

NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS
OCTOBER 29 AND 30, 1985

SUMMARY MINUTES

National Toxicology Program
Board of Scientific Counselors Meeting
October 29-30, 1985

Summary Minutes

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NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS MEETING
OCTOBER 29 AND 30, 1985

Summary Minutes

The National Toxicology Program (NTP) Board of Scientific Counselors met on October 29 and 30, 1985, 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. James Swenberg (Chairperson), Norman Breslow, Michael Gallo, Jerry Hook, Jeanne Manson, Mortimer Mendelsohn, Frederica Perera, and Henry Pitot. Dr. Hook was unable to attend the meeting.

Review of NIEHS/NTP Chemical Pathology Branch Programs

I. Overview: Dr. Gary Boorman, Branch Chief, briefly described the history of the pathology programs at NIEHS including involvement in the NTP beginning in 1978, and the background and special expertise of the professional staff. The Branch is organized into five units: electron microscopy and histology; tumor pathology; toxicologic pathology; experimental pathology; and laboratory animal management. Dr. Boorman stated there were three broad themes or goals of the Branch: (1) to improve quality assessment and validation of the pathology data from the rodent studies to assure that the data for which the potential toxicity and carcinogenicity of a chemical is evaluated is sound and accurate; (2) to make greater use of existing resources, e.g., the archives, to conduct more retrospective and in depth research studies; and (3) to attempt to better understand the natural history and biology of the tumor and non-tumor lesions observed using state-of-the-art techniques, e.g., immunoperoxidase assay, and nuclear magnetic imaging. He concluded by reporting on major research activities including research on myelotoxicity of environmental chemicals (in collaboration with the NIEHS/NTP Immunotoxicology Section), research on the effect of corn oil on the pancreas, and in-house studies on methylisocyanate and methyl bromide.

II. Tumor Pathology: Dr. Scot Eustis, Section Head, noted that 80 to 90% of the group's efforts are devoted to supporting the scientific activities of the Toxicology Research and Testing Program which is the NIEHS component of the NTP. A major part of their activity is involved with validating diagnoses and coordinating and tracking the flow of pathology materials and data from completion of the in-life study to preparation of the NTP Technical Report including entry into the computerized data base systems used by the NTP. The primary objectives of the Section are (1) to ensure the thoroughness and accuracy of the pathology data from chronic studies through a multi-stage review process, and (2) to ensure that the criteria used for diagnosis and evaluation of lesions are up-to-date and reflect

current knowledge regarding biological behavior of the lesions. Dr. Eustis discussed the stages of pathology data review culminating with Pathology

Working Group (PWG) review and resolution of diagnostic discrepancies and evaluation of treatment related lesions. With regard to (2), the materials in the archives have been used to conduct retrospective studies on specific types of tumors followed by sponsorship of international conferences to share information derived, e.g., on tumors of the exocrine pancreas, brain and ovary. Prospective goals of the Section are (1) to put the responsibility for data auditing and quality assurance back with the contractor laboratories, and (2) to exert a better effort to educate and provide guidelines to the contractor laboratory pathologists, e.g., appropriate diagnostic terminology.

There was support by the Board members and ad hoc reviewers for putting more responsibility on the contractor laboratories in data auditing and quality assurance but concern expressed that there be adequate monitoring by the NTP. They also supported use of the archives for indepth retrospective studies and called for more such studies.

III. Toxicologic Pathology: Dr. Charles Montgomery, Section Head, said their primary responsibility was the management and support of the anatomic pathology aspects of the acute, subchronic, and interim sacrifice studies conducted by the NTP. He noted that 125 studies had been reviewed since 1981. Other responsibilities include: (1) developing and managing the pathology portion of the Toxicology Data Management System (TDMS); (2) conducting collaborative and independent research; and (3) providing diagnostic pathology and training. He stated that information developed in the subchronic PWG had contributed to the decisions to go to three doses in chronic studies as well as decisions to initiate interim sacrifice and stop exposure studies. Dr. Montgomery described the computerization of pathology data.

Dr. Montgomery described several areas of independent research in the Section, including: (1) the renal pathology of chlorinated aliphatic chemicals; (2) the ovary as target organ for various chemicals in prechronic and interim sacrifice studies; (3) studies, using the archives as a resource, on gall bladder lesions and chordomas as well as using an immunoperoxidase technique to detect cell surface markers in mouse lymphomas. Future activities include a predominant emphasis on continuing pathology support of NTP toxicology and carcinogenesis studies, somewhat more time for research as a result of transfer of data computerization to the Tumor Pathology Section, and continuing review of the first 100 NTP studies with attempts to correlate nonneoplastic and neoplastic lesions in target organs and tissues. Discussion by the peer reviewers focused on the roles of in-house pathology contrasted with what can be done by pathologists in the contract laboratories, and on how the subchronic data is used in dose selection and design of the chronic studies.

IV. Laboratory Animal Management: Dr. Ghanta Rao, Section Head, said his program supervises and directs under contract production of genetically and microbiologically defined B6C3F₁ mice and F344 rats for NIEHS/NTP toxicology and carcinogenicity studies. He described the contracts that support rodent production, monitoring for rodent diseases, genetic monitoring, and

diet production and analyses. Monitoring of animal care in the toxicology studies is carried out by site visiting each of the toxicology testing laboratories at least once a year and evaluating the animal care and health by reviewing the procedures, inspecting the facility, and examining animals on test. Monthly reports from the laboratories are reviewed to assess further the quality of animal care and management. Diseases are investigated through services of two diagnostic laboratory contracts. A continuing goal is to reduce the prevalence of viral and microbial infections in rodents on the studies. Laboratory Animal Management participates in review of proposals and site visits for new laboratories, technical evaluation of proposals for prechronic and chronic studies, revision of the NTP General Statement of Work, and assisting and providing expertise to the laboratories and to NTP staff.

Dr. Rao spoke about recent workshops sponsored by Laboratory Animal Management which in part responded to recommendations in the report of the NTP Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation. One had to do with evaluating strengths and weaknesses of various strains of mice for toxicology and carcinogenesis studies concluding that the B6C3F₁ mouse was still the best model since a better alternative could not be identified. Other workshops concerned the use of hamsters as an alternative species and the role of animal diet in toxicology and carcinogenesis studies.

Future plans include continuation of primary activities in monitoring animal care at the testing laboratories and producing quality animals for the studies. Enhancing control and prevention of infections in the rodents will be a challenge. The hamster model will be evaluated, and various strains of mice and various types of diets will be evaluated for usefulness in toxicology and carcinogenesis studies.

V. Experimental Pathology: Dr. Robert Maronpot, Section Head, reported that the three major activities of the Section are (1) to support the NTP toxicology programs, (2) to provide diagnostic pathology and clinical pathology support for NIEHS scientists, and (3) to conduct independent research. Elaborating on the research activity to emphasized that the projects studied were considered to be relevant to program needs and primarily collaborative in nature. Underlying motivation for the research is to understand the biology of the toxicologic responses observed in NTP studies.

Among major research areas, first are the studies with in vivo rat liver tumor models, currently the partial hepatectomy (PH) model and the neonatal model. With the PH model, six different initiators have been used along with phenobarbital as a promoter to study oncogene activation and expression in the induced livers. An initiation-promotion-initiation model is being used to study the 2-hit hypothesis for the mechanism of chemical carcinogenesis. The promotional activity of sodium dodecyl sulfate was investigated in the neonatal model. Under three contracts, the two models are being refined, e.g., effects of diet, rat strain and sex on liver tumor response will be evaluated, and, subsequently, selected chemicals will be evaluated in the refined models. Dr. Maronpot discussed an in-house collaborative study investigating oncogene activation and expression in spontaneous and chemically induced rodent tumors. He said the NTP long-term studies provided a unique resource for obtaining various types of tumors.

Discussion by the Board and ad hoc reviewers was concerned with refinements in liver tumor models, whether or why other initiation/promotion models were not being used, future directions of the oncogene studies, and peer review of in-house projects.

VI. Clinical Pathology and NMR Studies: Dr. Morrow Thompson elaborated on three major objectives of the clinical pathology discipline, other than continuing direct support of NTP rodent studies. First is standardization of clinical pathology techniques. He discussed two factors in sample collection, bleeding site and type of anesthetic, which can affect or introduce variability into hematologic and clinical chemical values measured. Second is optimization of the application of techniques in the studies which means using the most appropriate test, e.g., total bile acids are more sensitive indicators of hepatobiliary function following animal exposure to hepatotoxins than are serum enzymes, and the most appropriate time, often in life rather than at terminal sacrifice. Third is evaluation and interpretation of the data from NTP studies. The evaluation is usually of unaudited data taken during the study while problems can still be corrected. An interpretation of the clinical pathology data from the completed study will be written and included in the final technical report.

Discussion by the Board and ad hoc reviewers centered on how clinical pathology data could be used in the absence of morphologic changes or clinical abnormalities, or how such data could or should be integrated with other data from prechronic studies, and on questions about indicators of immunologic, endocrine or genetic toxicologic effects.

