NATIONAL TOXICOLOGY PROGRAM

BOARD OF SCIENTIFIC COUNSELORS

November 14 and 15, 1988

Summary Minutes

National Toxicology Program

Board of Scientific Counselors Meeting

November 14 and 15, 1988

Summary Minutes

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NATIONAL TOXICOLOGY PROGRAM

BOARD OF SCIENTIFIC COUNSELORS MEETING

November 14 and 15, 1988

SUMMARY MINUTES

The National Toxicology Program (NTP) Board of Scientific Counselors met on November 14 and 15, 1988, in the Conference Center, Building 101, South Campus, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. (Attachment 1: Federal Register Meeting Announcement; Attachment 2: Agenda and Roster of Members and Expert Consultants.) Members of the Board are Drs. Michael Gallo (Chairman), John Little, Richard Miller, Frederica Perera, Adrianne Rogers, Robert Scala, and Arthur Upton. All were present.

Program Review of the Cellular and Genetic Toxicology Branch (CGTB), Division of Toxicology Research and Testing (DTRT), NIEHS

I. Introduction and Overview: Dr. Richard Griesemer, Director, DTRT, gave a brief overview of the Division, noting there were four branches. Dr. Raymond Tennant, Chief, CGTB, stated the mandate for the Branch and noted the five functional groups in the CGTB (Attachment 3: CGTB - Overview Material). He reported that at the last program review by the Board, the primary recommendation was that the Branch organize and conduct an in-depth evaluation of short-term in vitro genetic toxicity assays for rodent carcinogens. He said this had been done and reported. Dr. Tennant commented on current and future directions deriving from this evaluation including identifying genotoxic carcinogens based on a combination of a positive response in the Salmonella assay and 'structural alerts' (electrophilic potential). He discussed indirect genotoxic mechanisms of carcinogenesis and non-genotoxic mechanisms of carcinogenesis, and said a program goal was to develop methods for discriminating between them, and for identifying non-genotoxic carcinogens. He noted that most known human carcinogens have electrophilic properties and are mutagenic yet some important ones are not mutagenic, e.g., asbestos and diethylstilbestrol.

Dr. Tennant mentioned the various programmatic activities to be discussed by other members of the CGTB staff. The review format combined platform presentations with poster sessions which allowed for more informal and indepth interactions among reviewers and program staff. The Board was supplemented by six ad hoc consultants with expertise in the program areas being reviewed. The names and affiliations of the consultants are given in Attachment 2.

II. Evaluation and Application of Current In Vitro Methodology: Dr. Errol Zeiger summarized the in-depth evaluation of four in vitro genetic toxicity assays for their ability alone or in various combinations to discriminate between carcinogenic and noncarcinogenic chemicals identified in NTP two-year rodent studies. The four assays were: mutagenesis in Salmonella; mutagenesis in mouse lymphoma cells; chromosome aberrations in Chinese hamster ovary (CHO) cells; and induction of sister chromatid exchanges (SCEs) in CHO cells. The initial evaluations were of 73 chemicals; an additional 42

chemicals were evaluated subsequently with the findings supporting the conclusions drawn for the 73. For the four short-term tests (STTs), estimates were made of the sensitivity (proportion of carcinogens positive in the STT), specificity (proportion of noncarcinogens not positive in the STT), positive predictivity (proportion of STT positives that are carcinogens), negative predictivity (proportion of STT negatives that are noncarcinogens), and concordance (overall proportion of carcinogens and noncarcinogens correctly identified). From the evaluation four primary conclusions could be drawn: (1) none of the four STTs lived up to original expectations of identifying correctly 80-90% of carcinogens and noncarcinogens; (2) the four STTs, by themselves or in combination, do not improve upon the predictive ability of Salmonella, alone; (3) the data do not support use of the other three STTs for routine screening of chemicals for presumptive carcinogenicity; and (4) none of the four STTs can be used as a surrogate for chronic carcinogenicity studies in rodents. Finally, Dr. Zeiger reported that a study was planned in which Salmonella assays would be performed on a random sampling of 100 chemicals derived from a selected 'universe of chemicals' as reported to the NIEHS in a National Academy of Sciences' report. The findings for mutagenesis should give an estimate of how well the NTP data base approximates the 'universe of chemicals'.

III. Evaluation and Application of In Vivo Systems: Dr. Michael Shelby summarized efforts over the last 4 to 5 years on studying the genetic toxicity of chemicals in intact rodents, and developing information on those most likely to present a carcinogenic hazard to humans. Assays primarily evaluated include mouse bone marrow cytogenetics for both chromosome aberrations (CAs) and sister chromatid exchanges (SCEs), and a mouse bone marrow micronucleus (MN) assay. He described improvements in the protocols which enhanced sensitivity for CAs and SCEs. The 73 chemicals tested in the four STTs are being evaluated in the in vivo assays and for tests completed performances, as measured by sensitivity, specificity, positive and negative predictivity, and concordance, were comparable with the in vitro assays for CAs but less sensitive for SCEs. Dr. Shelby compared the capabilities of CAs and micronucleus assays to discriminate between rodent carcinogen and noncarcinogens. He noted several carcinogens which are negative in the Salmonella assay but positive for CAs or MN in mouse bone marrow; IARC Group I human carcinogens are almost uniformly positive in the in vivo bone marrow assay. Also presented were studies comparing the sensitivity of the in vivo-in vitro hepatocyte DNA repair (unscheduled DNA synthesis) assay with the S-phase assay (scheduled DNA synthesis) for detecting hepatocarcinogens. Dr. Shelby said future plans include more extensive use of the MN assay as an initial assay, further evaluation of the S-phase assay, and exploring the use of transgenic mice for determining mutations in specific tissues.

Projects Under Development

IV. Transgenic Mice Projects: Dr. Lawrence Boone described the development and use of transgenic technology. He said there were two projects under development in the Branch that involved transgenic animals, one designed for mutagenesis studies and the other for carcinogenesis studies. The first involves genetically engineering a mutation target suitable for in situ scoring or shuttle vector approaches to measure somatic or germ cell mutations in mice. The second is a collaborative project with researchers at Harvard which will

evaluate the use of transgenic mice carrying activated oncogenes in the early identification and analysis of chemical carcinogens. The exposure and pathology studies will be conducted by an NTP master agreement laboratory.

V. Induction of Aneuploidy: Dr. Michael Resnick noted that meiotic aneuploidy (primarily in sperm)contributes to the genetically based disease burden in humans and mitotic aneuploidy is implicated in the etiology of some human cancer. He described the development of model systems in yeast (Saccharomyces cerevisiae) and Drosophila for detection of spontaneous and chemically-induced aneuploidy. These systems are being used to relate aneuploidy to other genetic endpoints and to determine mechanisms for the induction of aneuploidy via DNA and non-DNA targets. Dr. Resnick discussed chemicals that induce or do not induce chromosome loss in mitotic yeast, noting the 100% agreement between test labs, and commenting on the enhancement by cold shock of aneuploidy induction by several aprotic solvents. He listed chemicals that induced meiotic aneuploidy (both chromosome loss and gain) in female Drosophila including some that were negative in the yeast system. Short exposures of Drosophila to aprotic solvents also resulted in induction of aneuploidy. In upcoming studies, emphasis will be given to looking at non-mutagenic carcinogens.

