NATIONAL TOXICOLOGY PROGRAM

BOARD OF SCIENTIFIC COUNSELORS

May 17, 1988

Summary Minutes

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NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS MEETING
May 17, 1988

SUMMARY MINUTES

The National Toxicology Program (NTP) Board of Scientific Counselors met on May 17, 1988, at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. (Attachment 1: Federal Register Meeting Announcement; Attachment 2: Agenda and Roster of Members and Expert Consultants.) Members of the Board are Drs. Michael Gallo (Chairman), Richard Griesemer, John Little, Frederica Perera, Adrianne Rogers, Robert Scala, and Arthur Upton. Drs. Little and Scala were unable to attend the meeting.

I. Report of the Director, NTP: Dr. David Rall reported that: (1) the President's budget allowed for about a 2 to 2 and 1/2 % increase in the NIEHS budget for 1988. He noted that funding for contracts has been level since 1982; one effect has been decreasing numbers of starts in toxicology and carcinogenesis studies; and (2) he had testified in Congress against the Boxer bill, legislation which could put severe limits on animal testing and would have adverse effects on animal research in general. Scientists should be aware of such bills and be willing to express their scientific judgements. Also, the National Association for Biomedical Research can be contacted and be very helpful in providing expert testimony.

II. Review of Chemicals Nominated for NTP Studies: The six chemical nominations had been reviewed previously by the NTP Chemical Evaluation Committee (CEC). Chemicals reviewed were anthraquinone, camphor, 1-chloro-2-bromoethane, glyoxal, lead oxide (Pbo), and lead sulfide (Pbs). (Summary data on the chemicals including CEC recommendations are provided in Attachment 3.) Dr. Dorothy Canter, NIEHS, Dr. William Allaben, NCTR, and Dr. Janet Haartz, NIOSH, CEC members, and Dr. Victor Fung, NIEHS, NTP Chemical Selection Coordinator, served as resource persons. Board members serve as principal reviewers for one or two chemicals, and following their presentation and discussion of each chemical, motions are made and voted on. Although not present, Dr. Scala had provided written comments to be read at the meeting on two chemicals, lead oxide and lead sulfide.

The Board's recommendations for the six chemicals are summarized in Attachment 4.

III. Overview of the NIEHS Division of Extramural Research and Training (DERT): Dr. Anne Sassaman, Division Director, said the DERT has responsibility for administering NIEHS research and training grants. Aided by the NIEHS's Advisory Council, research needs and opportunities are assessed and means developed to address them. For FY 1988, about $110 million or 42% of the NIEHS budget has been allocated to the grants program. She described the various kinds of grants and commented on the 10 university-based environmental health science centers and the five marine and freshwater biomedical science centers. The primary areas for support of training are environmental toxicology, pathology, mutagenesis, and epidemiology/biostatistics. Dr. Sassaman said

the major science areas supported were in characterization of environmental health hazards and mechanism of action studies with lesser support to applied toxicology and biometry. She noted the large extramural emphasis on applied toxicology through the contracts of the Division of Toxicology Research and Testing. She concluded by discussing activities associated with the Superfund Amendments and Reauthorization Act of 1986 (SARA) which authorized two new grant-supported programs in the NIEHS, one for university-based basic research and training and one for health and safety training of workers at hazardous waste sites and emergency response personnel.

IV. NTP Proposed Plans for Toxicological Evaluation of Ozone: Dr. Gary Boorman, NIEHS, said that ozone had been nominated to the NTP by the State of California and by the Health Effects Institute. In the fall of 1987, the NTP Chemical Evaluation Committee evaluated ozone and recommended carcinogenicity studies, a recommendation since affirmed by the Board and NTP Executive Committee. A group of scientists met at NIEHS, recommending a top exposure concentration of one part per million, inclusion of markers of lung damage, and consideration of a cocarcinogenesis study using a known pulmonary carcinogen. Dr. Boorman noted that EPA estimates that more than a hundred million people in the U.S. are exposed annually to levels exceeding the EPA standard. He said design protocols include three exposure levels with the top level of one ppm for durations of 24- and 30-months in F344 rats and B6C3F1 mice. Additionally, cocarcinogenesis studies are planned.

V. Species Correlation in Long-Term Carcinogenicity Studies in Rats and Mice: Implications on Possible Experimental Design Strategies: Dr. James Huff, NIEHS, began by noting that the 1984 Report of the NTP Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation had recommended there be an evaluation as to whether continued use of both a rat and mouse strain was needed for detection of carcinogens. Dr. Huff reported on retrospective studies done by himself, Dr. Joseph Haseman and others at NIEHS using the NCI/NTP database and conclusions drawn. The initial study, published in 1984 in response to suggestions by others that mice were "redundant", using results from 86 two-year NTP studies indicated that all of the 43 carcinogens would have been detected using only male rats and female mice while use of male and female rats only would have missed at least 10. More recent studies with a much larger number of studies supported this finding. Examination of species correlation in neoplastic response for 266 long-term studies showed an overall concordance between rats and mice of 74%, and within a species concordence of 87% (rats) and 89% (mice). Using male rats and female mice only would have correctly identified 255/266 (96%) of the experimental results. Dr. Huff discussed potential limitations including possible false negatives, lack of supporting evidence from the other two experiments, and possible lack of acceptability by regulatory agencies. Of the 12 chemicals "missed", 11 were positive in only one sex/species while using male rats/female mice would have reduced another 11 chemicals to being positive in only a single sex/species. Dr. Huff concluded by commenting that this modified protocol will be used only on a selective basis and perhaps in experiments supplemented with other species or strains. The basic Program protocol will continue to use both sexes of both species.