Dr. Thompson then discussed a collaborative project with the Radiology Department, Duke University Medical Center, to explore the uses of nuclear magnetic resonance (NMR) imaging in toxicology studies. After describing the physics of the system he talked about ongoing projects using the NMR to look at biological samples. He said they hoped to be able to detect pre-neoplastic foci (liver) before they can be detected by other means, and to follow regression/progression of neoplastic lesions. In following discussion, he was cautioned to define carefully the questions to be asked and seek peer review in protocol design so as to optimize the use of this unique analytical system.

VII. Report of the Director, NTP: Dr. David Rall reported that: (1) the conference on quality assurance entitled "Managing Conduct and Data Quality of Toxicology Studies" cosponsored by the NTP, American Industrial Health Council and other industry trade groups was to be held on November 18-20, 1985, in Raleigh, N. C. Dr. Frank Press, President, National Academy of Sciences, was to be keynote speaker; (2) the Technical Reports Review Subcommittee (Peer Review Panel) of the Board will meet on December 9, 1985, at NIEHS to review draft technical reports on the toxicology and carcinogenesis studies of ampicillin trihydrate, chlorpheniramine maleate, dimethylvinyl chloride, methyl methacrylate, oxytetracycline hydrochloride, and trichloroethylene; (3) the FY 1985 NTP Annual Plan has been printed and is being distributed; (4) the FY 1986 House and Senate appropriations bills for the NIH have passed, and the NIEHS seems to have fared fairly well. However, should the Deficit Reduction Act pass, substantial budgetary cuts might be effected; (5) the concern the Board had with the proposed methodol-

ogy in the concept proposal for an NTP/EPA interagency agreement, "Design for the Testing Phase of a Retrospective Study of PMN Health Hazard Predictions," reviewed on May 1, 1985, had been resolved and there would be a progress report on this project at the next Board meeting. Dr. Ernest McConnell, NIEHS, discussed his trip to Moscow as head of a scientific delegation investigating contamination and possible health effects on American diplomatic staff and their families of the chemical dust, NPPD, used as a surveillance agent.

VIII. Status Report on Reproductive and Developmental Toxicology Program Review Subcommittee Activities: Dr. James Lamb, NIEHS, said the Subcommittee (now chartered as a standing Subcommittee of the Board) held its first open meeting on September 27, 1985, in Cincinnati. Dr. Jeanne Manson is the Chair, and the membership comes from academia, industry and government. Among the Subcommittee's activities are concept review of new project proposals, review of technical proposals for contract renewal, review of draft reports and review of inhouse research. In summary, the group gives valuable feedback on the research and testing protocols at the three agencies.

IX. Strains of Mice for Chemical Carcinogenicity Studies: (Attachment 3) Dr. Rao, Section Head, Laboratory Animal Management, said a workshop on this subject was organized, in part in response to a recommendation of the Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation that the NTP "give serious consideration to replacement of the B6C3F₁ mouse with a strain having an established lower and less variable spontaneous incidence of important tumors that are induced by chemicals." Presentations at the workshop reviewed historical control tumor incidences for B6C3F₁ (B6) mice, compared tumor incidences for different sites and at different ages for B6 mice and other strains, and compared survival rates for other mouse strains with the B6. The conclusions evolving from the workshop were that: (1) there is no acceptable replacement for the B6 mouse at this time; (2) more information should be developed on liver tumor susceptibility of the B6; (3) other mouse hybrids should be further studied; and (4) an adequate data base should be developed with other rodents such as the hamster to use as a substitute where metabolism of a chemical would indicate an advantage. In discussion, the Board supported continuing use of the B6C3F₁ mouse, while continuing to evaluate other strains and species.

X. NIEHS/NTP Concept Review - Mouse Strain Differences in Hepatocarcinogenesis: (Attachment 4) Dr. Jeffrey Collins, Carcinogenesis and Toxicology Evaluation Branch, stated that the proposed project would be an experimental follow-up to the workshop described by Dr. Rao. The objective of the proposal will be to evaluate possible strain differences in hepatocarcinogenesis in genetically defined mice, particularly those related to the B6C3F₁ mouse. Specifically, two known liver carcinogens, will be used in parallel conditions in the various strains.

In a lengthy discussion, the Board raised several concerns, especially: (1) the unknown variability resulting from animal diet; (2) the need for data on the relative cross-strain biotransformation of the chemicals; (3) questions as to whether the two chemicals proposed (1,1,2,2-tetrachloroethane and 2,6-dichloro-p-phenylenediamine) were the

most appropriate; and (4) the projected cost is considerable - could the number of biologic endpoints measured be reduced, and perhaps the length of the studies? There was a consensus for approving the concept contingent on resolving these concerns. Dr. Gallo moved to accept the concept. Dr. Breslow seconded the motion and it was accepted by 4 affirmative to 2 negative (Dr. Manson, Dr. Pitot) votes. The NTP should revise the concept to address questions raised and return this to the Board either by mail or at the next Board meeting.

XI. Discussion of Levels of Evidence of Carcinogenicity: (Attachment 5) Dr. James Huff, TRTP, NIEHS, reported the five levels of evidence of carcinogenicity were an attempt by the NTP to provide descriptors which can be used to interpret the findings from long-term toxicology and carcinogenesis studies in rodents. They have been used for more than two years (since June 1983) to describe the results from 42 studies, and as requested the "levels of evidence" are being brought back to the Board for further evaluation. Primary modification proposed by NTP staff would be to add a preamble or explanatory introductory paragraph to assist Peer Review Panel members who review the draft Technical Reports as well as to promote further understanding for those who use them. He concluded by asking for the Board's comments and endorsement of their continued use.

In discussion by the Board, there was agreement that a preamble would be helpful. A major issue had to do with whether benign tumors by themselves were appropriate for classifying results as clear evidence of carcinogenicity; for example, benign tumors having no malignant counterpart or where there was no evidence of progression from benign to malignant would more likely fit into some evidence of carcinogenicity. Other discussion focused on the specific terminology used and on issues or influences which might be included in the new preamble, e.g., evidence of metastases, and whether or not the treatment decreased the average time-to-tumor (latency). The NTP will incorporate appropriate suggestions and bring a revised draft to the Peer Review Panel for discussion at their next meeting on December 9, 1985. Subsequently, a final draft will be brought back to the Board at their next meeting.

XII. Peer Review and Priority Ranking of Chemicals Nominated for NTP Testing: There were seven chemical nominations to be considered by the Board (Attachment 6). All had been reviewed previously by the NTP Chemical Evaluation Committee (CEC). Dr. Swenberg chaired the review and Dr. Dorothy Canter, NIEHS, and Dr. Barry Johnson, NIOSH, members of the CEC, and Dr. Victor Fung, NIEHS, NTP Chemical Selection Coordinator, served as resource persons. Each Board member had been asked to serve as principal reviewer for one chemical. As before, following oral presentation of each review and discussion, a motion was made and voted on by the Board members.

Of the seven nominations, two, ellagic acid and alpha terpineol, were nominated by the National Cancer Institute as a result of a class study on wood chemicals, and were reviewed by the CEC on February 5, 1985. The remaining five chemicals consisting of two glycol ethers (2-ethoxyethanol and 2-methoxyethanol) and three glycol ether acetates (2-butoxyethanol acetate, 2-ethoxyethanol acetate, and 2-methoxyethanol acetate) were nominated by the UAW International Union for mutagenicity and carcinogenicity testing. The

CEC reviewed these five chemicals on July 30, 1985. During discussion, it was noted that 2-butoxyethanol was not included because the Consumer Product Safety Commission had already designated this chemical as its priority chemical selection for FY 1984. The Executive Committee selected this chemical for carcinogenicity testing on March 7, 1985.

The Board's recommendations, priority for testing, and additional remarks and/or caveats for the seven chemicals reviewed are summarized in Attachment 7.

program listed in the *Catalog of Federal Domestic Assistance* are affected.

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 BILLING CODE 4160-01-01

Public Health Service

National Toxicology Program, Board of Scientific Counselors Meeting

Pursuant to Pub. L. 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, Research Triangle Park, North Carolina, on October 29 and 30, 1985.

The meeting will be open to the public from 9:00 a.m. until adjournment on October 29. The preliminary agenda with approximate times are as follows:

Review of NIEHS/NTP Chemical Pathology Branch Program:

9:00 a.m.-12:00 noon—Overview and presentations on intramural and extramural projects in tumor pathology and toxicologic pathology.

1:00 p.m.-4:30 p.m.—Presentation on intramural and extramural projects in laboratory animal management and experimental pathology. Concluding remarks.

The meeting on October 30 will be open to the public from 8:30 a.m. to 12:15 p.m. 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.—Status Report on Reproductive and Developmental Toxicology Program Review Subcommittee Activity.

10:00 a.m.-10:00 a.m.—NIEHS/NTP Concept Reviews.

10:15 a.m.-10:45 a.m.—Discussion of Levels of Evidence of Carcinogenicity.

10:45 a.m.-12:15 p.m.—Peer Review and Priority Ranking of Chemicals Nominated for NTP Testing. (Seven chemicals will be reviewed. Two, ellagic acid and alpha-terpineol, were nominated as a result of a class study on Wood Chemicals and Associated Industries, and are listed in the *Federal Register*, Volume 50, No. 66, p. 13666, April 5, 1985. Five chemicals are members of the class of glycol ethers and acetates, being: 2-ethoxyethanol; 2-methoxyethanol; 2-butoxyethanol acetate; 2-ethoxyethanol acetate; and 2-methoxyethanol acetate.)