VI. Mammalian Cell Transformation Systems: Dr. Judson Spalding said these systems are believed to be the most appropriate models for in vitro study of chemical carcinogenesis, and depending on the system used the three phases of neoplastic development, initiation, promotion and progression can be demonstrated. He reported on the validation study begun several years ago with three systems: Syrian hamster primary embryo (SHE) cell assay; chemical enhancement of simian adenovirus SA7 transformation of Syrian hamster embryo cells (SHE/SA7) assay; and the Rauscher leukemia virus (RLV)-infected Fischer rat embryo cell (RAT/RLV) assay. These studies involved several laboratories with initially uncoded model chemicals followed by evaluation of up to 40 coded chemicals (carcinogens and noncarcinogens). Conclusions of this "Phase I" study were that the capability of the assays to discriminate between carcinogens and noncarcinogens was no greater than for other less complex and labor intensive assays and offered no advantage for screening chemicals. Dr. Spalding then described an ongoing Phase II study in which three culture systems were being evaluated: the SHE assay which was the most promising of those from Phase I; human fibroblasts; and Balb/c 3T3 cells. To date, it has been possible to create altered phenotypes in human fibroblasts and use chemicals to further alter the expression of these phenotypes. These changes can be characterized, and plans are to create the phenotypes in the other two cell systems.

VII. Transfection of Human Genes for Drug Metabolizing Enzymes Into Human Cells: Dr. Robert Langenbach said the goals of this study were to improve the metabolic capability of human cells used in genetic toxicology and to determine the role of specific P450 enzymes in carcinogen metabolism. He characterized the genes or cDNAs transfected. He compared mutagenic activity of potent mutagens in transfected vs. control AHH-1 cells, and also the pattern of metabolites formed by one of these mutagens, benzo(a)pyrene. From the results obtained, Dr. Langenbach concluded that human genes for drug/chemical metabolizing enzymes can be transfected into target human cells, genetic damage can be measured, carcinogen/mutagen metabolism by specific human enzymes can be determined, and long-term low-dose protocols which minimize high dose toxic

effects can be utilized with such cells. Future studies will utilize other vectors and target cells, and for the P450s transfected, substrate specificities, DNA adducts and mutational spectrum will be compared.

VIII. Studies on Nonmutagenic Carcinogens: Dr. Langenbach reported that the first assay used to study nongenotoxic carcinogens was the V79 metabolic cooperation assay. This study was a collaborative effort with NIOSH consisting of a validation phase followed by an evaluation of 40 coded chemicals (both genotoxic and nongenotoxic) for which there were carcinogenicity results in NTP two-year rodent studies. Conclusions were: (1) there was no clear evidence that this assay could identify the nongenotoxic carcinogens which were negative in Salmonella; and (2) while inhibition of metabolic cooperation may be involved in the carcinogenesis process, it does not appear to be sufficient by itself or necessarily a requirement for tumor induction. Thus, a multi-endpoint approach has been initiated for determining biological mechanisms associated with nongenotoxic nonelectrophilic chemicals. The biological effects to be investigated include: enhancement of cellular transformation in vitro; inhibition of metabolic cooperation; protein kinase C activation; and altered cellular differentiation. Dr. Langenbach concluded by discussing expected outcomes.

IX. Evaluation of the Mouse Lymphoma Assay: Dr. William Caspary said the mouse lymphoma L5178Y $tk^{+/-}$ forward mutation assay was one of those examined by the Branch for its ability to predict carcinogenicity in rodents. He described the test protocol used, evaluation of intra- and interlaboratory reproducibility, and recent modification of the "standard" protocol which has resulted in a large increase in spontaneous and induced mutant frequencies. He noted that results obtained with the new protocol were being compared with those obtained using a series of chemicals previously tested using the standard protocol.

X. Chemical Effects on DNA Methylation: Dr. Caspary stated that evidence for a role of altered methylation in carcinogenesis originates from the hypomethylation patterns observed in some malignant colon cancers and hypomethylated -ras genes found in some human cancers. Altered methylation may affect a gene directly or the regulation and expression of the gene. Dr. Caspary said he proposed to examine DNA adduction and methylation at actively transcribed genes using chemicals for which results on carcinogenicity are available from NTP long-term studies in rodents.

XI. Germ Cell Mutation Projects: Dr. Shelby noted that the germ cell mutagenesis program of the Branch is one of the largest in the world and the primary one in the United States. The organisms used are Drosophila and the mouse. Over the last six years three mouse germ cell systems have been used for both testing of chemicals and research. Chemical induction of chromosomal effects are studied using a combination of dominant lethal (DL) and heritable translocation tests (HTT). The morphological specific locus test (MSLT) is the most well established test for germ cell mutagens while the electrophoretic specific locus test (ESLT) is newer and being compared with the other assays. He said that over the last six years using these systems, the world-wide data base on germ cell mutagens has been increased about 50%. Dr. Shelby discussed results with several chemicals including tetrahydrocannabinol, urethane, acrylamide, chlorambucil, and ethylene oxide, and patterns of genetic toxicity in vivo for a longer list of chemicals. He presented highlights from the germ cell chromosomal effects project and detailed indepth studies done with the

"supermutagen", ethylnitrosourea. He concluded by reporting on two genetic disease models that had derived from the electrophoretic specific locus test project: a beta-thalassemia model, and a carbonic anhydrase II deficiency syndrome.

XII. Overview of Intramural Research Projects: Dr. Tennant said the research projects were usually investigator initiated and not usually extensions of programmatic activities. These projects were to be reviewed in depth by the ad hoc expert consultants. Dr. Tennant briefly summarized the projects: (1) carcinogen metabolism (Langenbach et al.); (2) mammalian cell mutagenesis (Caspary et al.); (3) mutagenesis and chromosome structure in Drosophila (James M. Mason et al.); (4) Salmonella mutagenesis (Zeiger et al.); (5) studies on the mouse FV-1 gene: a model for the genetic control of retrotransposition (Boone et al.); (6) chromosome maintenance, metabolism and distribution in yeast (Resnick et al.); and (7) mammalian germ cell and somatic cell mutagenesis (Shelby et al.).

XIII. Report of the Director, NTP: Dr. David Rall reported that: (1)there were several recent changes in program leaders at the NIEHS. He introduced Dr. Richard Griesemer, new Director, Division of Toxicology Research and Testing. Dr. Martin Rodbell, Director, Division of Intramural Research (DIR), returned to the laboratory as senior investigator in the Laboratory of Cellular and Molecular Pharmacology, DIR. Recently, Dr. John McLachlan, Chief, Laboratory of Reproductive and Developmental Toxicology, DIR, was named new Director, DIR; (2) the FY 1989 NIEHS budget has been passed with an addon from the House of Representatives earmarked for indoor air pollution toxicology studies with emphasis on radon. The Division of Biometry and Risk Assessment has initiated some case control studies. Also, studies on the biological disposition of lead will be initiated; (3) a resolution was passed by the NIEHS national advisory council supporting the continuing use of laboratory animals in research with similar resolutions passed by advisory councils for the National Cancer Institute and the National Heart, Lung and Blood Institute (copies of these resolutions were given to Board members); (4) a conference was held at NIEHS on September 29-30 dealing with Chemically Contaminated Aquatic Food Resources and Human Cancer Risk (a summary of the conference will be sent to Board members), and another conference dealing with a serious public health problem will be held at NIEHS on January 9-11, 1989, and titled Advances in Lead Research with Related Implications for Environmental Health; and (5) a new environmental health sciences center has been established at the University of Medicine and Dentistry of New Jersey (UMDNJ) with Dr. Bernard Goldstein as Director.