VI. Rodent Model Validation Using Known Human Carcinogens: Dr. Huff acknowledged the contributions of Dr. Richard Irwin, NIEHS. The primary

objective of studies will be to evaluate toxicology and carcinogenesis responses in F344 rats and B6C3F1 mice exposed to chemicals known to cause cancer in humans. A second objective was to upgrade or strengthen the level of evidence of carcinogenicity in experimental animals. The major source of chemicals used for this analysis came primarily from the International Agency for Research on Cancer (IARC) lists of known human carcinogens (Group 1) and agents considered probably carcinogenic to humans (Group 2A). From these lists, the NTP would focus on 17 chemicals for which there was sufficient or limited evidence of carcinogenicity in humans but limited or inadequate evidence or no data in animals. He noted the chemicals from Groups 1 and 2A which have been studied or are currently under study by the NTP in F344 rats and B6C3F1 mice. Three chemicals: azathioprine, 1,4-butanediol dimethane sulfonate, and treosulfan; and two chemical mixtures: alcoholic beverages and conjugated estrogens, are being given serious consideration for study in the NTP rodent models. Dr. Huff said this research project was responsive to a recommendation of the Ad Hoc Panel, and the information developed would fill data gaps, undergird animal predictiveness, and strengthen human data. A modified experimental design would be considered for these studies.

VII. Prechronic Technical Reports and Peer Review: Dr. Ernest McConnell, NIEHS, announced that the NTP will begin to report the results of the prechronic studies on chemicals prior to and separate from the two-year study reports ("Blue Books"). The format of the prechronic reports will be similar to the current Technical Report series, although the archival data and pathology specimens will be unaudited and will include results primarily from 14- and 90-day studies as well as studies of up to six months when part of the design. The subsequent two-year reports will also contain prechronic study information but in a more highlighted and referenced form and not in as much detail as in the current Blue Books.

Peer review of the prechronic reports will be performed by mail to save Peer Review Panel meeting time and to expedite moving to the next step if the decision is made that chronic studies should be done. Most often three Panel members will be asked to peer review each report, although they will be sent to all members. If the written responses are uniformly in agreement, that will be considered the completion of the peer review. However, if the results of a given study appear to be debatable or any member of the Panel requests public review, that report will be submitted to the full Panel for discussion at the next open meeting. Also, the staff may bring a particular report to the Panel meeting if felt that it needs more indepth consideration. Dr. McConnell said the NTP will begin this procedure on a trial basis realizing that the prechronic reports will evolve as have the two-year study reports. To continue to allow for public input, completed prechronic studies will be listed in the Federal Register along with a contact person and an adequate time period for comments.

VIII. Disposition of Studies Conducted at Gulf South Research Institute: Dr. Douglas Bristol, Director, Quality Assurance, DTRT, NIEHS, reported that due in part to recognized deficiencies with certain toxicology and carcinogenesis studies at Gulf South Research Institute (GSRI), the NTP made a policy decision in 1983 to perform retrospective data audits on all studies that would be presented and many studies that had been presented previously to the NTP Board's Peer Review Panel. A special data audit subcommittee was formed from staff of agencies on the NTP Executive Committee to aid in setting priori-

ties for audits. The NIEHS decided that it would be necessary to audit all of the GSRI studies together as a special project, allowing a comprehensive assessment of the problems there before making decisions about the disposition of any one of the GSRI studies. The comprehensive audit of GSRI involved a total of 61 chronic studies on 29 chemicals. Dr. Bristol reviewed the steps in the comprehensive audit project, provided details of the QA procedures used to audit studies of an individual chemical, and discussed the relevant findings and indications. The audits were all completed and had undergone interdisciplinary review.

Dr. McConnell stated that, as result of the audits, the GSRI studies could be grouped into three categories: (1) studies where the data were not fully reliable or interpretable; (2) studies where the data were less reliable than in (1); and (3) studies that were not complete or not reliable (Attachment 5). For three chemicals in the first category, Technical Reports had been printed; these will be withdrawn. For all the chemicals in categories (1) and (2), there will be a short writeup, presenting details of materials and methods including doses and results with incidence tables for neoplastic and nonneoplastic lesions. Flaws and discrepancies will be fully noted along with a summary of the full audit report. However, no statistics, discussion, interpretation, or conclusions will be given. For the studies in category (3) nothing will be published other than a summary of the audit findings. All of the preceding, including an introductory historical background, will be published in a single report which will be announced in the Federal Register and then made available on request. The study records are stored in the NTP Archives and are available for examination on request. Dr. McConnell commented that lessons learned from this exercise have already been incorporated as improvements in study design, chemistry, pathology quality assessment, and other aspects of the process. New prechronic or chronic studies for 14 of the 29 chemicals have been planned, are underway, or have been completed. The other GSRI chemicals are being reevaluated as to whether new studies should be done. In discussion, Dr. Daniel Byrd, Halogenated Solvents Industry Alliance (HSIA), stated that his group differed with the NTP to some extent on uses for the data and inferences which could be drawn, and thought external peer review for some of the data would have been desirable. Although the HSIA thought some of the GSRI studies were publishable, they agreed with the announced course of action.