In accordance with the provisions set forth in section 552b(c)(6) Title 5 U.S. Code and section 10(d) of Pub. L. 92-463,

the meeting will be closed to the public on October 30 from approximately 1:00 p.m. to adjournment for further evaluation of NIEHS/NTP programs in chemical pathology, 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, Office of the Director, National Toxicology Program, P.O. Box 12233, Research Triangle Park, North Carolina 27702, 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: September 25, 1985.

David P. Rail,

Director, National Toxicology Program.

[FR Doc. 85-24393 Filed 10-10-85; 9:45 am]

BILLING CODE 4160-17-01

DEPARTMENT OF THE INTERIOR

Bureau of Land Management

Arizona; Filing of Plats of Survey

1. The plats of survey of the following described lands were officially filed in the Arizona State Office, Phoenix, Arizona, on the dates indicated:

A supplemental plat showing subdivision or original lots 1, 2 and 3 in section 4, Township 7 North, Range 2 East, Gila and Salt River Meridian, Arizona, was accepted August 13, 1985 and was officially filed August 14, 1985.

A plat representing a survey of Tract 37 in Township 8 North, Range 15 West, Gila and Salt River Meridian, Arizona, was accepted on September 13, 1985 and was officially filed September 13, 1985.

A plat representing a survey of Tract 37 in Township 9 North, Range 13 West, Gila and Salt River Meridian, Arizona, was accepted on September 13, 1985 and was officially filed September 13, 1985.

These plats were prepared at the request of the Bureau of Land Management, Phoenix District Office.

A supplemental plat showing amended lottings in section 27, Township 4 North, Range 3 East, Gila and Salt River Meridian, Arizona, was accepted July 2, 1985 and was officially filed July 3, 1985.

A plat representing a dependent resurvey of a portion of the south boundary (First Standard Paralled

South) and a portion of the subdivisional lines of Township 5 South, Range 22 East, Gila and Salt River Meridian, Arizona, was accepted July 24, 1985 and was officially filed July 26, 1985.

These plats were prepared at the request of the Bureau of Land Management, Arizona State Office, Branch of Lands and Minerals Operations.

A plat representing a dependent resurvey of portions of the south and north boundaries and the east boundary of section 7 and a survey of the subdivision of section 7 and the metes-and-bounds survey of lot 6, Section 7, Township 30 North, Range 7 East, Gila and Salt River Meridian, Arizona, was accepted July 24, 1985 and was officially filed July 26, 1985.

This plat was prepared at the request of the Bureau of Land Management, Arizona Strip District.

A plat representing a dependent resurvey of the east boundary of Tract 37 and a survey of Tract 38 in unsurveyed Township 3 North, Range 13 East, Gila and Salt River Meridian, Arizona, was accepted July 29, 1985 and was officially filed July 31, 1985.

A supplemental plat showing a subdivision of original lot 1, section 5, Township 10 North, Range 10 East, Gila and Salt River Meridian, Arizona, was accepted August 8, 1985 and was officially filed August 9, 1985.

These plats were prepared at the request of the U.S. Forest Service, Region 3.

A plat (in two sheets) representing a dependent resurvey of portions of the north and west boundaries, and subdivisional lines, and a survey of the subdivisions in sections 5 and 30, Township 17 North, Range 6 East, Gila and Salt River Meridian, Arizona, was accepted September 23, 1985 and was officially filed September 25, 1985.

A plat representing a dependent resurvey of a portion of the north and east township boundaries and subdivisional lines, and a survey of the subdivisions in section 1, Township 18 North, Range 6 West, Gila and Salt River Meridian, Arizona, was accepted August 2, 1985 and was officially filed August 6, 1985.

These plats were prepared at the request of the U.S. Forest Service, Coconino National Forest and Prescott National Forest, respectively.

2. These plats will immediately become the basic records for describing

AGENDA

BOARD OF SCIENTIFIC COUNSELORS
NATIONAL TOXICOLOGY PROGRAM
OCTOBER 29 AND 30, 1985

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

Tuesday, October 29, 1985

Open

Review of NIEHS/NTP Chemical Pathology Branch

9:00 a.m. - 9:30 a.m.	Overview of Branch Activities	Dr. G. A. Boorman
9:30 a.m. - 10:30 a.m.	Tumor Pathology	Dr. S. L. Eustis
10:30 a.m. - 10:45 a.m.	Break	
10:45 a.m. - 11:45 a.m.	Toxicologic Pathology	Dr. C. A. Montgomery
11:45 a.m. - 12:00 noon	Discussion	
12:00 noon - 1:00 p.m.	Lunch	
1:00 p.m. - 2:00 p.m.	Laboratory Animal Management	Dr. G. N. Rao
2:00 p.m. - 2:45 p.m.	Experimental Pathology	Dr. R. R. Maronpot
2:45 p.m. - 3:00 p.m.	Break	
3:00 p.m. - 3:45 p.m.	Clinical Pathology and NMR Studies	Dr. M. B. Thompson
3:45 p.m. - 4:00 p.m.	Discussion	

Wednesday, October 30, 1985

Open

8:30 a.m. - 8:45 a.m.	Report of the Director, NTP	Dr. D. P. Rall
8:45 a.m. - 9:00 a.m.	Status Report on Reproductive and Developmental Toxicology Program Review Subcommittee Activity	Dr. J. C. Lamb, IV
9:00 a.m. - 9:30 a.m.	Strains of Mice for Chemical Carcinogenesis Studies	Dr. G. N. Rao
9:30 a.m. - 10:00 a.m.	NIEHS/NTP Concept Review: Mouse Strain Differences in Hepatocarcinogenesis	Dr. J. J. Collins

10:00 a.m. - 10:15 a.m.	Break	
10:15 a.m. - 11:00 a.m.	Discussion of Levels of Evidence of Carcinogenicity	Dr. J. E. Huff
11:00 a.m. - 12:30 p.m.	Peer Review and Priority Ranking of Chemicals Nominated for NTP Testing	Board Dr. D. Canter
12:30 p.m. - 1:15 p.m.	Lunch	
		Closed
1:15 p.m. - 3:00 p.m.	Evaluation of Programs and Personnel in Chemical Pathology Branch	Board and Consultants

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS

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Ad Hoc Reviewers for Chemical Pathology Branch
on October 29 and 30, 1985

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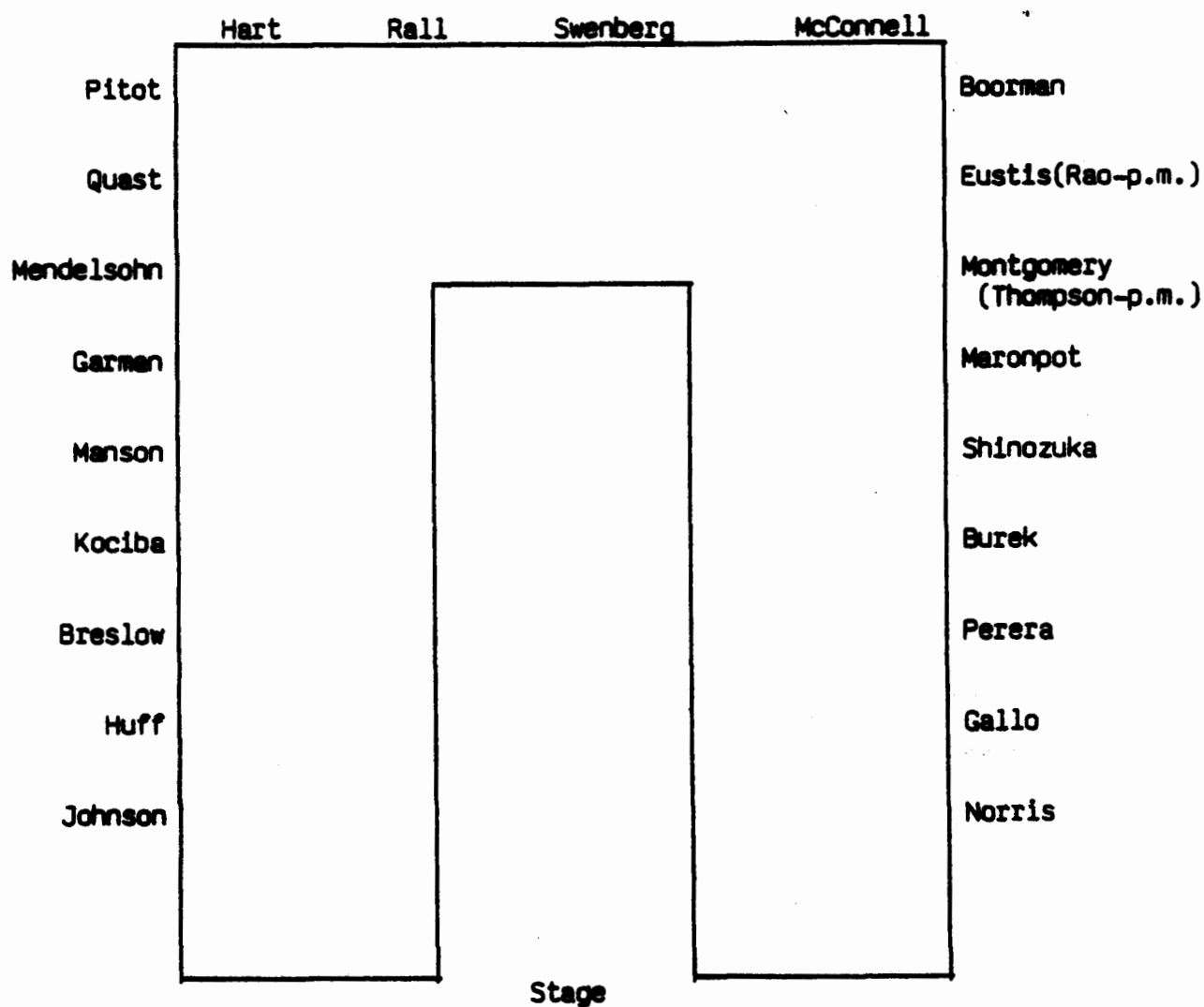
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NTP BOARD OF SCIENTIFIC COUNSELORS MEETING