XIV. Update on Activities of the Technical Reports Review Subcommittee: Dr. James Huff gave the Board a progress report on recent and upcoming activities of the Technical Reports Review Subcommittee and associated ad hoc Panel of Experts (Peer Review Panel). He discussed the levels of evidence used in making interpretive conclusions about carcinogenicity in the two-year rodent studies reviewed by the Peer Review Panel. Dr. Huff summarized the findings of the Panel's meetings on November 6, 1987, April 18-19, 1988, and October 3-4, 1988. He noted that of 23 chemicals reviewed, there was a positive finding (clear evidence or some evidence of carcinogenic activity) in at least one experiment for 13 of the chemicals (57%). Looking at all the experiments (each sex group of each species used was an experiment) for the 23

chemicals, there were positive findings in 24 of 86 experiments (28%). The Panel concurred with NTP staff recommendations on 94% of the levels of evidence. Dr. Huff summarized the Technical Reports expected to be reviewed at the next meeting of the Panel on March 13-14,1989. He handed out a listing of draft Technical Reports projected for review from June 1989 through October 1990.

Dr. Perera, Subcommittee member and Chair of the Panel meeting on October 3-4, commented that there was considerable discussion on possible mechanisms of carcinogenic action and some confusion, particularly among new Panel members, as to use of mechanistic considerations in setting the level of evidence. There was agreement that the Panel should restrict itself to the data available in evaluating the approriate level of evidence. Information on mechanisms would be useful to others having responsibility for conducting risk assessments.

XV. Status of Oncogene Studies: Dr. Robert Maronpot, Chemical Pathology Branch (CPB), DTRT, NIEHS, reported on completed, ongoing and proposed studies, noting that this was a collaborative effort primarily between the CPB and investigators in the Laboratory of Biochemical Risk Analysis, Division of Biometry and Risk Assessment, NIEHS, especially Drs. Marshall Anderson and Steven Reynolds. Input and experimental samples were provided by other investigators within the NIEHS and from the NTP. He said the approaches have been primarily transfection of tumor DNA into NIH 3T3 cells, and more recently, use of the more sensitive nude mouse assay. Initially, tumor samples for oncogene analysis were obtained at terminal sacrifice from NTP two year rodent studies. Also, provisions have been made in the design of specific studies for collection of samples, e.g., 1,3-butadiene.

Dr. Maronpot reported that activated oncogenes were found in only 1/37 spontaneous neoplasms in F 344 rats while there was a high incidence of activated oncogenes in chemically-induced tumors. He cited studies with benzidine congeners and lung tumors induced by tetranitromethane. In B6C3F1 mice, there is a high incidence of activated oncogenes (55%) in spontaneous liver tumors but a much lower incidence (8%) in spontaneous tumors at other sites. Citing studies with furan and furfural, he pointed out differences in the pattern of mutations in oncogenes between control and treated mice. Dr. Maronpot discussed other ongoing molecular biological studies with NTP chemicals as well as some with chemicals not studied by the NTP. He described studies in which oncogenes will be analyzed at different times during the course of tumor progression with liver tumors in B6C3F1 mice and lung tumors in strain A mice. Patterns of oncogene activation seem to be quite chemical specific. He concluded that information on oncogene activation and patterns obtained from the NTP studies should be helpful in understanding mechanisms of tumor response.

XVI. Review of Chemicals Nominated for NTP Studies: There were 13 chemical nominations considered by the Board. All had been reviewed previously by the NTP Chemical Evaluation Committee (CEC). (Summary data on the chemicals including CEC recommendations are provided in Attachment 4.) Dr. Gallo chaired the review. Dr. William Allaben, NCTR, Dr. Dorothy Canter, NIEHS, and Dr. Janet Haartz, NIOSH, CEC members, and Dr. Victor Fung, NIEHS, NTP Chemical Selection Coordinator, served as resource persons. Board members served as principal reviewers for one or two chemicals, and following their presentation and discussion of each chemical, motions were made and voted on.

The Board's recommendations for the 13 chemicals are summarized in Attachment 5.

[Billing Code 4140-01]

PUBLIC HEALTH SERVICE

NATIONAL TOXICOLOGY PROGRAM

NATIONAL TOXICOLOGY PROGRAM, BOARD OF SCIENTIFIC COUNSELORS' MEETING

Pursuant to Public Law 92-463, notice is hereby given of a meeting of the National Toxicology Program (NTP) Board of Scientific Counselors, U. S. Public Health Service, in the Conference Center, Building 101, South Campus, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina on November 14 and 15, 1988.

The meeting will be open to the public from 8:30 a.m. until adjournment on November 14. The preliminary agenda with approximate times are as follows:

Review of Cellular and Genetic Toxicology Branch (CGTB), Division of Toxicology Research and Testing, NIEHS

8:30 a.m.- 9:00 a.m. - Introduction

9:00 a.m.-12:15 p.m. - Current CGTB Programmatic Activities

Activities

1:00 p.m.- 3:15 p.m. - Initiatives on Non-Mutagenic Carcinogens

3:30 p.m.- 4:15 p.m. - Germ Cell Mutation Projects including Concept Review

4:15 p.m.-Adjournment - Intramural Research Projects

The meeting on November 15 will be open to the public from 8:30 a.m. to 12:00 noon. The preliminary agenda with approximate times are as follows:

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

8:45 a.m.- 9:00 a.m. - Update on Activities of the Technical Reports Review Subcommittee

9:00 a.m.- 9:30 a.m. - Status of NIEHS Oncogene Studies

9:30 a.m.-10:30 a.m. - CGTB Poster Session

10:30 a.m.-12:00 noon - Review of Chemicals Nominated for NTP Studies. Thirteen chemicals will be reviewed. Seven of the chemicals were evaluated by the NTP Chemical Evaluation Committee (CEC) on May 10, 1988, and are (with CAS Nos. in parenthesis): (1) β-Cadinene (523-47-7); (2) Diphenylamine (122-39-4); (3) Firemaster 680 (37853-59-1); (4) Isobutene (115-11-7); (5) Methacrylonitrile (126-98-7); (6) Phenylpropanelamine Hydrochloride (154-41-6); and (7) Trichloromelamine (7673-09-8, 12379-38-3). Six of the chemicals were evaluated by the CEC on July 27, 1988, and are: (1) Acrolein (107-02-8); (2) Acrylic Acid (79-10-7); (3) Aldicarb Oxime (1646-75-9); (4) Butanal Oxime (110-69-0); (5) Cyclohexanone Oxime (100-64-1); and (6) 1,1,2,2-Tetrabromoethane (79-27-6).

In accordance with the provisions set forth in section 552b(c)(6) Title 5 U. S. Code and section 10(d) of Public Law 92-463, the meeting will be closed to the public on November 14 from 7:45 a.m. to 8:30 a.m. and November 15 from 12 noon to 3:00 p.m. for further evaluation of research activities in the NIEHS Cellular and Genetic Toxicology Branch, including the consideration of personnel qualifications and performance, the competence of individual investigators, and similar items, the disclosure of which would constitute a clearly unwarranted invasion of personal privacy.