IX. Toxicology of Chemical Mixtures:

(1) NAS Report on Complex Mixtures--Dr. Bernard Schwetz, NIEHS, said the Institute provided support to the National Academy of Sciences (NAS) to form a committee to study the issue of toxicity testing of complex chemical mixtures and recommend strategies for the in vivo testing of mixtures. The committee formed four groups to deal with: (a) relationship of human diseases with exposure to chemical mixtures; (b) strategies for testing toxicity of complex mixtures in animals; (c) sampling and chemical characterization; and (d) aspects of modeling applicable to complex mixtures. These efforts resulted in a NAS book, Complex Mixtures-Methods for In Vivo Toxicity Testing. Dr. Schwetz discussed some of the issues examined. Some situations which lead to synergistic or antagonistic effects of mixtures in humans are hard to reproduce in animals, e.g., drinking of alcohol or tobacco smoking. A key to designing strategies and design protocols is to better define the questions to be answered, e.g., which toxic endpoint, which component to test, or which mixture

to study and for what purpose. In assessing exposure vs. dose, more information on bioavailability is needed. Among key conclusions were: (1) methodology that is available for use with single chemicals can often be used with mixtures; (2) strategies for studying complex mixtures can be clear only if the questions to be answered are well defined; and (3) better animal models are needed for certain human diseases. He said the NTP work in progress seemed to be 'on track' with recommendations of the committee.

(2) NTP Initiative and Ongoing Experiments- Dr. Raymond Yang, NIEHS, said the NTP role in the initiative derives from an interagency agreement with the Agency for Toxic Substances and Disease Registry (ATSDR). There are about 25,000 hazardous waste sites, yet no fully representative one. The NTP strategies were to: complement the activities of other agencies; start by focusing on groundwater where there may be commonalities with certain chemicals; and work with a defined mix of chemicals. Experimental designs received peer review from an interdisciplinary inhouse group and an external review panel experienced with mixture toxicity studies. Dr. Yang described the types of toxic endpoints being assessed in the groundwater contamination study by scientists from NIEHS and other agencies. Included were evaluations of immunotoxicology, biochemical toxicology, mutagenicity, and reproductive and developmental toxicity. He talked about how the 25 chemicals were selected reflecting a consensus of groundwater contaminants from the EPA's 10 different regions. Dr. Yang concluded by reporting results from several immunotoxicology assays which suggested at least additive effects of the mixture compared with the individual chemicals.

X. Concept Reviews- NIEHS Cellular and Genetic Toxicology Branch: Dr. Raymond Tennant, NIEHS, Branch Chief, said the three concept proposals to be presented were aimed at addressing, using available technology, distinctions between nonmutagenic carcinogens and noncarcinogens. He noted that recent publications of his own group and others reported increasing numbers of substances classified as nonmutagenic carcinogens. These agents are characterized by absence of an electrophilic structure and by absence of an induced response in the Salmonella mutagenesis assay.

(1) In Vitro and In Vivo Short Term Test Characterization of the Toxicity of Rodent Nonelectrophilic Carcinogens-- (Attachment 6) PART A. Dr. Judson Spalding said these nongenotoxic, nonelectrophilic carcinogens generally gave negative results in currently available short-term genetic toxicity assays. One group of these chemicals induces liver tumors and these chemicals also have the ability to induce S-phase or scheduled DNA synthesis. The objectives are to test the hypothesis that chemical induction of scheduled DNA synthesis in rodent liver cells is an indicator of the potential hepatocarcinogenicity of that chemical, and to develop the S-phase endpoint as an assay to detect hepatocarcinogens, especially those that are nongenotoxic. Dr. Spalding indicated some carcinogens and noncarcinogens that might be studied. Questions to be addressed are: (1) specificity of the assay among the four sex/species groups; (2) the specificity and sensitivity for carcinogens and noncarcinogens; (3) attempt to develop the S-phase endpoint into a short-term in vivo assay that detects potential hepatocarcinogens and is especially useful for detecting nongenotoxic hepatocarcinogens; and (4) compare single vs. repetitive dosing protocols for S-phase induction.

The Board stressed obtaining mechanistic data about the action of nonelectrophilic (nongenotoxic) carcinogens on target cells. The technology is available and the methodology proposed appears adequate to achieve the goals of the project. Interactions with other laboratories working in this area were encouraged.

PART B. Dr. Robert Langenbach, NIEHS, said the second approach to the concept is designed to obtain information about biological effects of the nonelectrophilic carcinogens. To gain insight into mechanisms of action, five different biological effects shown to be associated with tumor promoters will be studied: (1) protein kinase C activation; (2) interruption of cell-to-cell communication in V79 cells; (3) enhancement of an initiation-promotion cell transformation system; (4) induction of cytochrome P-450 activity in liver of rats and mice; and (5) oxygen radical production. Major outcomes expected are: (1) ability to group the nonelectrophilic chemicals by biological effects/mechanisms; (2) identification of structure/activity relationships (3) possible association of activity in each of the five systems with tumor sites in rodents will be determined; and (4) better mechanistic understanding of nonelectrophilic carcinogens may emerge.

The Board affirmed the importance of the proposed studies but questioned whether too much emphasis was being placed on cell culture models. Dr. Langenbach said the in vivo studies from Part A. would complement the studies in Part B. Concern was expressed as to whether the resources allocated were sufficient to achieve the goals.

Dr. Upton moved that the concept be approved. Dr. Rogers seconded the motion, which was approved unanimously (4 Yes votes) by the Board. Dr. Upton then moved to recommend giving Part A moderate to high priority, and Part B moderate priority. Dr. Rogers seconded these motions, which were approved unanimously (4 Yes votes) by the Board.

(2) DNA Adducts in Rodent and Human Tissues-- (Attachment 7) Dr. Langenbach stated that DNA adduct formation can serve as a relevant dosimeter of tissue exposure and biologically effective dose of a chemical. The goals of this proposal are to: (1) measure DNA adducts of genotoxic carcinogens in target and nontarget tissues of rodents; (2) study dose-responses of chemicals including doses below those which give carcinogenic responses; (3) measure tissue and species differences in DNA repair; (4) compare DNA adduct levels in target tissues with those in circulating lymphocytes; (5) compare adducts in rodent liver, in vivo and in vitro, and human liver, in vitro; and (6) use the 'parallelogram' approach to estimate human adduct levels in vivo. He expected the major outcomes to include: (1) development of DNA adduct dosimetry as it relates to species and target tissue differences; (2) obtain data relevant to low dose extrapolation; (3) determine the relationship between adducts in target tissues and adducts in lymphocytes for rodents and humans; and (4) develop models for extrapolation from rodents to humans involving DNA adducts.