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Research Triangle Park, North Carolina

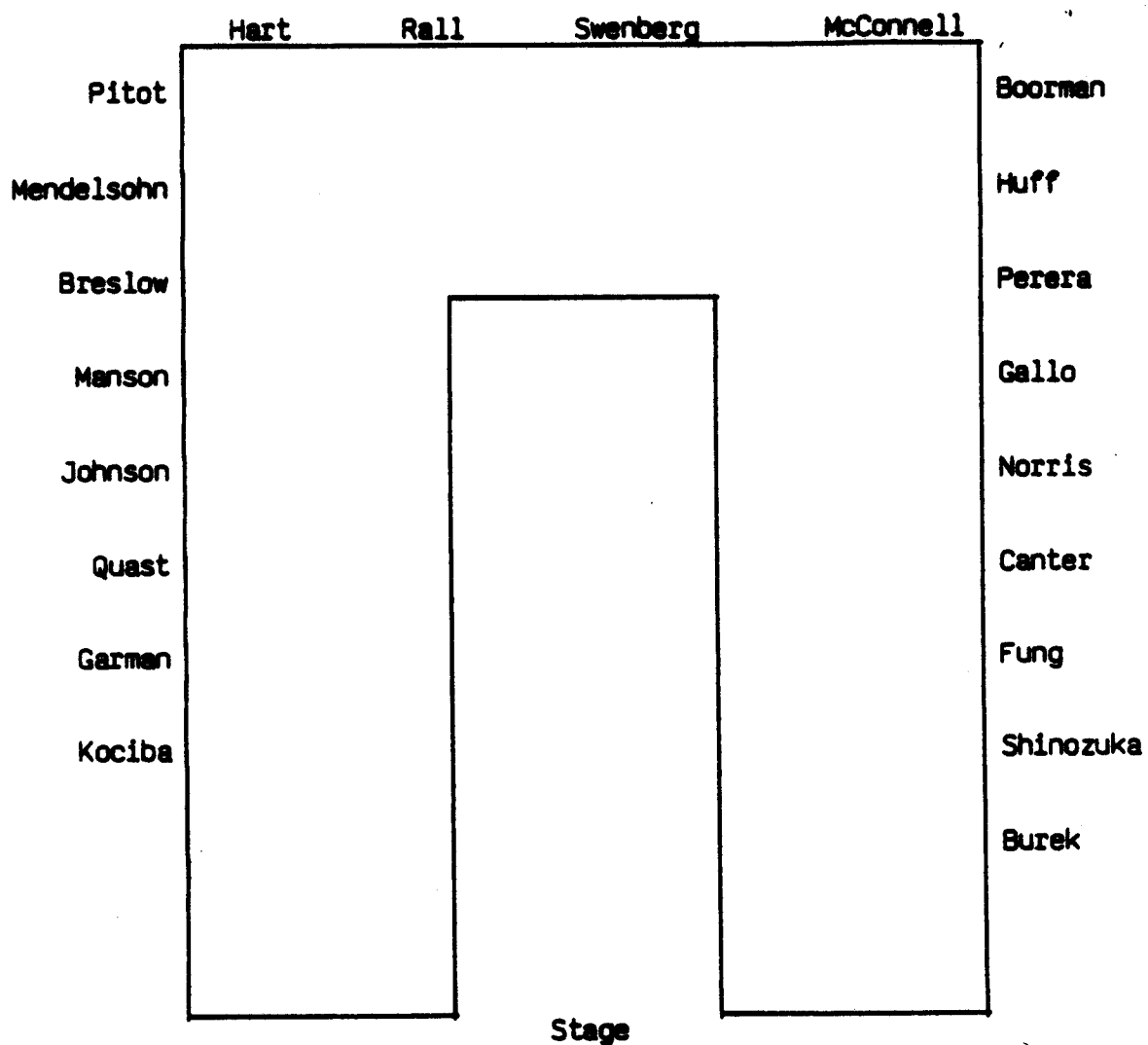
October 29, 1985



NTP BOARD OF SCIENTIFIC COUNSELORS MEETING

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

October 30, 1985



STRAINS OF MICE FOR
CHEMICAL CARCINOGENCITY STUDIES

A workshop held at NIEHS/NTP
on 4-17-85

Summary By
G. N. Rao, D.V.M., M.S., Ph.D.
NIEHS/NTP

TUMOR INCIDENCES (%) IN CONTROL B6C3F1 MICE
OF NTP STUDIES*

<u>Tissue</u>	<u>Tumor</u>	<u>Males</u>		<u>Females</u>	
		<u>Mean</u>	<u>Range</u>	<u>Mean</u>	<u>Range</u>
Liver	Adenoma	10	0-44	4	0-18
	Carcinoma	21	8-32	5	0-15
Lung	Adenoma	12	2-28	5	0-14
	Carcinoma	5	0-17	2	0- 6
Hematopoietic System	Lymphoma	12	2-32	27	10-62
Circulatory System	Hemangioma	1	0-14	2	0- 6
	Hemangio- sarcoma	3	0-10	2	0- 8
Harderian Gland	Adenoma	3	0-12	1	0- 6
Pituitary	Adenoma	1	0- 6	9	0-30

*36 Feed studies with approximately 1800 animals on control NIH-07 diet.

SITE-SPECIFIC CARCINOGENICITY FOR B6C3F₁ MICE
IN 86 NTP STUDIES*

<u>Tissue</u>	No. Chemicals Showing Carcinogenic Effects		
	<u>M</u>	<u>F</u>	<u>Total</u>
Liver	15	20	21
Lung	5	6	6
Stomach/ Forestomach	4	5	5
Hematopoietic System	2	4	4
Circulatory System	1	4	4
Thyroid Gland	2	3	4
Mammary Gland	0	3	3
Nasal Cavity	2	3	3

*31 of these 86 studies are positive in mice.
Only 8 of these 31 are positive due to liver
tumors only.

INCIDENCES (%) OF LIVER TUMORS IN ICR
OR CD-1 MICE AT DIFFERENT AGES COMPARED WITH OTHER STRAINS

<u>Age</u> (Months)	<u>N</u>	<u>Males</u>		<u>Females</u>	
		<u>Adenoma</u>	<u>Carcinoma</u>	<u>Adenoma</u>	<u>Carcinoma</u>
19	100	8 (8-8)*	9 (2-16)	3 (0-6)	1 (0-2)
22	500	11	14	2	1
19-24	300	6 (0-16)	14 (6-28)	2 (0-6)	2 (0-12)
25	540	9 (2-22)	10 (2-28)	1 (0-3)	1 (0-5)
	100	14	11	4	1
	800	25 (11-39)	10 (0-24)	5 (1-13)	1 (0-4)
BALB/c 24	760	NA	NA	3	
B6C3F1 26	1800	10 (0-44)	21 (8-32)	4 (0-18)	5 (0-15)

*-Range, NA-Not available

INCIDENCES (%) OF LUNG TUMORS IN
ICR OR CD-1 MICE AT DIFFERENT AGES
COMPARED WITH OTHER STRAINS

<u>Age</u> (Months)	<u>N</u>	<u>Males</u>		<u>Females</u>	
		<u>Adenoma</u>	<u>Carcinoma</u>	<u>Adenoma</u>	<u>Carcinoma</u>
19	100	7 (6-8)*	5 (4-6)	0	0
22	500	16	8	11	2
19-24	300	13 (0-24)	5 (2-15)	7 (0-18)	7 (3-21)
25	540	18 (12-25)	5 (0-11)	13 (3-23)	3 (0-7)
	100	21	NA	19	NA
	800	14 (4-26)	18 (4-26)	15 (5-31)	12 (6-20)
BALB/c 24	760	NA		37	
B6C3F1 26	1800	12 (2-28)	5 (0-17)	5 (0-14)	2 (0-6)

*-Range, NA-Not available

INCIDENCES (%) OF LYMPHORETICULAR TUMORS
IN ICR OR CD-1 MICE AT DIFFERENT AGES
COMPARED WITH OTHER STRAINS

<u>Age</u> (Months)	<u>N</u>	<u>Males</u>	<u>Females</u>
19	100	4	5
22	500	7	15
19-24	2100	9	17
	350	3	7
25	540	9 (3-13)*	24 (12-37)
	800	8 (2-15)	21 (10-34)
BALB/c 24	760	NA	58
B6C3F1 26	1800	12 (2-32)	27 (10-62)

*-Range, NA-Not available

INCIDENCE (%) OF AMYLOIDOSIS IN ICR OR CD-1 MICE

<u>Site</u>	<u>23 Months of Age^(a)</u>		<u>25 Months of Age^(b)</u>	
	<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>
Liver	40	34	5-14	7-21
Kidney	55	57	13-33	17-52
Spleen	19	12	2-16	7-19
Heart	33	28	5-19	3-20
Thyroid	24	36	2-33	8-40
Adrenal	45	42	10-26	15-40
G.I. Tract/ Stomach	48	52	0-19	0-25
Lymph node	15	10	0	0-11
Ovary	-	41	-	12-21

(a) Data on 500 animals from MSDRL - mean of 5 studies.