The Executive Secretary, Dr. Larry G. Hart, National Toxicology Program, P. O. Box 12233, Research Triangle Park, North Carolina 27709, telephone (919) 541-3971; FTS 629-3971, will have available a roster of Board

members and expert consultants and other program information prior to the meeting, and summary minutes subsequent to the meeting.

Dated: 10/5/88

[redacted]

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

ATTACHMENT 2

AGENDA

BOARD OF SCIENTIFIC COUNSELORS

NATIONAL TOXICOLOGY PROGRAM

November 14 and 15, 1988

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

Monday, November 14, 1988

CLOSED MEETING

7:45 a.m. - 8:30 a.m.	Evaluation of Personnel in the Cellular and Genetic Toxicology Branch, NIEHS	Board and Consultants

OPEN MEETING

Review of the Cellular and Genetic Toxicology Branch (CGTB)
Division of Toxicology Research and Testing, NIEHS

8:30 a.m. - 8:45 a.m.	Welcome	Dr. M. Gallo
8:45 a.m. - 9:00 a.m.	Introduction	Dr. D. Rall Dr. R. Griesemer
9:00 a.m. - 9:50 a.m.	Overview of Current Branch Programmatic Activities	Dr. R. Tennant
9:50 a.m. - 10:30 a.m.	Evaluation and Application of Current *In Vitro* Methodology	Dr. E. Zeiger
10:30 a.m. - 11:00 a.m.	Break	
11:00 a.m. - 11:30 a.m.	Evaluation and Application of *In Vivo* Systems	Dr. M. Shelby
11:30 a.m. - 11:35 a.m.	Introduction to Projects Under Development	Dr. R. Tennant
11:35 a.m. - 11:50 a.m.	Transgenic Mice Projects	Dr. L. Boone
11:50 a.m. - 12:05 p.m.	General Discussion	
12:05 p.m. - 1 p.m.	Lunch	
1:00 p.m. - 1:25 p.m.	Induction of Aneuploidy	Dr. M. Resnick
1:25 p.m. - 2:00 p.m.	Mammalian Cell Transformation Systems	Dr. J. Spalding

2:00 p.m. - 2:25 p.m.	Studies on Nonmutagenic Carcinogens	Dr. R. Langenbach
2:25 p.m. - 2:50 p.m.	Chemical Effects on DNA Methylation	Dr. W. Caspary
2:50 p.m. - 3:15 p.m.	General Discussion	
3:15 p.m. - 4:00 p.m.	Break/Viewing of Posters	
4:00 p.m. - 4:45 p.m.	Germ Cell Mutation Projects	Dr. M. Shelby
4:45 p.m. - 5:00 p.m.	Overview of Intramural Research Projects	Dr. R. Tennant

Tuesday, November 15, 1988

8:30 a.m. - 8:45 a.m.	Report of the Director, NTP	Dr. D. Rall
8:45 a.m. - 9:00 a.m.	Update on Activities of the Technical Reports Review Subcommittee	Dr. J. Huff
9:00 a.m. - 9:30 a.m.	Status of Oncogene Studies	Dr. R. Maronpot
9:30 a.m. - 10:30 a.m.	Break/Viewing of Posters	
10:30 a.m. - 12 noon	Review of Chemicals Nominated for NTP Studies	Board Dr. D. Canter
12:00 noon - 1:00 p.m.	Lunch	
		CLOSED MEETING
1:00 p.m. - 2:30 p.m.	Evaluation of Programs and Personnel in the Cellular and Genetic Toxicology Branch, NIEHS	Board and Consultants

Adjourn

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS

November 14-15, 1988

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CELLULAR AND GENETIC TOXICITY BRANCH, NIEHS

November 14-15, 1988

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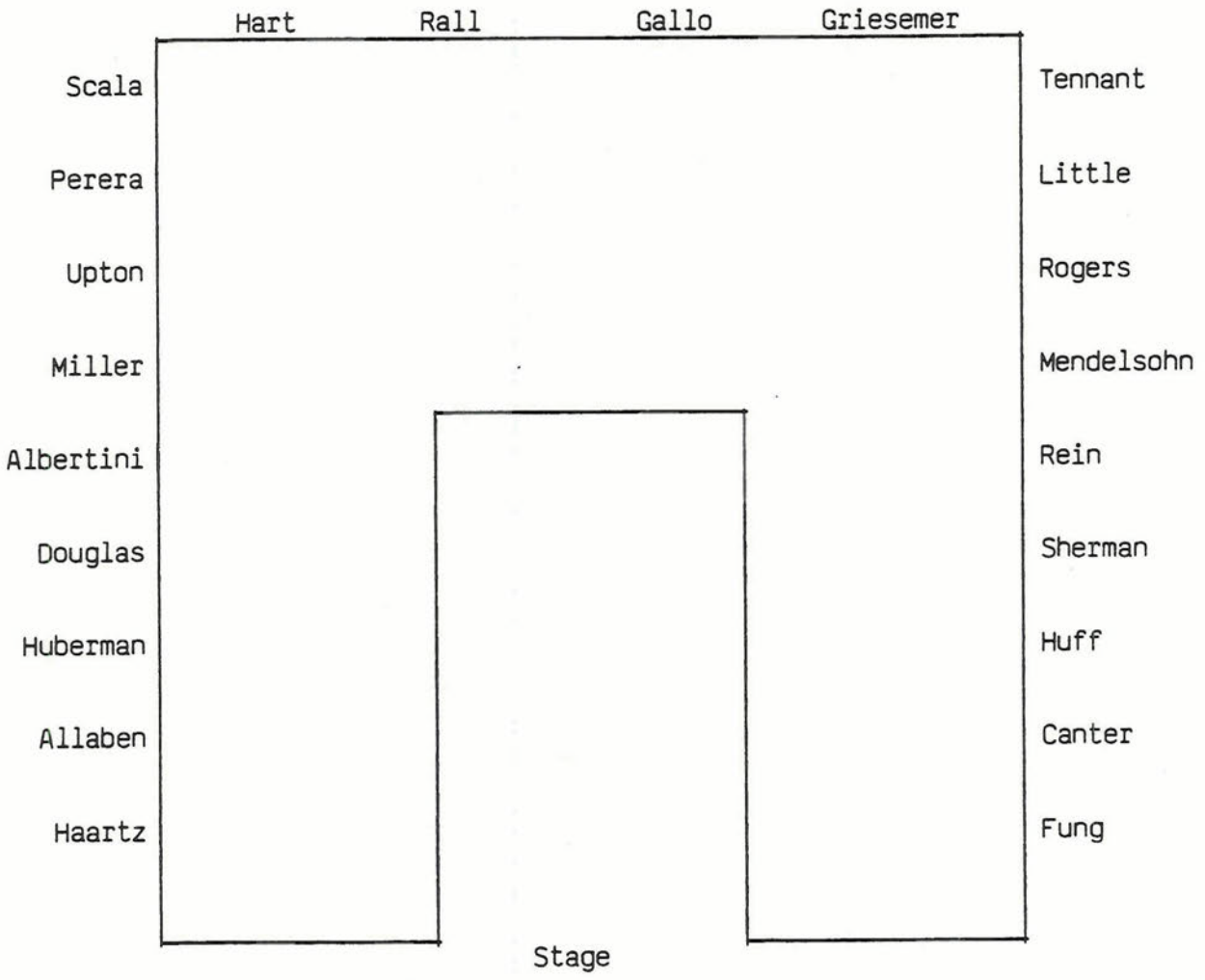
NTP BOARD OF SCIENTIFIC COUNSELORS MEETING

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

November 14-15, 1988



CELLULAR AND GENETIC TOXICOLOGY BRANCH

OVERVIEW MATERIAL

Prepared for:

National Toxicology Program
Board of Scientific Counselors Meeting

November 14 - 15, 1988

CELLULAR AND GENETIC TOXICOLOGY BRANCH - POSTER SESSION
NTP BOARD OF SCIENTIFIC COUNSELORS MEETING
NOVEMBER 15 - 9:30-10:30 A.M.
BUILDING 101 LOBBY

Abu-Shakra, A., and Zeiger, E. The mutagenic activity of hydrogen peroxide in Salmonella typhimurium.

