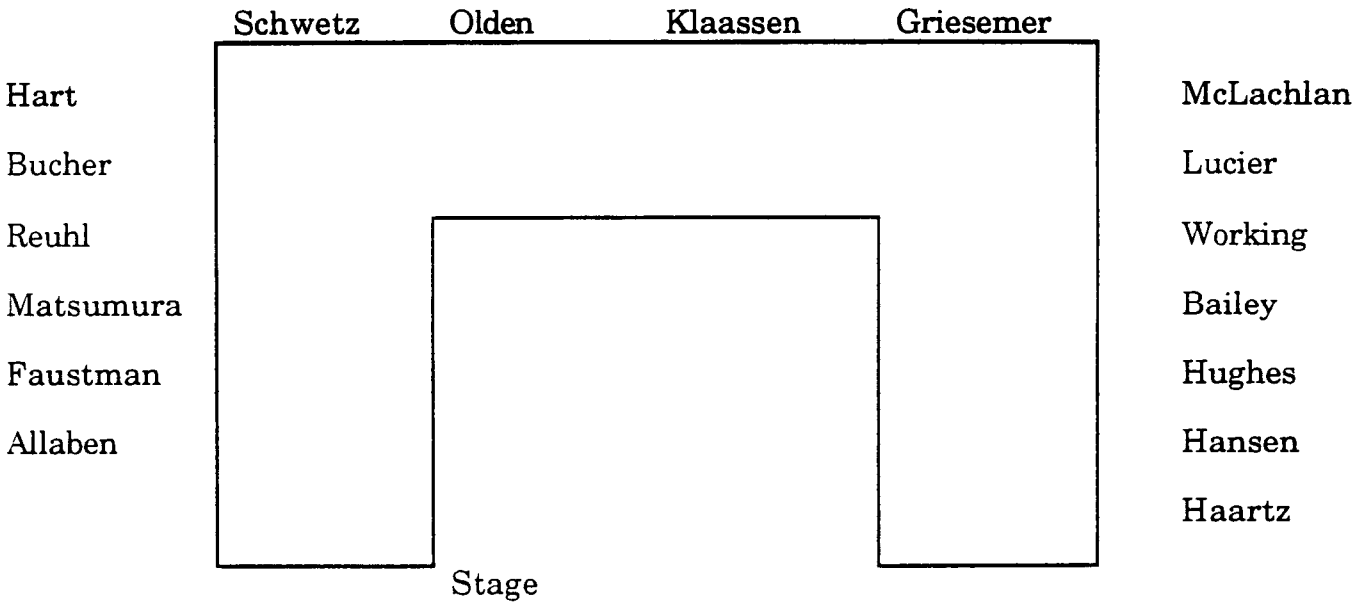
(Reproductive Toxicology, Genetics)

* 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

November 2, 1993



CHEMICALS RECOMMENDED FOR TESTING BY THE ICCEC, Sept. 1, 1993				
CHEMICAL (CAS NUMBER)	Nomination Source	Testing Recommendations (Priority)	NTP Chemical Selection Principles	Rationale/Remarks
1. **Malachite green** (chloride: 569-64-2) (oxalate: 18015-76-4)	FDA	-Carcinogenicity studies in rodent and fish bioassays -Mechanistic studies (High)	2, 3, 7	-FDA's FY93 priority chemical for NTP carcinogenicity testing -High potential for human exposure -High potential for misuse as an antibacterial and antifungal agent in the aquaculture industry -Potential for contamination of drinking water and recreational facilities, and food fish -Preliminary disposition studies in fish indicate potential for bioaccumulation -FDA needs data to regulate the chemical -Suspicion of carcinogenicity
2. **Allyl acetate** (591-87-7)	NCI	-Short-term and disposition studies (if necessary) (Low to moderate)	1, 2, 6, 7	-Study as a pair of chemicals -Potential for human exposure due to presence in the food chain -Use as a synthetic food flavoring agent -NCI's interest in chemicals in food chain -Lack of adequate toxicity data
3. **Allyl alcohol** (107-18-6)	NCI	-Carcinogenicity (Low to moderate)	1, 2, 6, 7	-High production -Potential for human exposure due to presence in the food chain -Allyl alcohol selected over allyl acetate for carcinogenicity because it has the higher production and the acetate is metabolized to the alcohol