(b) Data on approximately 540 animals from P and G.

Range in 9 studies conducted at different laboratories.

SURVIVAL OF ICR OR CD-1 MICE
COMPARED WITH BALB/C AND B6C3F1 MICE

<u>Age</u> (Months)	<u>N</u>	<u>Males</u>	<u>Females</u>
19 (CD-1)	150	79	73
22 (CD-1)	500	45	62
24-26 (ICR, CD-1)	550	46 (27-67)*	45 (28-53)
	480	39 (27-60)	29 (15-42)
	1070	32	39
	100	47	45
BALB/c 24	553	30	49(N-2313)
B6C3F1 25-26	640	68 (43-93)	74 (62-87)
	1800	74 (48-88)	73 (48-88)

* Range

INCIDENCES (%) OF NEOPLASTIC LESIONS IN
B6CF1/An1 MICE* UP TO 44 MONTHS OF AGE**

<u>Site of Tumor</u>	<u>Males</u> (N-698)		<u>Females</u> (N-734)	
	<u>Mean</u>	<u>Range+</u>	<u>Mean</u>	<u>Range</u>
Lung	69	62-72	39	38-42
Lymphoreticular	54	50-62	72	66-76
Vascular	17	15-21	19	11-27
Liver	8	7- 8	3	3

Mean Survival++

Months	33	32-35	32	31-33
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* C57B1/6J An1 X BALB/cJ An1

** As reported by Dr. Grahn, Argonne National Laboratories

+ Range of 3 studies

++ 7 to 8 studies

GENETIC CONSIDERATIONS IN SELECTION OF
STOCKS/STRAINS FOR CHEMICAL CARCINOGENICITY STUDIES

Select genotypes that are
representative of mouse specie

or

Identify and use a genotype that
is uniquely suitable for the type
of toxicology (types of chemicals
or types of tumors) to be done.

Taylor: Genetic Relationships Between Inbred Mouse Strains

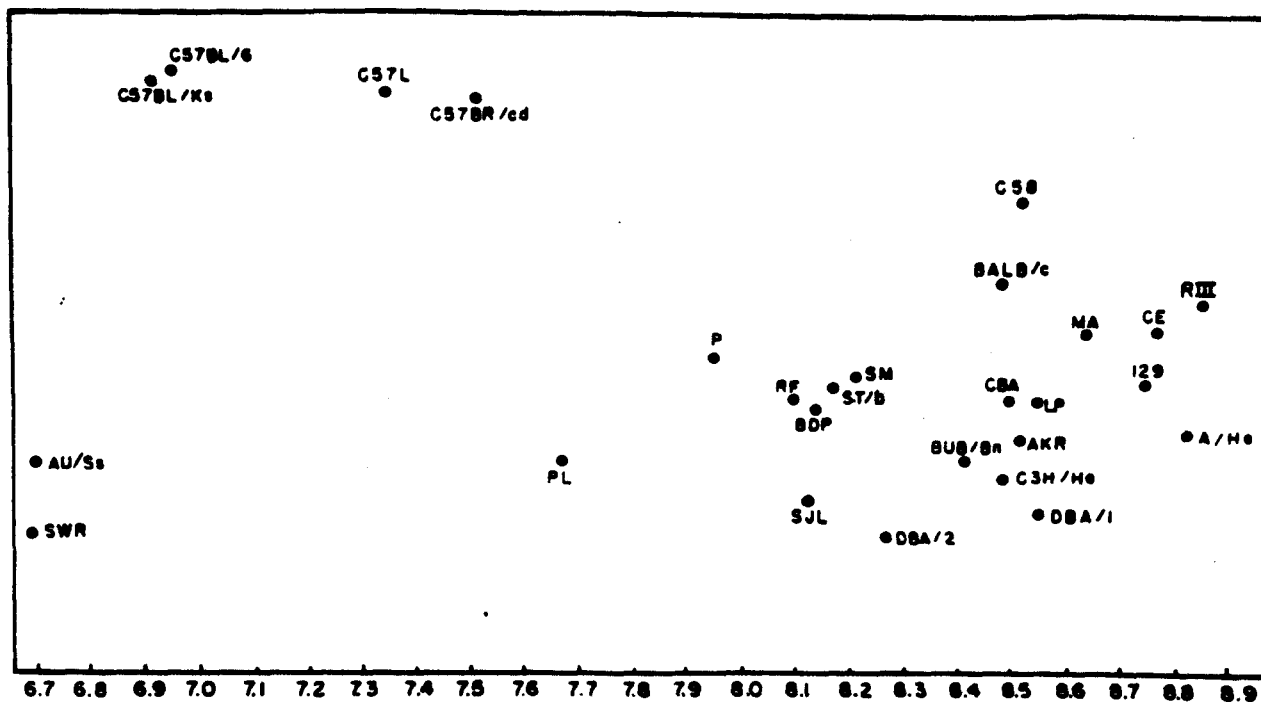


FIGURE 1—Positions of 27 inbred strains in two dimensions as determined by an eigenvector analysis of similarity matrix. See text for details of the scale of coordinates and the linear distance between strains.

"If a determination is made to maintain
a two species bioassay protocol
GIVE SERIOUS CONSIDERATION TO REPLACEMENT
OF THE B6C3F1 MOUSE WITH A STRAIN HAVING
AN ESTABLISHED LOWER AND LESS VARIABLE
SPONTANEOUS INCIDENCE OF IMPORTANT TUMORS
THAT ARE INDUCED BY CHEMICALS."

DISCUSSION

Q.1 Is the Swiss Albino, ICR or CD-1 mouse an acceptable replacement for the B6C3F1?

Consensus No. There is no advantage in changing to CD-1 mouse.

Reasons 1. We should stay away from a random bred (or outbred) as there is the problem of genetic drift between colonies of CD-1 mice or same colony overtime. There may be marked differences in genotype or array of genotype of this random bred mouse in different colonies.

 2. High incidence of amyloidosis in the liver, kidney, spleen, thyroid, adrenal, etc.

 3. Low survival (less than 50%) at 24 months into the study.

 4. Variable and high incidence of lung, liver and lymphoreticular tumors.

DISCUSSION

Q.2 How about using an inbred like BALB/C or C57BL?

Consensus No.

- Reasons
1. An inbred is a single genotype and a hybrid is better than an inbred to represent mouse specie.
 2. High incidence of lymphorecticular and lung tumors in BALB/C and high incidence of lymphorecticular tumors in C57BL mice.
 3. Survival of BALB/C mice is less than 50% at 24 months and C57BL is difficult to produce in large numbers.

DISCUSSION

Q.3 If we have to select a different hybrid or more than one hybrid what are your recommendations.

- Consensus
1. Should continue to use B6C $\times$ F1 hybrid because we have a lot of experience with it and there is more information on this hybrid than any other hybrid. If we use other hybrids in hundreds of studies in several laboratories, we will have similar (if not the same) concerns about the high background neoplasms.
 2. We should evaluate other hybrids for their suitability to replace B6C $\times$ F1 or as substitutes for B6C $\times$ F1 with selected class(es) of chemicals.
 3. Use of more than one hybrid for each chemical may not be an answer because, if a chemical is tested in enough number of hybrids, one organ of one sex of one hybrid may give you a positive response. Such a result will complicate interpretation for regulatory purposes and risk assessment.

DISCUSSION

Q.4 Should we consider another rodent species or an alternate rodent species to the mouse or rat?

Response Not as a routine procedure. But if hamster or other rodents metabolize a chemical more similar to human than the rat or the mouse, then that rodent should be used instead of the rat or the mouse.

However, we do not have adequate experience and historical data base to use hamster or gerbil in chronic studies.

CONCLUSIONS

1. There is no acceptable replacement for the B6C3F1 mouse at this time and this hybrid should be retained as the mouse to be used in the NTP chemical carcinogenicity studies.
2. More information should be developed on the liver tumor susceptibility of B6C3F1 mouse.
3. Other mouse hybrids should be evaluated for their suitability in chemical carcinogenicity studies.
4. Adequate data base should be developed with other rodents such as hamster to use such a rodent as a substitute for the rat or the mouse where metabolism of a chemical indicates an advantage.