Anderson, B. Procedures for conducting data audits of genetic toxicology assays.

Bennett, C., and Resnick, M. Consequences of unrepaired double-strand breaks in yeast.

Boone, L.R., Innes, C.L., and Glover, P.L. Development of a retrovirus packaging system to study Fv-1 restriction.

Daston, D.S., and Caspary, W.J. Spontaneous mutation in the mouse lymphoma (MOLY) cell assay.

Dresser, M. E., Tiano, H. F., Giroux, C.N. Genetic control of chromosome synapsis in yeast: The SPO11 gene but not the RAD52 gene is required for formation of the synaptonemal complex.

Heitman, C.K., Innes, C.L., Jetten, A.M., and Boone, L.R. Differential expression of the Fv-1 gene in fibroblasts derived from embryonal carcinoma cells.

Kari, F.W., Driscoll, S.M., Strom, S.C., Jenkins, W.L., Volosin, J.S., Rudo, K.M., and Langenbach, R.L. Comparative metabolism and genotoxicity of two structurally similar nitrophenylenediamines (HC blue 1 and HC blue 2) in mouse hepatocytes.

Langenbach, R., Crespi, C., Davies, R., Rudo, K., and Turner, T. Transfection of drug metabolizing genes as a method of increasing the metabolic capability of cells used in genotoxicity assays.

Langenbach, R., Spalding, J., Bohrman, J.S., Burg, J., Elmore, E., McGregor, D., and Toraason, M. An interlaboratory evaluation of 34 chemicals in the V79 metabolic cooperation assay.

Mason, J.M., Scobie, N.N., Yamamoto, A.H., and Champion, L. Genetic Characterization of the mei-41 locus of Drosophila melanogaster.

Pagano, D.A., and Zeiger, E. Autoxidation and mutagenicity in Salmonella of sodium bisulfite.

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Stark, A-A., Zeiger, E., and Pagano, D.A. Mechanisms of mutagenicity of glutathione and other thiols.

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CELLULAR AND GENETIC TOXICOLOGY BRANCH

OVERVIEW
1980-1988

The Cellular and Genetic Toxicology Branch (CGTB) was formed in 1980 in conjunction with the establishment of the National Toxicology Program (NTP) in the National Institute of Environmental Health Sciences (NIEHS). The mandate for the CGTB is the development, evaluation and application of short-term test (STT) methods that can be used to characterize the toxicity of chemicals, identify potential carcinogens and germ cell mutagens and reduce the dependence upon animal models currently used for those purposes. The CGTB developed around five functional groups (Table 1) and the number and identity of the senior staff has been essentially stable since the last Board of Scientific Counselors (BSC) review of the CGTB in 1984.

Evaluation of Short-term Assays

The primary recommendation arising from that BSC review was that the CGTB organize and conduct an in-depth evaluation of short-term in vitro genetic toxicity test methods for the detection of rodent carcinogens. This project was organized and initiated in the year following the review. The focus of the project was the inter-test comparison of four in vitro systems as prototypes of genetic toxicity assays to determine their ability to discriminate between rodent carcinogens and noncarcinogens. The four in vitro assays were selected for three principal reasons. First, they represented assays that had been developed over the previous years in contract laboratories by CGTB staff and they had been subject to rigorous validation with an emphasis on establishing protocols that yielded reproducible results both within and among the contract laboratories. Second, these were assays which were used in other laboratories throughout the world. Third, they were representative of many other in vitro assays proposed to for the identification of carcinogens; the endpoints included microbial and mammalian mutagenicity as well as cytogenetic changes.

In order to evaluate the four assays (mutagenesis in Salmonella and in mouse lymphoma (L5178Y) cells and chromosome aberrations (ABS) and sister chromatid exchanges (SCE) in Chinese hamster ovary (CHO) cells), 73 chemicals were selected that had been tested in chronic assays in rodents by the National Cancer Institute (NCI) and NTP. These chemicals were chosen only on the basis that the assays had been completed within a fixed period of time. The decision to limit the evaluation to chemicals tested by the NCI or the NTP was based on the following: 1) These assays had been conducted in a consistent manner using generally the same strains of mice and rats, a common protocol, "biologically active" doses ("maximum tolerated dose") of chemicals that allow for comparisons between assays; and 2) a sufficient number of animals had been exposed to active doses of chemical for a significant portion of their lifespan such that chemicals that showed no evidence of carcinogenicity could be identified. This latter issue was particularly important since a major impediment to previous

attempts to evaluate short-term tests (e.g. the EPA Genetox project) has been the inadequate number of noncarcinogens tested. By having comparable numbers of both carcinogens and noncarcinogens available for evaluation, it was possible not only to estimate the sensitivity (i.e., the proportion of carcinogens correctly identified) of each assay, or combination of assays, but to also estimate the specificity (i.e., the proportion of noncarcinogens correctly identified) and the concordance (i.e. the proportion of carcinogens and noncarcinogens correctly identified).

We developed several imortant procedures listed in Table 2 to assume a high quality of analysis. The results of the evaluation were published (Science: 236 (1987) copy enclosed) and have had a significant impact on the field of genetic toxicology. However, in order to challenge the conclusions from these 73 chemicals, we recently completed an evaluation of an additional 42 chemicals that have been tested in rodents since completion of the first evaluation. The results of this evaluation, presented in the enclosed summary, support the general conclusions of the study published in Science.

By virtue of sensitivity and specificity characteristics, the Salmonella mutagenesis assay has the best overall utility for detecting potential carcinogens. This utility is further established by the efficiency, relatively low cost, and worldwide use of this assay. The inability of the other assays to complement the Salmonella assay has some important implications. It supports the view that the assays are primarily confirmatory, i.e. they generally tend to detect properties of chemicals indicative of DNA interaction, and leads to the conclusion that, for routine screening, the other 3 assays or any combinations of the 4 assays, do not improve the predictivity for carcinogenicity of the Salmonella assay alone. The relatively low specificity of some of the assays can be viewed as a response to properties of some chemicals that do not relate to DNA interactions (e.g. cell membrane or effector molecule interactions, or dead cell effects).