The Board noted that this proposal responded to a recommendation of the Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation. Attainment of the objectives would provide information that could allow more rational extrapolation of data to humans from carcinogenicity studies in rodents. Caution was expressed about the large interindividual variability in humans. The technology to perform these studies is available and adequate.

Dr. Upton moved that the concept be approved. Dr. Rogers seconded the motion, which was approved unanimously (4 Yes votes) by the Board. Dr. Upton then moved to recommend giving the concept high priority. Dr. Rogers seconded the motion, which was approved unanimously (4 Yes votes) by the Board.

(3) Chemically Induced DNA Modifications In Vivo and In Vitro--(Attachment 8) Dr. William Caspary, NIEHS, explained that the rationale for this proposal is to look at damage at the gene level by measuring methylation patterns and DNA adducts at specific genes from chemicals for which there are discordant results between short-term mutagenicity tests and long-term animal bioassays. Methylation patterns and DNA adducts in specific genomic sites will be examined in cells from mutation studies and from target and nontarget tissues in rodents. Classical carcinogens will be studied first and then chemicals which have demonstrated discordant results in NTP studies. This study should provide further information on the validity of such terms as nongenotoxic carcinogens and genotoxic noncarcinogens.

The Board supported the adduct studies and considered the resources adequate but thought it was perhaps premature for the NTP to get involved in very complex methylation studies. Dr. Tennant noted that methylation is the one aspect of DNA association not involving major sequence change that is chemically-induced and results in heritable genetic changes. To what extent can adduction vs. methylation help us to prospectively discriminate between chemicals?

Dr. Rogers moved that the concept be approved with high overall priority but with the note that the concept needs more focus and refining. Dr. Upton seconded the motion, which was approved unanimously (4 Yes votes) by the Board.

(Room 117) Building 30, National Institutes of Health, Bethesda, Maryland. This meeting will be open to the public from 9 a.m. to recess on May 25 for program presentation and dedication of the H. Trendley Dean Conference Room. Attendance by the public will be limited to space available.

In accordance with the provisions set forth in secs. 552b(c)(4) and 552b(c)(6), Title 5, U.S.C. and sec. 10(d) of Pub. L. 92-463, the meeting of the Council will be closed to the public on May 26 from 9 a.m. to 2:30 p.m. for the review, discussion and evaluation of individual grant applications. These applications and the discussions could reveal confidential trade secrets or commercial property such as patentable material, and personal information concerning individuals associated with the applications, the disclosure of which would constitue a clearly unwarranted invasion of personal privacy.

In accordance with the provisions set forth in section 552b(c)(9)(B), Title 5, U.S. Code, the Council meeting will be closed to the public from 2:30 p.m. to adjournment on May 26 for discussion and preparation of comments Council wishes to submit to the Director, NIH, for inclusion in the biennial report to Congress.

Dr. Preston A. Littleton, Jr., Executive Secretary, National Advisory Dental Research Council, and Deputy Director, National Institute of Dental Research, National Institutes of Health, Building 31, Room 2C39, Bethesda, Maryland 29892, (telephone 301-496-9469) will furnish a roster of committee members, a summary of the meeting, and other information pertaining to the meeting.

(Catalog of Federal Domestic assistance Program Nos. 13.121—Diseases of the Teeth and Support Tissues; Caries and Restorative Materials; Periodontal and Soft Tissue Diseases; 13.122—Disorders of Stucture, Function, and Behavior; Craniofacial Anomalies, Pain Control, and Behavioral Studies; 13.845—Dental Research Institute; National Institutes of Health)

Dated: April 12, 1988.

Betty J. Beveridge,

Committee Management Officer, NIH

[FR Doc. 88-8977 Filed 4-22-88; 8:45 am]

BILLING CODE 4140-01-M

National Institute of Dental Research; Special Grants Review Committee; Meeting

Pursuant to Pub. L. 92-463, notice is hereby given of the meeting of the Special Grants Review Committee, National Institute of Dental Research, June 7-8, 1988, in the Holiday Inn Chevy Chase, 5520 Wisconsin Avenue, Chevy Chase, Maryland 20815. The Committee will be open to the public from 9 a.m. to 9:30 a.m. on June 7 for general discussions. Attendance by the public is limited to space available.

In accordance with provisions set forth in secs. 552b(c)(4) and 552b(c)(6), Title 5, U.S.C. and sec. 10(d) of Pub. L. 92-463, the meeting will be closed to the public on June 7 from 9:30 a.m. to recess and on June 8 from 9 a.m. to adjournment for the review, discussion and evaluation of individual grant applications. The applications and the discussions could reveal confidential trade secrets or commercial property, such as patentable material, and personal information concerning individuals associated with the applications, the disclosure of which would constitute a clearly unwarranted invasion of personal privacy.

Dr. Rose Marie Petrucelli, Executive Secretary, NIDR Special Grants Review Committee, NIH, Westwood Building, Room 519, Bethesda, MD 20892, (telephone 301/496-7658) will provide a summary of the meeting, roster of committee members and substantive program information upon request.