Jeffrey J. Collins, Ph.D.
NTP/TRTP/CTEB
October 30, 1985

National Toxicology Program Concept Review

Title: Mouse Strain Differences in Hepatocarcinogenesis

Period of Award: 4 years

Funding: \$4,100,000

Funding Mechanism: Contract

Objective:

The objective of this proposal is to evaluate possible strain differences in hepatocarcinogenesis in genetically-defined mice, particularly those closely related to the standard NTP mouse strain, B6C3F₁. This will be done by comparing the carcinogenic activity of two established inducers of liver tumors, 1,1,2,2-tetrachloroethane and 2,6-dichloro-p-phenylenediamine, in B6C3F₁, reciprocal cross C3B6F₁, hybrid B6D2F₁, parental C57Bl/6N, C3H and DBA/2N, and unrelated Balb/c mice. Selection of doses for chronic testing will depend upon prior establishment of maximum tolerated doses (MTD) for these chemicals by means of 90-day subchronic toxicity testing.

Background:

It has been suggested (1,2) that the most reliable toxicologic data is obtained from tests carried out in animals of several unrelated reproducible genotypes (i.e., inbred strains) rather than using a single inbred strain as is commonly done. Given the utilization by the NTP of the B6C3F₁ mouse as the standard murine host for toxicologic testing, the following study is proposed to provide preliminary data as to the relative susceptibility of B6C3F₁ and related inbred mice to hepatocarcinogens. It should be noted that of the 86 chemicals tested by the NTP which have recently been reviewed by Haseman et al. (3), 31 were positive in mice and 21 of these induced liver tumors. Of these 21 chemicals, 13 also induced tumors at other sites, thus only 8 of the 86 chemicals have been positive for the mouse liver only. In light of the concerns which have been expressed with respect to the high spontaneous incidence of liver tumors in (male) B6C3F₁ mice, it is important to determine whether carcinogenesis limited to the B6C3F₁ mouse liver accurately reflects hepatocarcinogenicity of a given chemical or rather is particular to this mouse hybrid. It is, therefore, important to conduct a comparative evaluation of selected chemicals for hepatocarcinogenicity in mouse strains which will allow an analysis of the genetic influence of the C3H parental strain (which is responsible for introducing the high spontaneous liver tumor incidence that characterizes the B6C3F₁ mouse) at the same time that the chemical effects are compared. A closely related proposal, utilizing short-term prechronic studies and directed primarily at a determination of the mechanism(s) responsible for any differences which may be revealed, will be submitted if the prechronic studies indicate substantial strain differences in toxic response.

Approach:

Of major importance in ensuring the reliability of the results obtained in this type of genetic screening is to use well-defined carcinogens. For this purpose, two chemicals were initially selected for a variety of reasons from a group of hepatocarcinogens which have demonstrated unequivocal carcinogenic activity in B6C3F₁ mice in previous NTP chronic bioassays, namely pentachloroethane and 2,6-dichloro-p-phenylenediamine (4,5). However, it was subsequently decided to replace pentachloroethane with 1,1,2,2-tetrachloroethane, also a proven hepatocarcinogen in B6C3F₁ mice (6), because of the latter's greater economic importance and more timely health concerns. The original suggestion of 5 mouse strains, including the standard B6C3F₁ and the reciprocal cross C3B6F₁, the parental strains C57Bl/6N and C3H, and the unrelated Balb/c (characterized by a low level of spontaneous hepatocarcinogenesis) has been expanded in this updated proposal to also include the B6D2F₁ hybrid and the corresponding DBA/2N parental strain. The latter two strains have been added based on information provided at the NIEHS-sponsored workshop on "Strains of Mice for Chemical Carcinogenicity Studies" held in April, 1985.

Given the fact that the toxicity of neither 1,1,2,2-tetrachloroethane nor 2,6-dichloro-p-phenylenediamine has been examined in any of the strains to be used other than the B6C3F₁ hybrids, it will first be necessary to establish the MTD for both chemicals in C3B6F₁, C57Bl/6N, C3H, B6D2F₁, DBA/2N and Balb/c mice (B6C3F₁ mice will also be included as a control). MTD's will be derived in 90-day subchronic toxicity testing using dosages based on previous subchronic testing in B6C3F₁ mice (5,6), but modified slightly so as to include the pre-designated standard doses to be used in the chronic test, which are also based on the previous NTP chronic testing in B6C3F₁ mice (5,6) [1,1,2,2-tetrachloroethane - 140 mg/kg, 2,6-dichloro-p-phenylenediamine - 3000 ppm; see below].

Selected doses for 90-day subchronic testing to establish MTD's are: 1,1,2,2-Tetrachloroethane (Gavage in corn oil) - 0, 35, 70, 140, 280, 560, and 1120 mg/kg; 2,6-dichloro-p-phenylenediamine (Feed) - 0, 750, 1500, 3000, 6000, and 9000 ppm. The toxicologic parameters normally evaluated by the NTP in 90-day subchronic studies, including clinical chemistry, hematology, micro-nuclei determinations, sperm morphology and vaginal cytology, will also be examined in these subchronic studies.

The number of animals required for the proposed subchronic studies are:

Chemical	Mice/Group	Sexes	Strains	Dose Levels	Total
1,1,2,2-Tetrachloroethane	10	x 2 x	7	x 7	= 980
2,6-Dichloro-p-phenylenediamine	10	x 2 x	7	x 6	= 840
					1820

Regardless of whether or not both chemicals demonstrate toxicity in all mouse strains comparable to that seen in B6C3F₁ mice, the subsequent chronic tests will utilize two dose levels. The pre-designated doses indicated above, namely 140 mg/kg 1,1,2,2-tetrachloroethane and 3000 ppm 2,6-dichloro-p-phenylenediamine, are derived directly from the results of the previous NTP

chronic testing of these chemicals (5,6). The former, representing the low dose from the previous NTP chronic test, demonstrated significant hepatocarcinogenesis in both male and female B6C3F₁ mice (6); the high dose from the earlier study (284 mg/kg) was not selected because of the considerable mortality observed in both sexes. The pre-designated dose of 3000 ppm 2,6-dichloro-p-phenylenediamine represents the high dose from the previous NTP chronic test and was selected because only this concentration induced statistically significant hepatocarcinogenesis in both male and female B6C3F₁ mice (5).

In those cases in which the 90-day subchronic results indicate that the MTD is higher or lower than that seen in B6C3F₁ mice, the two dose levels selected for the subsequent chronic studies will include the MTD and the pre-designated standard dose. This will eliminate possible problems related to either usage of an equivalent dose in mice of differing toxic susceptibilities or of comparing the carcinogenic effects in various mouse strains treated with different concentrations of a single chemical in which the pharmacokinetic characteristics may vary significantly. All animals will be subjected to complete necropsy and histopathological evaluation as specified for current NTP chronic studies. Because of the limited background tumor incidence information available for nearly all of the strains being used, the number of mice in control groups has been doubled. However, these animals will be divided into two groups for placement in each test chemical room.

The number of animals required for the proposed chronic studies are:

	Chemicals		Mice/Group		Sexes		Strains		Dose Levels	Total
Test Groups	2	x	50	x	2	x	7	x	2	= 2800
Controls	2	x	50	x	2	x	7	x	1	= 1400
										4200

It is recommended that both the 90-day subchronic and 104-week chronic studies for both chemicals be performed by the same laboratory.

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LEVELS OF EVIDENCE OF CARCINOGENICITY

Report of Program Staff

to the

Board of Scientific Counselors

30 October 1985

In June 1983, the National Toxicology Program began using five categories of interpretative conclusions (Attachment 1, see definitions) in their Toxicology and Carcinogenesis Studies Technical Report Series (Attachment 2, list of chemicals and summary information). The use of these categories was implemented largely in an attempt to better differentiate and evaluate the "strength of evidence" of the experimental findings and to replace the restrictive classifications in common use that a chemical "was" or "was not" carcinogenic under the conditions of the particular study.

The levels of evidence were formulated with the underlying need to allow considerable scientific flexibility and to promote better understanding and usefulness not only among the Board of Scientific Counselors Peer Review Panel members and Program Staff but significantly as well for those who subsequently must rely on these findings. Thus, five categories of evidence of carcinogenicity seemed to represent a reasonably optimal number to meet these objectives; that is, two categories for positive results ("Clear Evidence" and "Some Evidence"), one category for uncertain findings ("Equivocal Evidence"), one category for no observable effects ("No Evidence"), and one category for experiments considered seriously flawed ("Inadequate Study").

As used since June 1983 one of these five categories has been selected to describe the findings for each individual study. A study has been defined to mean data collected from a single species/sex; thus, in Program studies this usually means four separate experiments: male rats, female rats, male mice, female mice. The system used in our Program should not be considered either new or fully unique, since others have defined for their own particular needs similar categories of evidence (IARC, 1978-1985; Griesemer and Cueto, 1980; LSRO, 1984; Nesnow, et al., 1985; OTA, 1981), which have been used by these groups with success. Still others have suggested means for considering overall toxicology evidence per se in arriving at more of a "risk assessment"-type evaluation whereby in some cases numerical values are proposed (NCAB, 1977; Squire, 1981; Weisburger and Williams, 1981; Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation 1984; OSTP, 1985; AIHC, 1984). None of these approaches seemed to fit fully our needs.

Further, the Peer Review Panel and the Program have not attempted to formulate a composite evaluation as is done by the International Agency for Research on Cancer, by the Regulatory Agencies, or by others. These all-available-data-type interpretations that help determine potential human health hazards extend beyond the Program purview, or the necessary risk assessment/risk management expertise. Importantly, however, the Program experimental findings from long-term carcinogenesis studies are most valuable for identifying potential human health hazards, which is the first step in the risk assessment process.