Chemical Structure/Activity Considerations

In a further effort to understand more comprehensively the relationships between chemical structure, effects in the Salmonella assay, and patterns of rodent carcinogenicity, data from 222 chemicals were compiled and evaluated in collaboration with Dr. John Ashby (ICI, Ltd., England). The results of this evaluation (published in Mutation Research 204 (1988)) were the subject of a commentary by Dr. Alister Hay in Nature: 332: 782-783 (1988) (see copies enclosed). We believe that these results substantiate the following: 1) There is a high correlation between chemical structure (electrophilic groups/structural alerts) and a positive response in the Salmonella assay; 2) approximately 70% of chemicals mutagenic in Salmonella have the potential for inducing neoplasia in rodents; 3) approximately half of the rodent carcinogens lack an electrophilic structure and are negative in the Salmonella assay. Therefore, we propose that chemicals capable of electrophilic interaction with DNA (directly or via metabolism) are good candidates for genotoxic carcinogens. Operationally, genotoxic carcinogens can generally be identified based on electrophilic potential and a positive response in the Salmonella assay.

However, based upon NTP results, approximately 30% of chemicals identified as genotoxic are not carcinogenic in rodents. In addition, approximately 10% of chemicals show discordance between structural alerts and Salmonella results. We are, therefore, currently evaluating the ability of in vivo cytogenetic assays (micronucleus (MN) and chromosomal aberrations (ABS)) to complement the Salmonella assay in discriminating such chemicals. These systems are currently being evaluated using the group of 73 carcinogens and noncarcinogens tested previously under code.

Nongenotoxic Carcinogens

Identification of nongenotoxic carcinogens poses some difficult problems. Included in this group, could be a number of substances with the capacity to alter DNA indirectly or to alter chromosomal metabolism or mechanics. It is likely that these chemicals operate via several mechanisms to heritably alter cellular phenotypes. As will be discussed during the review, we have begun to evaluate several systems for their ability to distinguish between nonmutagenic carcinogens and noncarcinogens. Because of the potential diversity of possible mechanisms of action and the diversity and specificity of their neoplastic effects, it is unlikely that one or even a few in vitro methods will be sufficient to prospectively discriminate between these two categories of chemicals. A major and continuing effort is in the further development of mammalian cell transformation systems. The problems and progress on this project are described in a subsequent section. Projects directed to two other mechanisms of mutagenesis include chemically induced gene transposition in Drosophila and in mouse cells and induction of aneuploidy in Drosophila and yeast also are described in the next section.

Transgenic Mice

The development of the ability to physically induce specific genes into single cell mouse embryos has opened up significant opportunities to improve the characterization of the genetic effects of chemicals. The Branch has initiated two projects for the development and evaluation of transgenic mouse strains. The projects specifically relate to evaluation of chemical-oncogene interactions and development of strains for measurement of tissue specific somatic and germ cell mutagenesis. These projects are described in the next section.

Germ Cell Mutagenesis

Because a potentially serious consequence of chemical exposure is genetic damage transmitted to future generations, the Branch conducts a multi-component project directed to the identification and characterization of germ cell mutagens. The section describing this project also includes a concept statement describing a new approach and consolidation of effort.

TABLE 1

CELLULAR AND GENETIC TOXICOLOGY BRANCH

Raymond W. Tennant, Ph.D. Chief
Contract Coordinator (Mr. Stan Stasiewicz)
Secretary (Ms. Karen Cowardin)
Clerk-Typist, Part-time (Ms. Crystal Wynn)

Cancer Biology Group - Dr. Raymond Tennant, Leader

Coordinated Testing Group - Dr. Judson Spalding, Leader

Molecular Genetics Group - Dr. Lawrence Boone, Leader
Biologist (Ms. Katyna Borroto-Esoda)
Microbiologist (Ms. Cynthia Innes)
IRTA Fellow (Dr. Catherine Heitman)

Chemical Mutagenesis Group - Dr. William Caspary, Leader
Biologist (Ms. Diane Daston)

Carcinogen Metabolism Group - Dr. Robert Langenbach, Leader
Biologist (Dr. Ken Rudo)
Guest Researcher (Dr. Peter Smith)

Yeast Genetics/Molecular Biology Group - Dr. Michael Resnick, Leader
Biologist (Mr. James Westmoreland)
Visiting Fellow (Dr. Craig Bennett)
Guest Worker (Dr. Edward Perkins)

Senior Staff Fellow (Dr. Craig Giroux)
Biologist (Mr. Howard Tiano)
Biological Aid, Part-time (Mr. David Wiggins)
NRC Research Associateship (Dr. Michael Dresser)

Environmental Mutagenesis Group - Dr. Errol Zeiger, Leader
Microbiologist (Mr. Dennis Pagano)
Visiting Fellow (Dr. Amal Abu-Shakra)
Visiting Scientist (Dr. Avishay-Abraham Stark)
Contract Coordinator (Ms. Beth Anderson)

Drosophila Genetics - Dr. James Mason, Leader
Biologist (Mr. Larry Champion)
Visiting Fellow (Vacant)

Mammalian Mutagenesis Group - Dr. Michael Shelby, Leader
Research Geneticist (Dr. Jack Bishop)
Guest Worker (Dr. Kristine Witt)
Secretary (Ms. Sharon Bolen)

TABLE 2

CHARACTERISTICS OF THE NTP OBJECTIVE EVALUATION OF GENETIC TOXICOLOGY TESTS

- Use of defined protocols that have been peer reviewed and published
- Intra- and interlaboratory reproducibility
- Use of statistical inference in evaluation
- Contract testing laboratories selected by competitive process and reviewed periodically
- Chemicals tested under code
- Chemical selection - guided by NTP two-year studies
- Publication of raw data
- Confirmation of findings

Cellular and Genetic Toxicology Branch Highlights in 1988

- Overall, a significant achievement of the staff of the Branch has been to continue to **influence the field of genetic toxicology** to shift away from the tacit acceptance of any and all short-term tests toward the critical acceptance of a few select in vitro and in vivo assays as predictors of potential carcinogenicity. In recognition of their achievements, members of the Branch received a 'Special Achievement' award.

- **Publication of a major evaluation** of structure, mutagenicity and carcinogenicity of over 200 NTP chemicals and human carcinogens (Mutation Res. 204, 17-115, 1988). This publication was the subject of a commentary in Nature 322: 782 (1988).

- **Publication of 40 unique papers** (i.e. papers authored by Branch staff but counted only once if more than one staff member was an author), and publication of 12 unique papers from groups supported by contract from the Branch, but on which branch staff were not authors.

- **Completed a project to verify conclusions** reached on the evaluation of short-term in vitro assays that were published in 1987 (Science 236: 933-941).

- **Initiated three projects to develop transgenic mouse strains** for evaluation of chemical effects. This is a significant new scientific initiative with the potential to markedly improve our ability to study chemical effects.

- One of the laboratories under contract to the Branch **successfully transfected two human P450 genes and an epoxide hydrolase gene** into human lymphoblastoid cells to create a stable phenotype with enhanced metabolic capability for detecting promutagenic substances.

- Two laboratories under contract with the Branch have identified at least one mechanism by which chemicals can induce changes in chromosomal number (aneuploidy) in yeast cells. They have **identified a specific class of solvents that interfere with tubulin aggregation.**

- In the mammalian germ cell mutagenesis projects, **a new mouse model for a human genetic disease**, carbonic anhydrase deficiency syndrome, resulted from an ENU experiment in the electrophoretic specific locus assay (PNAS 85: 1962-1966, 1988). The model presents another opportunity to conduct detailed investigations of the molecular basis and therapies for a genetic disease. Second, high frequencies of visible specific locus mutants were observed in offspring derived from post-meiotic germ cells treated with **chlorambucil**, a nitrogen mustard-containing chemotherapy agent and human carcinogen. An induced frequency of more than 1000X the spontaneous frequency was observed in offspring conceived in the third week following paternal treatment, making feasible, in-depth studies of the mutagenesis process.