Attachment 3

CHEMICALS RECOMMENDED FOR TESTING BY THE ICCEC, Sept. 1, 1993

CHEMICAL (CAS NUMBER)	Nomination Source	Testing Recommendations (Priority)	NTP Chemical Selection Principles	Rationale/Remarks
4. **p-tert-Butyl-catechol** (98-29-3)	NCI	-Class study of p-substituted 1,2-dihydroxybenzenes -Comparative metabolism, short-term toxicity, and skin tumor promotion studies of members of this class -Select best class representative for carcinogenicity testing (High)	1, 6, 7	-Potential for human exposure -Interest in evaluating the toxicity of the dihydroxybenzenes class of antioxidants -Suspicion of carcinogenicity -Interest in the role of oxidative changes in the carcinogenic process -Have an interagency committee select members of the chemical class for testing
5. **Cyclohexene oxide** (286-20-4)	NCI	-Mechanistic carcinogenicity studies -Synergism studies or two-stage carcinogenicity studies; e.g., test with other carcinogens deemed appropriate by NTP	3, 6, 7	-High annual production -Potential for human exposure -Representative cycloalkene oxide -Suspicion of epoxide's carcinogenicity -Perform mechanistic studies to provide insight as to whether cyclohexene oxide (CO) poses a human risk and whether further studies are necessary -Interest in the ability of CO to deplete GSH and its effect on the body's detoxication systems
6. **Ecdysterone** (5289-74-7)	NCI	-Carcinogenicity -Complete toxicological evaluation (High)	2, 3, 6	-Unregulated over-the-counter drug, available in health food stores, and advertised in body-building magazines -Concern of CDC, FDA, and NCI about the use of ecdysterone by athletes, body builders, and teenagers, and its potential abuse -Suspicion of carcinogenicity -Drug is currently under evaluation in some countries as an antiarrhythmic, accelerator of bone healing, wound healing, and skin regeneration

CHEMICALS RECOMMENDED FOR TESTING BY THE ICCEC, Sept. 1, 1993

CHEMICAL (CAS NUMBER)	Nomination Source	Testing Recommendations (Priority)	NTP Chemical Selection Principles	Rationale/Remarks
7. Tetralin (119-64-2)	NCI	-Carcinogenicity -Chemical disposition (Low)	3, 4, 6, 7	-High production and import volumes -Widely-used solvents; substitute for turpentine in lacquers, paints, and varnishes
8. Decalin (91-17-8)	NCI	-Carcinogenicity -Chemical disposition (Low) -Study tetralin and decalin as a pair of chemicals	3, 4, 6, 7	-Potential for human exposure -Environmental pollutants -Assign higher priority for carcinogenicity studies to tetralin, which has a greater suspicion of carcinogenicity -Both compounds are derived from naphthalene
9. Dicyclohexylcarbodiimide (DCC) (538-75-0)	NCI	-General toxicity studies (Moderate to high)	3, 6, 7	-Potential for human exposure, especially research scientists in bioenergetics field and DNA industry -Widely used reagents in chemical and pharmaceutical industries; increasing use in field of bioenergetics
10. Diisopropylcarbodiimide (DIC) (693-13-0)	NCI		3, 6, 7	-Representatives of the carbodiimide chemical class -Lack of general toxicity data -Study DCC and DIC as a pair of chemicals -Include ophthalmic examinations in toxicity studies
11. 2,4-Hexadienal (142-83-6)	NCI	-Full toxicological evaluation -Consider for carcinogenicity studies after completion of toxicity studies (High)	1, 6, 7	-Potential for human exposure -Used as food flavoring agents -Naturally occurring products present in food chain
12. 2,4-Decadienal (25152-84-5)	NCI		1, 6, 7	-Oxidation products of unsaturated fats -Suspicion of carcinogenicity of α,β-unsaturated aldehydes -Test 2,4-hexadienal and 2,4-decadienal as a pair -Interest in hazardous natural products -Examine mechanisms of toxicity

Attachment 4

Environmental Toxicology Program

Division of Intramural Research

National Institute of Environmental Health Sciences

CONCEPT REVIEWS

Prepared for:

National Toxicology Program
Board of Scientific Counselors

November 2, 1993

CONCEPT REVIEWS

Table of Contents

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: Tumor Incidence in the Offspring of Mutagen-Treated Mice
PRESENTER: Michael D. Shelby, Reproductive Toxicology Group, ETP

OBJECTIVES: The objective of the proposed study is to investigate reported observations that induced germ cell mutations can lead to an increased risk of cancer in subsequent generations. The project will investigate tumor frequencies in the offspring of mice treated with known germ cell mutagens. These studies offer to 1) improve our ability to predict the impact of exposure to germ cell mutagens on human health in generations immediately following exposure, 2) increase our understanding of the genetics of cancer, and 3) further elucidate the bases for individual susceptibilities to both spontaneous and induced cancer.

BACKGROUND: Human genetic risk estimations are based on mammalian germ cell mutagenesis studies. Although methods are available to detect dominant mutations, the systems most widely used to determine frequencies of induced mutations detect recessive mutations (e.g., visible phenotypic changes such as coat color or enzyme activity/ electrophoretic mobility changes) or chromosomal aberrations (e.g. dominant lethality or heritable translocations). These systems are well suited for detecting germ cell mutagens and for determining induced mutant frequencies. However, to predict human health effects from an increased rate of such mutations requires major assumptions that diminish the confidence that can be placed in the resulting risk estimates. In order to more accurately assess the health effects that might result from human exposure to germ cell mutagens, it is necessary to develop animal systems that detect effects directly related to human disease states and that are expressed in the first generations following exposure to a mutagenic agent.