The Board of Scientific Counselors Ad Hoc Peer Review Panel members (Attachment 3), who helped evaluate the studies reported beginning June 1983 (Attachment 2), have given the Program staff considerable insight and constructive comments about the categories of evidence by their indepth discussions during the Panel meetings as well as individually on other occasions.

During Peer Review of Draft Technical Reports beginning June 1983, the key areas that seem to consistently gather the most attention regarding the levels of evidence are:

- i) whether benign neoplasia (alone) should be considered as being evidence of carcinogenicity given that cancer means "malignancy" (see definition of chemical carcinogenesis, attachment 1);
- ii) whether "substantial increases" in benign neoplasia was sufficient to evoke the highest level of evidence;
- iii) whether neoplasia without a "benign" counterpart (that is, leukemia) necessitates "clear evidence";
- iv) whether "common" or "uncommon" occurring neoplasia should influence the selected level of evidence;
- v) whether "some evidence of carcinogenicity" and "clear evidence of carcinogenicity" were really distinctive.

Nonetheless the Panel members were in consensus agreement that the levels of evidence of carcinogenicity as used for the 42 Technical Reports beginning June 1983 was a considerable advancement. And the Panel members (and the Board of Scientific Counselors Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation) urged continued use of these categories of evidence, with minor adjustments made where necessary to accommodate Panel concerns and advances in knowledge.

The major addition proposed for the levels of evidence centers on a more explanatory introductory paragraph (see Attachment 4, J. A. Swenberg letter) that should assist Panel members who review the Technical Reports

as well as to promote further understanding for those who use these Technical Reports and are not involved as deeply in the overall process. A proposed addition to the current Note to the Reader (attachment 1), as given in all Technical Reports, attempts to address these concerns, and will become a permanent part of the Note section to be placed immediately before the definitions:

Five categories of evidence of carcinogenicity are used in the Technical Reports series to summarize the strength of the evidence observed in each experiment: two categories for positive results ("Clear Evidence" and "Some Evidence"), one category for uncertain findings ("Equivocal Evidence"), one category for no observable effects ("No Evidence"), and one category for experiments that because of major flaws cannot be evaluated ("Inadequate Study").

While selecting a conclusion statement for a particular experiment, ample and appropriate consideration must be given to key influences in data interpretation coming from other than the actual brief collection of words that form the basis of an individual category of evidence. This extends the overall tone of the categories to take into proper account scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, and should be useful in placing results into a category of evidence; especially those that may be on the borderline between two adjacent levels. These considerations, among others, should include the overall experimental design and conduct; occurrence of common versus uncommon neoplasia; progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions; combining benign and malignant tumor incidences known or thought to represent stages of progression in the same organ or tissue; the malignant lesion may or may not (leukemia) have a benign counterpart; the presence or absence of dose response relationships; supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in the other experiments (same lesion in other sex or species); the historical control rate and variability for a specific neoplasm; survival adjusted analyses and false positive or false negative concerns; structural activity correlations; and in some cases even the laboratory where the studies were conducted. These factors together with the definitions as written should be used as composite guidelines for selecting one of the five categories.

In our experience, the underlying tenet to continued use and acceptance of these categories of evidence centers on scientific and judgemental flexibility (Attachment 5); attempts to "spell out" details and specifics

quickly disuades the conceptual (and opinionated) "feeling about the data" and frequently leads to narrow and often obligatory confinement to "definitional boxes". Thus, the words used to render our levels of evidence may seem vague in some instances, yet importantly these definitions must remain flexible and should be considered as guidelines to assist in choosing one of the five categories.

Nonetheless, some rearrangements or wording modifications have been made in the individual categories of evidence to allow further clarity of thought, yet continues to maintain flexibility for selection (word additions have been underlined and word deletions are shown by a lined strikeover). The comment notations are provided here for further exposition, and will not be shown in the Technical Reports.

Clear Evidence of Carcinogenicity is demonstrated by studies that are interpreted as showing a chemically related dose response increased incidence of malignant neoplasms, studies that exhibit an increased incidence of a combination of malignant and benign neoplasms in the same organ or tissue where each increases with dose, or studies that exhibit a substantially increased incidence of benign neoplasms.

Comment: The weight of evidence in this category indicates conclusively an unequivocal carcinogenic response due to chemical exposure. Generally yet not exclusively, this level is reserved for chemicals causing dose related increases in malignant neoplasia. A rearrangement of second and third phases was done to place in order of "decreasing" evidence.

Some Evidence of Carcinogenicity is demonstrated by studies that are interpreted as showing a chemically related increased incidence of ~~benign~~ neoplasms (malignant, benign, or combined), studies that exhibit marginal increases in neoplasms of several organs/tissues, or studies that exhibit a slight increase in uncommon malignant or benign neoplasms.

Comment: The major differentiation between "clear evidence" and "some evidence" hinges on the degree or strength of the response. Both categories represent positive evidence of carcinogenicity, but separate largely with respect to the nuances of the overall response: clear evidence being the "higher degree of evidence" and some evidence being the "lower degree of evidence".

Equivocal Evidence of Carcinogenicity is demonstrated by studies that are interpreted as showing a ~~chemically related~~ marginal increase of neoplasms that may be chemically related.

Comment: The strength of the evidence is considered insufficient to permit a conclusion of a definitive positive association between the response and the chemical, yet some correspondence seems to exist that prevents placement in the "no evidence" category. In essence, the findings are considered somewhat uncertain.

No Evidence of Carcinogenicity is demonstrated by studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms.

Comment: Given the relatively small number of animals used in each control and dose group coupled with a maximally optimal exposure regimen and the two-year duration, the "no evidence" level labels those experiments as exhibiting no neoplastic responses as being related to chemical exposure under the conditions of the study. No change in definition.

Inadequate Study of Carcinogenicity is demonstrated by studies that because of major qualitative or quantitative limitations ~~the studies~~ cannot be interpreted as valid for showing either the presence or absence of a carcinogenic effect.

Comment: Assigned to studies frequently on the basis of poor survival, due at times to bacterial/viral influence or to chemical toxicity, resulting in not enough animals surviving long enough with sufficient numbers to be considered "no evidence". Positive studies suffer less from reduced survival. Also, major scientific or technical flaws may render a study uninterpretable. Changes only reflect an attempt at clarity.

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
National Institutes of Health**Memorandum**

Date October 7, 1985

From NTP Chemical Selection Coordinator

Subject Review of Seven Chemicals Nominated to the NTP for Toxicological Testing

To National Toxicology Program Board of Scientific Counselors

As part of the NTP chemical selection process, the Board of Scientific Counselors evaluates and makes recommendations on chemicals nominated to the NTP for toxicological testing. This assessment takes place following review of the chemicals by the NTP Chemical Evaluation Committee (CEC).

The Board of Scientific Counselors will review seven chemicals from 10:30 a.m. to 12:15 p.m. on Wednesday, October 30, 1985. Two of these chemicals, ellagic acid and α -terpineol, were nominated as a result of a class study on wood chemicals and associated industries that was conducted by the National Cancer Institute (NCI). Ellagic acid was nominated for *in vitro* cytogenetics testing and for testing as a carcinogenic inhibitor. α -Terpineol was nominated for carcinogenicity testing and for tumor initiation promotion studies. The CEC reviewed these chemicals on February 5, 1985. The remaining five chemicals, consisting of two glycol ethers (2-ethoxyethanol and 2-methoxyethanol), and three glycol ether acetates (2-butoxyethanol acetate, 2-ethoxyethanol acetate, and 2-methoxyethanol acetate) were nominated by the UAW International Union for mutagenicity and carcinogenicity testing. The CEC reviewed these five chemicals on July 30, 1985.

Table 1 contains the seven chemicals to be reviewed by the Board, the source of nomination, production, worker exposure, NTP testing status, CEC recommendations and priority assigned.

The following materials are enclosed in order to assist you in your review of the seven chemicals:

1. NCI Summary Sheets on ellagic acid and α -terpineol. Attached to each of the NCI Summary Sheets is an addendum prepared by NTP staff containing information relating to physical and chemical properties, acute toxicity of the chemical, nomination history and NTP Chemical Evaluation Committee review. These chemicals were nominated after the National Center for Toxicological Research had stopped preparing Executive Summaries for the NTP but before a contractor was hired to perform this task as well as other tasks. Since the NCI

2.

Summary Sheets contain summaries of available data pertaining to use, exposure, chemical disposition, carcinogenicity and mutagenicity of the chemicals, NTP staff concluded that it would be appropriate, in this unique situation, to add sections on acute toxicity, nomination and selection history to the Summary Sheets and use them in the evaluation of these chemicals.

2. Set of five NTP Executive Summaries on the two glycol ethers and three glycol ether acetates. These Executive Summaries are the first group of summaries prepared by the new NTP chemical nomination and selection support contractor.
3. Summary Data Table on the seven chemicals.
4. List of NTP chemical selection principles.