(Catalog of Federal Domestic Assistance Program Nos. 13.121—Diseases of the Teeth and Supporting Tissues: Caries and Restorative Materials; Periodontal and Soft Tissue Diseases; 13-122—Disorders of Structure, Function, and Behavior: Craniofacial Anomalies, Pain Control, and Behavioral Studies; 13-845—Dental Research Institute; National Institutes of Health)

Dated: April 12, 1988.

Betty J. Beveridge,

Committee Management Officer, NIH.

[FR Doc. 88-8975 Filed 4-22-88; 8:45 am]

BILLING CODE 4140-01-M

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 May 17, 1988.

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

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

8:45 a.m.-9:45 a.m.—Review of Chemcials Nominated for NTP Studies.

Six chemicals will be reviewed. The chemicals were evaluated by the NTP Chemcial Evaluation Commitee on December 9, 1987, and are (with CAS Nos. in parentheses): (1) Anthraquinone (84-65-1); (2) Camphor (76-22-2); (3) Chloro-2-bromoethane (107-04-0); (4) Glyoxal (107-22-2); (5) Lead (II) Oxide (1317-36-8); and (6) Lead (II) Sulfide.

9:45 a.m.-10:15 a.m.—Overview of the NIEHS Extramural Program.

10:30 a.m.-11:00 a.m.—NTP Proposed Plans for Toxicological Evaluation of Ozone.

11:00 a.m.-11:30 a.m.—Rodent Model Validation Using Known Human Carcinogens.

11:30 a.m.-12:00 noon—Proposed Selective Reduction in Sex/Species Groups for Long-Term Carcinogenesis Studies.

12:00 noon-12:15 p.m.—Prechronic Technical Reports and Peer Review.

1:00 p.m.-2:30 p.m.—Disposition of Studies Conducted at Gulf South Research Institute.

2:30 p.m.-3:00 p.m.—Toxicology of Chemical Mixtures:

I. NAS Report on Complex Mixtures.

II. ATSDR/NTP Initiative and Ongoing Experiments.

3:00 p.m.-4:30 p.m.—Concept Reviews—NIEHS Cellular and Genetic Toxicology Branch:

I. Characterizing and Developing Detection Methods for Nonelectrophilic Carcinogens.

II. DNA Adducts in Rodent and Human Tissues.

III. Chemcially Induced DNA Modifications.

The Executive Secreatary, Dr. Larry G. Hart, National Toxicology Program, P.O. Box 12233, Research Triangle Park, North Carolina 27609, 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: April 19, 1988.

David P. Rall,

Director, National Toxicology Program.

[FR Doc. 88-9037 Filed 4-22-88; 8:45 am]

BILLING CODE 88-9037-M

Social Security Administration

Supplementary Agreement on Social Security Between the United States and the Federal Republic of Germany; Entry Into Force

The Commissioner of Social Security gives notice that on March 1, 1988, a supplementary agreement entered into

AGENDA

NTP BOARD OF SCIENTIFIC COUNSELORS
NATIONAL TOXICOLOGY PROGRAM

May 17, 1988

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

8:30 a.m. - 8:45 a.m.	Report of the Director	Dr. D.P. Rall, NIEHS
8:45 a.m. - 9:45 a.m.	Review of Chemicals Nominated for NTP Studies	Board Dr. D.A. Canter, NIEHS
9:45 a.m. - 10:15 a.m.	Overview of the NIEHS Division of Extramural Research and Training	Dr. A.P. Sassaman, NIEHS
10:30 a.m. - 11:00 a.m.	NTP Proposed Plans for Toxicological Evaluation of Ozone	Dr. G.A. Boorman, NIEHS
11:00 a.m. - 11:30 a.m.	Species Correlation in Long-Term Carcinogenicity Studies in Rats and Mice: Implications on Possible Experimental Design Strategies	Dr. J.E. Huff, and Dr. J.K. Haseman, NIEHS
11:30 a.m. - 12:00 noon	Rodent Model Validation Using Known Human Carcinogens	Dr. J.E. Huff, NIEHS
12:00 p.m. - 12:15 p.m.	Prechronic Technical Reports and Peer Review	Dr. E.E. McConnell, NIEHS
1:15 p.m. - 2:00 p.m.	Disposition of Studies Conducted at Gulf South Research Institute	Dr. E.E. McConnell and Dr. D.W. Bristol, NIEHS
2:00 p.m. - 2:45 p.m.	Toxicology of Chemical Mixtures	
	I. NAS Report on Complex Mixtures	Dr. B.A. Schwetz, NIEHS
	II. NTP Initiative and Ongoing Experiments	Dr. R.S.H. Yang, NIEHS
3:00 p.m. - 4:30 p.m.	Concept Reviews - NIEHS Cellular and Genetic Toxicology Branch:	Dr. R.W. Tennant, NIEHS
	I. <u>In Vitro</u> and <u>In Vivo</u> Short Term Test Characterization of the Toxicity of Rodent Nonelectrophilic Carcinogens	Dr. J.W. Spalding, NIEHS

II.	DNA Adducts in Rodent and Human Tissues	Dr. R.J. Langenbach, NIEHS
III.	Chemically Induced DNA Modifications *In Vivo* and *In Vitro*	Dr. W.J. Caspary, NIEHS

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS

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AD HOC REVIEWERS FOR NTP BOARD OF