The case for conducting this proposed study using cancer as the genetic endpoint is supported by several lines of evidence. First, the fact that the risk of cancer in humans is strongly influenced by genetic factors is well established. More than 300 genetic traits in humans are associated with an increased risk of cancer (Mulvihill, in Genetics of Human Cancer, 1977 and personal communication); over 200 of these are dominant traits. Further, there are clear differences in the sites and frequencies of tumors among strains of laboratory rodents. These are naturally occurring, genetically determined effects on sites and incidences of spontaneous tumors. Early attempts to induce such genetic differences with x-rays were unsuccessful (Green, Annual Review of Genetics (1968) 2, 87-120), but successes have been reported in more recent experiments that used either x-rays or chemicals as germ cell mutagens.

Nomura (Nature (1982)296, 575-577) reported that, with x-rays, urethane, and 4-NQO, the offspring of treated parental mice exhibit increased tumor frequencies and that this genetic predisposition to cancer is transmitted to subsequent generations. Tomatis et al. (Int. J. Cancer (1981)28, 475-478) reported an increased frequency of neurogenic tumors in the offspring of male rats treated with ethylnitrosourea (ENU) but the effect was not reproduced in a subsequent experiment (Mutation Res. (1990) 229, 231-237).

Although epidemiology studies designed to detect increased incidences of cancer in the offspring of mutagen-exposed humans have been generally negative, the recent report by Gardner et al. (Br. Med. J. (1990) 300, 423-429) of an increased incidence of leukemia in

children of radiation-exposed workers has renewed scientific interest and controversy in this topic.

In sum, results suggesting that induced germ cell mutations might lead to elevated tumor frequencies, taken with the evidence for a clear genetic component in the susceptibility to cancer, make this an important area of research, both for the development of a model animal system designed to predict the health effects of exposure to germ cell mutagens and for a better understanding of the genetics of cancer.

APPROACH: The initial phase of the proposed study would be designed to confirm or refute the phenomenon of an increased cancer incidence resulting from induced germ cell mutations in mice. With regard to earlier studies, Nomura's experiments did not employ the most effective germ cell mutagens and the animals were sacrificed too early (eight months) to obtain complete tumor information. Tomatis et al. employed an extremely effective mutagen (ENU) but at a suboptimal dose and then sampled germ cell stages that are not demonstrated to be of maximum sensitivity to mutation induction by ENU.

The proposed experiments would maximize the probability of observing an effect by using B6C3F1 mice on which the most is known about spontaneous tumor incidences, would employ a treatment regimen of ENU known to induce extremely high frequencies of mutations, would test germ cell stages with demonstrated sensitivity to ENU, would allow animals to be followed for up to two years, and would employ the same thorough histopathology practices used in the NIEHS/NTP rodent carcinogenesis studies.

If these experiments confirm the phenomenon of increased tumor frequency in the offspring of mutagen treated parents, additional studies would be conducted. These would include nvestigations of the relationship between mutant frequencies and the incidences of tumors. This would be accomplished by determining the two frequencies in the same F_1 population. Such information would be needed in order to predict tumor frequency from a determined induced mutation frequency. Further studies could be conducted to extend the observations to germ cell mutagens other than ENU.

In addition, experiments will be carried out to determine if progeny of mutagen-treated parents are more susceptible to chemical-induced cancers and whether such susceptibility is concomitant with or independent of a predisposition to spontaneous tumors.

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 ensure 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 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 F344/N rats, B6C3F1 mice and other strains of rodents produced 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

5289-74-7

Skip Sorry for not copying you earlier. John

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health
National Institute of
Environmental Health Sciences
P. O. Box 12233
Research Triangle Park, NC 27709

Memorandum

Date: October 27, 1993

From: Head, Toxicology Group, TB, ETP JB

Subject: New Chemical Assignments

To: Addressees

At the ICCEC meeting of September 1, 1993, 12 new chemicals were approved for study. These chemicals will be presented to the BSC on Nov. 2 for their information, and will go to the Executive Committee on Dec. 1. It is unlikely that the decision to study these chemicals will be reversed at the Nov. 2 or Dec. 1, meeting. Therefore, I would very much appreciate it if the listed people would serve as study scientists for the indicated chemicals, and begin to develop study plans, and formulate appropriate review teams.

Allyl alcohol/ allyl acetate	Dr. Irwin
p-Tert-butyl catechol	Dr. Dunnick
Cyclohexene oxide	Dr. Cunningham
Ecdysterone	Dr. Wilson
Tetralin/decalin	Dr. Chan
Dicyclohexylcarbodiimide/ Diisopropylcarbodiimide	Dr. Chhabra
2,4-Hexadienal/ 2,4-Decadienal	Dr. Chan

Attached please find copies of the executive summaries and a copy of the ICCEC meeting minutes. For assistance in determining appropriate agency contacts I will be glad to help, as will Drs. Eastin and Zeiger. Please submit suggestions for research teams to me or Dr. Stokes. We would like to begin considering new study designs for these chemicals soon after the first of the year. Thanks much.

cc:
Dr. Boorman
Dr. Matthews
Dr. Cunningham
Dr. Chan
Dr. Chhabra
Dr. Dunnick
Dr. Irwin
Dr. Wilson
Dr. Stokes
Dr. Lucier
Dr. Schwetz