- Understanding of the mechanisms of recombination in yeast have been advanced by the joint efforts of Dr. Mike Resnick of the Branch staff and Dr. Akio Sugino (LG/DIR). They have **identified a protein involved in exchange of DNA between DNA duplexes.** In addition, Dr. Resnick and his group have characterized a nuclease that is involved in recombination and repair.

- Using a yeast model for germ line chromosome behavior and inheritance, Drs. Giroux and Dresser have **molecularly isolated the first meiosis specific gene**, SPO11, and shown that it is essential both for a proper meiotic chromosome behavior at the cytological level and for production of viable gametes (spores). This work is an important first step in being able to describe chromosome mechanics at the molecular level and, eventually, to understand how chemicals may interfere with the process.

- Dr. Larry Boone's Group has recently developed a retrovirus vector packaging system as a new approach to study the mechanism by which a mouse gene, Fv-1, blocks some preintegration event in the retrovirus/retrotransposon pathway. Some fundamentally important properties of Fv-1 are emerging from studies using this new system. Also, this group has demonstrated differential expression of Fv-1 in a cell culture system that responds to retinoic acid and other inducers of differentiation. This observation underlies the group's strategy for cloning and characterizing Fv-1. Understanding its mode of action has **intriguing possibilities for defining new antiviral targets and therapy strategies** for other retroviruses, possibly including HIV, the cause of AIDS.

- Dr. Jim Mason's group has recovered a number of chromosomes that are deleted for the naturally occurring telomeres. A telomere is the structure on each end of eukaryotic chromosome that is responsible for (a) identifying the chromosome terminus as distinct from the end of a broken chromosome, and (b) faithfully replicating the end of the linear chromosome. In collaboration with Dr. Harald Biessmann (UC Irvine) they have shown that the neotelomeres on the ends of the deleted chromosomes are losing DNA at a rate of 75 base pairs per generation (EMBO J 6: 1081-1086, 1988). The termini of the deleted chromosomes are being cloned and sequenced in order to identify possible structural components that convey the residual telomeric activity.

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Summary Data on Chemicals for Review by the NTP Board of Scientific Counselors
on November 15, 1988

Chemical (CAS Number)	Nomination Source	Domestic Production lbs.	Worker Exposure	NTP Testing Status	Other	Chemical Evaluation Committee Recommendation (Priority)	Chemical Selection Principles	Rationale/Remarks
I. Chemicals Reviewed by the Chemical Evaluation Committee on May 10, 1988								
1. ß-Cadinene (523-47-7)	NCI	No definitive production data found[a,b,c]	--	-Negative in Salmonella -Negative in mouse lymphoma -Negative for chromosomal aberrations and equivocal for sister chromatid exchanges in CHO cells	--	No testing	--	Lack of significant toxicity in previous studies
2. Diphenylamine (122-39-4)	NCI	Listed in TSCA Inventory but no production volume provided[a]	4,204[d]	-Negative in Salmonella	--	-Carcinogenicity -Reproductive effects (Moderate)	8	-Current use on apples -Potential for increased use as pesticide -Potential for human exposure -Evidence of toxicity from previous studies
3. Firemaster 680 (37853-59-1)	NIEHS	1.0×10^5-1.0×10^6 (1977)[a]	--	-Negative in Salmonella	--	-Chemical disposition -Subchronic studies (Low)	2,3,8	-Potential for increased use as flame-retardant -Subchronic studies pending results of chemical disposition studies -Several polybrominated biphenyls already tested by NTP

ATTACHMENT 4

Chemical (CAS Number)	Nomination Source	Domestic Production lbs.	Worker Exposure	NTP Testing Status	Other	Chemical Evaluation Committee Recommendation (Priority)	Chemical Selection Principles	Rationale/Remarks
4. Isobutene (115-11-7)	NCI	8.0×10^8-3.6×10^9 (1977)[a] 1.0×10^9 (1985)[b]	545[d]	--	--	-Toxicity studies -Carcinogenicity (High)	3,8	-Extensive use -Significant human exposure -Lack of toxicity and carcinogenicity data -Structural interest
5. Methacrylonitrile (126-98-7)	NCI	1.0×10^6-1.0×10^7 (1977)[a]	28[d]	-Negative in Salmonella in two independent studies -Negative for sex-linked recessive lethal mutations in Drosophila	--	-Chemical disposition -Carcinogenicity (High)	3,8	-High production and extensive use -Lack of chronic and carcinogenicity data -Structural similarity to acrylonitrile -Chemical disposition studies to precede carcinogenicity testing (determine extent of absorption and formation of cyanide)
6. Phenylpropanolamine hydrochloride (154-41-6)	NCI	4.7×10^5 (1984)[b]	--	--	--	-Salmonella -In vivo cytogenetics -Subchronic studies (High)	3,8	-Extensive pharmaceutical use in U.S. -Lack of chronic toxicity data -NTP has tested or is testing several structurally related compounds

Chemical (CAS Number)	Nomination Source	Domestic Production lbs.	Worker Exposure	NTP Testing Status	Other	Chemical Evaluation Committee Recommendation (Priority)	Chemical Selection Principles	Rationale/Remarks
7. Trichloro-melamine (7673-09-8)	NCI	Listed in TSCA Inventory but production volume not provided[a]	1,822[d]	-On test in Salmonella -Stability studies completed	--	-No testing	--	-NTP stability studies indicated that trichloromelamine is expected to metabolize to melamine in a biological medium; melamine already tested for carcinogenicity by NTP
II. Chemicals Reviewed by the Chemical Evaluation Commitee on July 27, 1988								
1. Acrolein (107-02-8)	NCI	$1.0\text{-}5.1\times10^{7}$ (1977)[a] 6.6×10^{7} (1985)[c]	64[d]	-Weakly positive in Salmonella -Negative for sex-linked recessive lethal mutations in Drosophila in two independent studies -Negative for chromosomal aberrations and sister chromatid exchanges in CHO cells	--	-Carcinogenicity (inhalation) (High) -Teratogenicity (Moderate to High)	3,8	-Widespread human exposure -Lack of adequate carcinogenicity and teratogenicity data -Suspicion of carcinogenicity as member of aliphatic aldehydes class
2. Acrylic acid (79-10-7)	NCI	$2.2\text{-}11.1\times10^{8}$ (1977)[a] 9.7×10^{8} (1986)[b]	6,041[d]	-Negative in Salmonella	-Drinking water carcinogenicity study in rats in progress[e]	-Carcinogenicity (Inhalation) (Moderate)	3,4,5,8	-Potential for worker exposure -Lack of carcinogenicity data