SCIENTIFIC COUNSELORS REVIEW OF CONCEPTS

NIEHS CELLULAR AND GENETIC TOXICITY BRANCH

May 17, 1988

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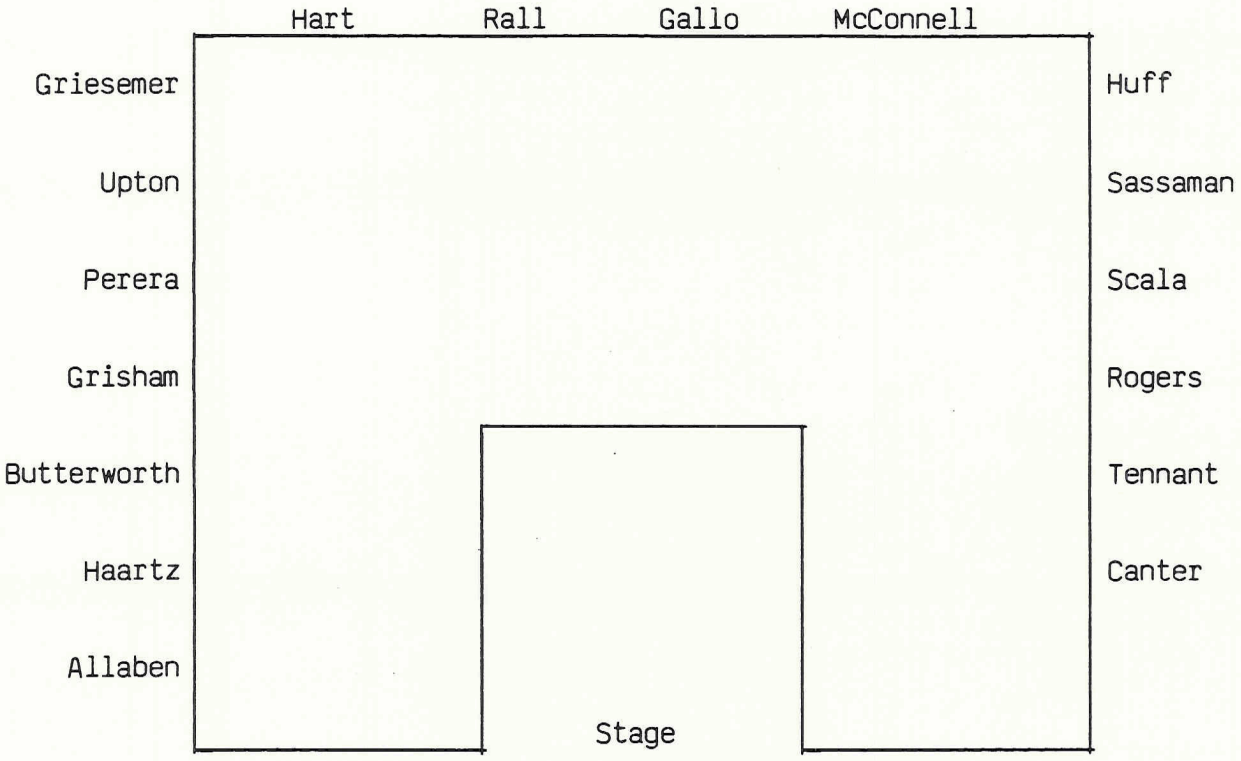
NTP BOARD OF SCIENTIFIC COUNSELORS MEETING

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

May 17, 1988



Summary Data on Chemicals for Review by the NTP Board of Scientific Counselors
On May 17, 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
1. Anthraquinone (84-65-1)	NIEHS	1.0×10^5-1.0×10^6 (1977)[a]	28[b]	Positive in Salmonella	--	-Chronic toxicity -Carcinogenicity (Moderate)	3,4	-Potential for increasing human exposure -Structural interest - parent compound for anthraquinone chemical class
2. Camphor (76-22-2)	NCI	2.2×10^5-2.2×10^6 (Imports, 1977)[a] 1.1×10^6 (Imports, 1985)[c]	4,400[b]	--	--	-Carcinogenicity -Teratology and reproductive effects studies (Moderate - High)	8	-Extensive use -Significant human exposure -Lack of carcinogenicity data
3. 1-Chloro-2-bromoethane (107-04-0)	NCI	Listed in TSCA Inventory but no definitive production data available[a,d]	--	--	--	-Carcinogenicity (Low)	3	-Structurally related to the carcinogens, 1,2-dichloroethane and 1,2-dibromoethane -Lack of carcinogenicity data -Recommendation dependent on commercial availability of chemical
4. Glyoxal (107-22-2)	TSCA Interagency Testing Committee	Listed in TSCA Inventory but no production data given 1.6×10^8 (Imports, 1985)[c]	9,649[b]	--	--	-Carcinogenicity (Inhalation) -Teratogenicity (High)	1,3,4	-Increasing use -Potential for exposure -Widespread environmental occurrence -EPA, OSHA interest in testing -Structurally similar to formaldehyde

ATTACHMENT 3

Chemical (CAS Number)	Nomination Source	Domestic Production (lbs)	Worker Exposure	NTP Testing Status	Other	Chemical Evaluation Committee Recommendation (Priority)	Chemical Selection Principles	Rationale/Remarks
5. Lead oxide (PbO) (1317-36-8)	NIOSH	3.0-14.2x10^{8} (1977)[a]	513[b]	--	--	-Acute and subchronic comparative toxicity studies of lead oxide and lead sulfide (Moderate)	3,8	-Data needed by Mining Safety and Health Administration to set TLV for mine workers -Studies should include determination of blood lead levels and investigation of blood parameters
6. Lead sulfide (PbS) (1314-87-0)	NIOSH	1.0-10.0x10^{6} (1977)[a]	7[b] 1,600[e]	--	--	-Acute and subchronic comparative toxicity studies of lead oxide and lead sulfide (Moderate)	3,8	-Data needed by Mining Safety and Health Administration to set TLV for mine workers -Studies should include determination of blood lead levels and investigation of blood parameters

Footnotes

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

b) National Occupational Exposure Survey (1980-1983). Department of Health and Human Services, National Institute for Occupational Safety and Health.

c) U.S. Department of Commerce. U.S. Imports for Consumption and General Imports. Washington D.C.: Bureau of the Census. Report FT 246/Annual 1986.