As at past meetings each of the Board members who will be in attendance is being requested to review one chemical for the purpose of leading the Board's discussion and presenting testing recommendations. The list of chemicals and reviewers follows:

<u>Name</u>	<u>Chemicals</u> (in order of review)
Dr. Mortimer L. Mendelsohn	Ellagic acid
Dr. Henry Pitot	α -Terpineol
Dr. Frederica Perera	2-Ethoxyethanol
Dr. James A. Swenberg	2-Methoxyethanol
Dr. Norman Breslow	2-Butoxyethanol acetate
Dr. Michael A. Gallo	2-Ethoxyethanol acetate
Dr. Jeanne Manson	2-Methoxyethanol acetate

If you wish to receive references for any of the chemicals, please contact me and we will send them by express mail.

If you will be unable to assume the responsibility for discussing the assigned chemicals, please call me at (301) 496-3511 or FTS 496-3511 so that other arrangements can be made.

With best regards.

Victor A. Fung
Victor A. Fung, Ph.D.

Attachments

3.

Addresses: Dr. Mortimer L. Mendelsohn
Dr. Norman Breslow
Dr. Michael Gallo
Dr. Jerry B. Hook
Dr. Jeanne Manson
Dr. Frederica Perera
Dr. Henry Pitot
Dr. James A. Swenberg

cc: Dr. David P. Rall
Dr. Eugene McConnell
Dr. Larry Hart
Dr. James Huff
Ms. Florence Jordan
Dr. Raymond Tennant
Dr. Douglas Bristol
Dr. Dorothy Canter

Table 1

Summary Data on Chemicals for Review by the NTP Board of Scientific Counselors
on October 30, 1985

Chemical (CAS No)	Nominating Source	Production (lbs)	Worker Exposure	NTP Testing Status	Other	Chemical Evaluation Committee Testing Recommendation (priority)	Chemical Selection Principles	Rationale/ Remarks
A. Wood Chemicals								
1. Ellagic acid (476-66-4)	NCI	Not listed in TSCA Inventory ^a	--	--	NCI studies: -Negative in Salmonella -Negative in mouse lymphoma	In vitro cytogenetics	3	-Need for further toxicity data -Potential for exposure -Several carcino- genesis inhibition studies already done; others in progress
2. α -Terpineol (98-55-5)	NCI	4.2x10 ⁶ (1981) ^b	16,411 ^c	--	NCI studies: -Negative in Salmonella -Negative in mouse lymphoma	-Carcinogenicity -Mouse skin tumor initiation promotion assay	3,8	-Wide exposure -Structural interest. Chemical is struc- turally related to α -pinene and δ -limonene, which were tumor promoters in mouse skin tests

Chemical (CAS No)	Nominating Source	Production (lbs)	Worker Exposure	NTP Testing Status	Other	Chemical Evaluation Committee Testing Recommendation (priority)	Chemical Selection Principles	Rationale/ Remarks
B. Glycol ethers and Glycol ether acetates								
1. 2-Ethoxy- ethanol (110-80-5)	UAW International Union	6.2x10 ⁷ - 1.71x10 ⁸ (1977) ^a 1.87x10 ⁸ (1983) ^d 0-10 ³ (Imports, 1977) ^a	411,982 ^c 6,804 ^e	-Gavage car- cinogenicity study in histopathol- ogy phase -Negative in Salmonella -Negative for sex-linked re- cessive lethal mutations in Drosophila -Positive for both chromosom- al aberrations and sister chromatid ex- changes in CHO cells <u>in vitro</u> -On test in mouse lymphoma assay -Conventional teratology study completed -Dominant lethal and continuous breeding studies completed	--	-Inhalation comparative chemical disposition studies of ethylene glycol ether acetates and parent glycol ethers -Toxicity and carcino- genicity studies by inhalation route, including testing for hematological, immuno- logical, and neurological effects. (High)	3,8	-High production -Significant ex- posure -Lack of carcinogeni- city data -Known reproductive toxicity in animals -Consider performing subchronic studies by both dermal and inhalation routes.
2. 2-Methoxy- ethanol (109-86-4)	UAW International Union	3.1x10 ⁷ - 1.6x10 ⁸ (1977) ^a 8.3x10 ⁷ (1983) ^d 2.7x10 ⁵ (Imports, 1984) ^f	103,424 ^c 13,834 ^e	-Continuous breeding study com- pleted -Continuous breeding study in progress -Short-term <u>in</u> <u>vivo</u> repro- ductive toxicity study completed	--	-Inhalation comparative chemical disposition studies of ethylene glycol ether acetates and parent glycol ethers -Toxicity and carcino- genicity studies by inhalation route, in- cluding testing for hematological, immuno- logical, and neurological effects. (High)	3,8	-High production -Significant exposure -Lack of carcino- genicity data -Known reproductive toxicity in animals

Chemical (CAS No)	Nominating Source	Production (lbs)	Worker Exposure	NTP Testing Status	Other	Chemical Evaluation Committee Testing Recommendation (priority)	Chemical Selection Principles	Rationale/ Remarks
3. 2-Butoxy- ethanol acetate (112-07-2)	UAW International Union	1.0-10x10 ⁶ (1977) ^a	36,396 ^c 4206 ^e	--	--	Inhalation comparative chemical disposition studies of ethylene glycol ether acetates (2-butoxyethanol acetate, 2-ethoxyethanol acetate, and 2-methoxyethanol acetate) and the parent compounds (2-butoxyethanol, 2-ethoxyethanol, and 2-methoxyethanol) (High)	3,8	-Potential for exposure -Investigate whether the glycol ether acetates are hydro- lyzed in the test animals to the parent glycol ethers, and then distributed, metabolized, excreted equivalently to the parent ethers. -Ascertain the need for further testing of the glycol ether acetates upon completion of chemical disposition studies
4. 2-Ethoxy- ethanol acetate (111-15-9)	UAW International Union	2.0x10 ⁷ -1.0x10 ⁸ (1977) ^a 1.26x10 ⁸ (1983) ^d 0-3x10 ³ (imports, 1977) ^a	321,096 ^c 22,113 ^e	-Conventional -- teratology study completed -Continuous breeding study completed	--			
5. 2-Methoxy- ethanol acetate (110-49-6)	UAW International Union	In TSCA Inventory but production volume not reported ^a ~1.0x10 ⁶ (1983) ^g	436,879 ^c 2,026 ^e	Short-term in-vivo reproductive toxicity study completed	--			

- a) U.S. Environmental Protection Agency, Public File of the TSCA Inventory of Chemicals in Commerce.
- b) U.S. International Trade Commission, Synthetic Organic Chemicals, U.S. Production and Sales, U.S. Government Printing Office, Washington, D.C., 1982
- c) National Occupational Hazard Survey (1972-1974), NIOSH, Cincinnati, OH 1976.
- d) U.S. International Trade Commission, 1984, Synthetic Organic Chemicals, 1983. U.S. Production and Sales, Publication no. 1588.
- e) National Occupational Exposure Survey (1980-1983), NIOSH, Cincinnati, OH 1984.
- f) U.S. Department of Commerce, Bureau of the Census, 1984, Report No. IM 146, Section 428-4740.
- g) Leaf, D.A., 1985. Glycol Ethers: An Overview. Unpublished report dated February, 1985. Regulatory Impacts Branch Economics and Technology Division, Office of Toxic Substances, U.S. Environmental Protection Agency, Washington, D.C.

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 commercial 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 for 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 October 30, 1985

Chemical (CAS Number)	Nomination Source	Testing Recommendations (Priority)	Remarks
A. <u>Wood Chemicals</u>			
1. Ellagic acid (476-66-4)	NCI	No testing	-Limited exposure
2. α -Terpineol (98-55-5)	NCI	-Defer	-Defer until completion of NTP carcinogenicity study on δ -limonene, a structural analogue. -Review α -terpineol as part of a class study on structurally related terpenes tested by NTP
B. <u>Glycol Ethers and Acetates</u>			
1. 2-Ethoxyethanol (110-80-5)	UAW International Union	-Comparative <u>in vitro</u> chemical disposition study of this compound and its acetate -Toxicity and carcinogenicity studies by inhalation route, including testing for hematological, immunological, and neurological effects (High)	-High production -Potential for increased usage -Known reproductive toxicity in animals
2. 2-Methoxyethanol (109-86-4)	UAW International Union	-Toxicity and carcinogenicity studies by inhalation route (High)	-High production -Significant worker exposure -Lack of carcinogenicity data

Chemical (CAS Number)	Nomination Source	Testing Recommendations (Priority)	Remarks
3. 2-Butoxyethanol acetate (112-07-2)	UAW International Union	-Comparative <u>in vitro</u> esterase activities of ethylene glycol ether acetates -Genotoxicity studies (--)	-Potential for human exposure -Ascertain extent to which the ethylene glycol ether acetates are hydrolyzed to parent glycol ethers
4. 2-Ethoxyethanol acetate (111-15-9)	UAW International Union	-Comparative <u>in vitro</u> chemi- cal disposition study of this compound and its parent glycol ether -Comparative <u>in vitro</u> esterase activities of ethylene glycol ether acetates -Genotoxicity studies (--)	-Potential for human exposure -Ascertain extent to which the ethylene glycol ether acetates are hydrolyzed to the parent glycol ethers
5. 2-Methoxyethanol acetate (110-49-6)	UAW International Union	-Comparative <u>in vitro</u> esterase activities of ethylene glycol ether acetates -Genotoxicity studies (--)	-Potential for human exposure -Ascertain extent to which the ethylene glycol ether acetates are hydrolyzed to parent glycol ethers.