Chemical (CAS Number)	Nomination Source	Domestic Production lbs.	Worker Exposure	NTP Testing Status	Other	Chemical Evaluation Committee Recommendation (Priority)	Chemical Selection Principles	Rationale/Remarks
3. Aldicarb oxime (1646-75-9)	NCI	$1.0x10.0x10^6$ $1.0-10.0x10^6$	--	-Negative in Salmonella -Negative for chromosomal aberrations and sister chromatid exchanges in CHO cells in two independent studies	--	-Immunotoxicity (Moderate to high)	3	-Lack of toxicity data -Scientific interest
4. Butanal oxime (110-69-0)	NCI	$1x10^5$-$1x10^6$ (1977)[a]	7,627[d]	--	--	-Carcinogenicity (Moderate)	3	-Potential for worker exposure -Scientific interest in toxicity of oximes -Representative acyclic oxime
5. Cyclohexanone oxime (100-64-1)	NCI	$2.0x10^8$-$1.0x10^9$ (1977)[a]	--	--	--	-Carcinogenicity (Low to moderate)	3	-High production -Scientific interest in toxicity of oximes -Representative alicyclic oxime
6. 1,1,2,2-Tetrabromoethane (79-27-6)	NCI	$1.0x10^6$-$1.0x10^7$ (1977)[a] $2.0x10^6$ (1987)[f]	419[d]	--	--	-Carcinogenicity (Inhalation) (Moderate)	3,8	-High production and use -Scientific interest in haloalkanes

Footnotes

a) U.S. EPA Toxic Substances Control Act (TSCA) Inventory of Chemicals in Commerce, public file

b) U.S. International Trade Commission, Synthetic Organic Chemicals, United States Production and Sales

c) SRI International, Chemical Economics Handbook

d) National Occupational Exposure Survey (1980-1983), National Institute for Occupational Safety and Health

e) Information provided by Drs. Dietrich and Gelbke, BASF, to Dr. D. Canter, NTP. Letter dated April 14, 1988.

f) Information provided by Dr. G. Ter Haar, Ethyl Corporation, to Dr. D. Canter, NTP. July 13, 1988.

NTP CHEMICAL SELECTION PRINCIPLES

The NTP Executive Committee operates under the principle that industry will test chemicals for health and environmental effects as intended and mandated by the Congress under legislative authorities. Therefore, the NTP, acting under its chemical selection principles, will test:

1. Chemicals found in the environment that are not closely associated with commercial activities;

2. Desirable substitutes for existing chemicals, particularly therapeutic agents, that might not be developed or tested without Federal involvement;

3. Chemicals that should be tested to improve scientific understanding of structure-activity relationships and thereby assist in defining groups of chemicals that should be tested by industry;

4. Certain chemicals tested by industry, or by others, the additional testing of which by the Federal Government is justified to verify the results;

5. Previously tested chemicals for which other testing is desirable to cross-compare testing methods;

6. "Old chemicals" with the potential of significant human exposure which are of social importance but which generate too little revenue to support an adequate testing program (some of these may be "grandfathered" under FDA laws);

7. Two or more chemicals together, when combined human exposure occurs (such testing probably cannot be required of industry if the products of different companies are involved); and

8. In special situations, as determined by the Executive Committee, marketed chemicals which have potential for large-scale and/or intense human exposure, even if it may be possible to require industry to perform the testing.

The selection of a chemical by the Executive Committee does not automatically commit the NTP to testing the chemical. The NTP is committed to ascertain the specific toxicologic and regulatory concerns; evaluate the adequacy of existing data or current efforts in government, academic, or private laboratories; and then propose and conduct specific tests that are needed. Occasionally new information is obtained that answers the questions posed in the nomination and selection process. Sometimes testing is not done because chemicals are withdrawn by the nominator, because others are or will be testing the chemical, or because the chemical is not available, or no longer produced.

Testing Recommendations for Chemicals Reviewed by Board of Scientific Counselors on November 15, 1988

Chemical (CAS Number)	Nomination Source	Testing Recommendations (Priority)	Rationale/Remarks
I. Chemicals Reviewed by the Chemical Evaluation Committee on May 10, 1988			
1. β-Cadinene (523-47-7)	NCI	- No testing	- Not used in significant amounts - Data on structure activity relationships not persuasive
2. Diphenylamine (122-39-4)	NCI	- Carcinogenicity - Reproductive effects (High)	- Potential for human exposure - Used as antioxidant in vide range of products - Test commercially available product - Contamination by 4-aminobiphenyl is significant issue
3. Firemaster 680 (37853-59-1)	NIEHS	- Chemical disposition - Subchronic studies (Low)	- Potential for increased use - Potential for bioaccumulation based upon lipophilicity - Structurally related to other polybrominated biphenyls tested by NTP - Subchronic studies pending results of chemical disposition studies
4. Isobutene (115-11-7)	NCI	- Genotoxicity - Inhalation toxicity and carcinogenicity studies (High) - Reproductive effects (Moderate)	- Widespread use - Significant human exposure - Lack of toxicity and carcinogenicity data - Structural interest

ATTACHMENT 5

Chemical (CAS Number)	Nomination Source	Testing Recommendations (Priority)	Rationale/Remarks
5. Methacrylonitrile (126-98-7)	NCI	- Chemical disposition - Carcinogenicity (High) - Reproductive effects (Moderate)	- High production and exposure - Component in many plastics - Lack of chronic toxicity data - Concern about reproductive toxicity based on potential for cyanide formation
6. Phenylpropanolamine hydrochloride (154-41-6)	NCI	- Salmonella - In vivo cytogenetics - Subchronic studies - Reproductive effects (High)	- Significant pharmaceutical use - Lack of chronic toxicity data - Structural interest - Reproductive effects studies subject to literature review
7. Trichloromelamine (7673-09-8)	NCI	- No testing	- NTP stability studies indicated that trichloromelamine would be transformed to melamine in a biological medium; NTP has already tested melamine for carcinogenicity
II. Chemicals Reviewed by the Chemical Evaluation Committee on July 27, 1988			
1. Acrolein (107-02-7)	NCI	- Inhalation carcinogenicity - Reproductive effects (Moderate to high)	- Significant human exposure - Lack of adequate data on carcinogenicity and teratogenicity - Review ongoing industry studies prior to finalizing testing decisions

	Chemical (CAS Number)	Nomination Source	Testing Recommendations (Priority)	Rationale/Remarks
2.	Acrylic acid (79-10-7)	NCI	- Inhalation carcinogenicity - Teratogenicity and reproductive effects studies (Moderate)	- Potential for worker exposure - Lack of carcinogenicity data - Review ongoing industry drinking water chronic study in rats prior to designing carcinogenicity studies - Reproductive effects testing dependent on evaluation of industry inhalation reproductive study - Industry pharmacokinetics studies by inhalation, dermal, and oral routes in progress
3.	Aldicarb oxime (1646-75-9)	NCI	- Defer	- Consideration for testing deferred pending results of toxicological studies on butanal oxime and cyclohexanone oxime - Provide Board with rationale for immunotoxicity testing at next meeting
4.	Butanal oxime (110-69-0)	NCI	- Subchronic studies (High) - Carcinogenicity - Reproductive and developmental toxicity (Moderate)	- Potential for exposure - Structural interest - Perform subchronic studies of butanal oxime and cyclohexanone oxime in tandem
5.	Cyclohexanone oxime (100-64-1)	NCI	- Subchronic studies (High) - Carcinogenicity (Low to moderate)	- High production - Structural interest - Perform subchronic studies of butanal oxime and cyclohexanone oxime in tandem
6.	1,1,2,2-Tetrabromoethane (79-26-6)	NCI	- Genotoxicity - Carcinogenicity (High) - Reproductive effects (Moderate to high)	- High production and use - Structural interest; several haloalkanes were positive in NTP carcinogenicity studies - Reproductive effects studies subject to literature review