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

f) Personal communication from Dr. J. Haartz, NIOSH, to the Chemical Evaluation Committeee. December 9, 1987

Testing Recommendations for Chemicals Reviewed by NTP Board of Scientific Counselors
on May 17, 1988

Chemical (CAS Number)	Nomination Source	Testing Recommendation (Priority)	Rationale/Remarks
1. Anthraquinone (84-65-1)	NIEHS	-Chronic toxicity -Carcinogenicity (Moderate)	-Environmental contaminant -Potential for human exposure -Structural interest
2. Camphor (76-22-2)	NCI	-Percutaneous absorption study -Dermal subchronic studies -Dermal neonatal toxicity study (High) -Carcinogenicity (Moderate) -Teratology and reproductive effects (Moderate to high)	-Widespread use -Significant human exposure by various routes -Lack of toxicity data -Chemical to be re-evaluated by Board prior to initiation of carcinogenicity studies
3. 1-Chloro-2-bromoethane (107-04-0)	NCI	-Carcinogenicity (Low)	-Structural interest -Chemical no longer used as a fumigant -Raise priority if use of chemical is increased
4. Glyoxal (107-22-2)	TSCA Interagency Testing Committee	-Carcinogenicity (Moderate to high) -Defer teratogenicity testing	-Potential for exposure -Significant environmental occurrence -Structurally related to formaldehyde -Board to reconsider chemical for teratogenicity testing when further information on rationale is available
5. Lead oxide (PbO) (1317-36-8) 6. Lead sulfide (PbS) (1314-87-0)	NIOSH NIOSH	(-Acute comparative toxicity studies (of lead oxide and lead sulfide ((High) (((	(-Concern over lead toxicity (-Studies should include determination of blood (lead levels, and investigation of biomarkers (-NTP should propose a study to investigate the neuro- (toxicity of lead and its salts, in particular, their (effect on brain development; proposed study (to be submitted to Board for review

ATTACHMENT 4

5/13/88

SHORT SUMMARY OF GSRI STUDIES BY PROPOSED CATEGORY, COMPLETION STATUS AND OTHER STUDY STATUS

Chemical and study by proposed reporting category	No Slides prepared	Slides prepared	SP evaluation	PWG review	Draft TR	TR peer reviewed	TR printed	Other Studies
1. Data Not Fully Reliable or Interpretable.								
Vinylidine chloride, R&M							X	
1,1,1,2-Tetrachloroethane, R&M							X	
Pentachloroethane, R&M							X	
Tetrachloroethylene, female M					X			TR 311, inhl.
Sodium 2-Ethylhexyl sulfate, R&M						X		
Ethoxylated dodecyl alcohol, R&M						X		
Fluorescein sodium, R&M						X		
Sodium dodecyl sulfate, R&M					X			
Gilsonite, R&M						X		
Hamamelis Water, R&M						X		
1,2-Epoxyhexadecane, R&M					X			
2. Data Less Reliable Than One.								
Tetrachloroethylene, R, four strains					X			TR 311, inhl.
Dichloromethane, R&M						X		TR 306, inhl.
1,1,1,-Trichloroethane, R&M						X		C planned
Pyridine, R&M					X			C designed
DMBA/TPA, R					X			
3. Studies Not Reliable Or Not Complete.								
DMBA/TPA, M&CD-1 strains					X			C complete
Ethylene glycol monoethyl ether, R&M			X					RD underway
Trichlorfon, R&M					X			SC underway
t-Butanol, R&M			X					C underway
Castor oil, R&M					X			SC underway
Sodium xylene sulfonate, R&M			X					SC underway
Diethanolamine, R&M			X					SC underway
Glutaraldehyde, R&M			X					SC underway
Coconut diethanolamide, R&M			X					
Oleic acid diethanolamide, R&M		X						
Lauric acid diethanolamide, R&M		X						
Methyl ethyl ketone peroxide, R&M		X						SC complete
Chloramine, R&M	X							C complete
p-Nitrophenol, CFW mouse	X							C complete
Maleic hydrazide diethanolamide, R&M	X							IARC & industry

Abbreviations: R, F344 rat; M, B6C3F$_1$ mouse; RD, 14-day repeated dose studies; SC, 90-day subchronic studies; C, 2-year chronic studies; SP, study-pathologist; PWG, pathology working group; TR, NTP Technical Report

ATTACHMENT 5

Dr. Judson Spalding &
Dr. Robert Langenbach
Cellular and Genetic
Toxicology Branch
919/541-7936 (JS)
919/541-7558 (RL)
May 2, 1988

CONCEPT REVIEW

TITLE: In vitro and in vivo short term test characterization of the toxicity of rodent nonelectrophilic carcinogens

PERIOD AND TYPE OF AWARDS: 5 years - Research and Development Contract;
Part A: Two awards anticipated; $200,000/yr.
Part B: One award anticipated; $125,000/yr.

FUNDING MECHANISM: Competitive Contract Award

PART A.

Objective

A significant number of the chemicals which are classified as carcinogenic in the rodent bioassay appear to be nongenotoxic (Weisburger and Williams, 1981; Tennant et al., 1987). In 1984, an ICPEMC committee considered the basis for distinguishing between genotoxic and nongenotoxic carcinogens (ICPEMC 1984). More recently, Ashby and Tennant (1988) using the NCI/NTP bioassay database reported that a common characteristic of the nongenotoxic chemicals was their nonelectrophilicity. As a result of the characteristics of this class of carcinogens, they cannot be detected nor well studied in the currently available short-term genetic toxicity assays. Therefore, in the present concept two approaches are proposed to investigate these nonelectrophilic chemicals which will better define their modes of biological activity and also, hopefully, lead to the development of short-term tests that will permit their detection.

A. One group of these nonelectrophilic chemicals induces liver tumors as a major tumor type, yet these chemicals can be defined as nongenotoxic both in in vitro and in vivo assays which have utilized hepatocytes from the same sex/species in which the liver tumors were induced. However, among the limited number of these same hepatocarcinogens which have been studied so far, it has been demonstrated that they have the ability to induce scheduled DNA synthesis (SDS) which is a prerequisite for hepatocellular proliferation. It appears likely that for most of these latter chemicals, the induction of SDS occurs in response to hepatotoxicity, (Mirsalis et al., 1985; Spalding et al., 1988; and Mirsalis, 1988). But at least one of these nonelectrophilic chemicals, di(2-ethylhexyl) phthalate, has proven to be a liver cell mitogen (Butterworth, 1987). The mechanism of hepatotoxicity expression and the relevance of possible chronic hepatocellular proliferation to the induction of liver tumors by these

chemicals is unknown. Using similar protocols of chronic dosing and chemical administration that were used in the chronic bioassay, we propose under a regimen of repetitive dosing to: (1) confirm that these nonelectrophilic chemicals are not genotoxic in the target organ (liver); (2) measure the extent of liver toxicity by serological endpoints; and (3) determine whether or not the chemicals induce chronic cell proliferation over a dose range that includes that used in the rodent carcinogenesis bioassay.

The purpose of measuring these endpoints is to increase our understanding of how the proliferative or mitogenic response may be related to the hepatocarcinogenicity of these chemicals. Further, in order to increase our knowledge of the the biological properties of these chemicals, the small group of chemicals selected for study in Part A, will also be included in the larger group of chemicals selected for study in Part B.

Summary

Goal 1. To determine the patterns of hepatotoxicity and hyperplasia for a selected group of rodent nonelectrophilic hepatocarcinogens which are representative of the group of nonelectrophiles identified by Ashby and Tennant, 1988.

Goal 2. To determine whether several different in vivo endpoints, e.g., DNA damage/repair - or the lack of it - and the initiation of scheduled DNA synthesis (S-phase) are relevant to, or associated with, the initiation and progression of rodent tumorigenesis under a multiple dosing protocol that includes the dosing range used in the rodent carcinogenesis assay.

Outcome

1. Confirm that the selected group of chemicals do not interact directly with DNA.

2. Determine the temporal relationships between S-phase and hepatoxicity under conditions of multiple dosing.

3. Possibly determine that the sex/species specificity for hepatocarcinogenicity correlates with the sex/species patterns of hepatotoxicity and hyperplasia using the endpoints described.

4. The hepatocarcinogens studied will be characterized for the four different biological effects associated with tumor promoters that have been described in Part B of the concept proposal.

PART B.

The second approach is designed to obtain information about the biological/ biochemical effects of the nonelectrophilic carcinogens in biological systems which have been shown to be responsive to tumor promoters. This approach is based on the observations to date that show that different nonelectrophilic carcinogens participate in carcinogenesis by diverse mechanisms and that no single mechanism (or assay system) has been shown to account for the activities of all of these chemicals (Langenbach et al., 1988b in press). Therefore, to gain insight into their possible modes of action, four different biological effects which have been shown to be associated with tumor promoters will be studied.

These four systems are: (1) foci enhancement in an initiation-promotion cell transformation system; (2) interruption of cell-to-cell communication in the V79 cell system (Bohrman et al. 1988); (3) direct activation of protein kinase C or indirect activation via the induction of second messenger activity; and (4) induction of cytochrome P-450 activity (Nims et al. 1987) in the liver of F344 rats and B6C3F_1 mice. These systems were discussed at a recent meeting at NIEHS (Langenbach et al. 1988a). It is anticipated that some nonelectrophiles will show activities in varying numbers of these systems. However, some may not show any of these activities, a finding which in itself will be informative. Chemicals which do show biological activity in one or more of the above systems will then be analyzed for structure/activity relationships and the possible interrelationships of the observed biological effects. It is hoped that a classification of these chemicals based on the biological effects and the known bioassay results (including tumor sites) will result in an improved understanding of their biological mechanisms.

The following major outcomes are expected. First, it will be possible to group the nonelectrophilic chemicals by biological effects/mechanisms in these systems. Second, possible structure/activity relationship may be identified. Third, the possible association of activity in each of the four systems with tumors site(s) in the rodent will be determined. Finally, it is hoped that a better mechanistic understanding(s), including possible common underlying or interrelated mechanisms, of this class of chemicals will emerge.

The studies described in Parts A and B of this proposal will be coordinated in a manner such that the chemicals studied in Part A will be included among the chemicals studied in Part B.

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Concept Review: Revision and Summary May 12, 1988

Title: In vitro and in vivo short term test characterization of the toxicity of Rodent nonelectrophilic carcinogens

Dr. Robert Langenbach &
Dr. Judson Spalding
Cellular and Genetic
Toxocology Branch

Period and Type of Awards:
5-years - Research and Development Contract:
Part A: Two awards anticipated: $200,000/yr.
Part B: One award anticipated: $125,000/yr.

Funding Mechanism: Competitive Contract Award

Part A.

Objectives:

1. To test the following hypothesis:
   The chemical induction of scheduled DNA synthesis (SDS or S-phase) in rodent liver cells is an indicator of the potential hepatocarcinogenicity of that chemical

2. To develope the S-phase endpoint as an assay to detect hepatocarcinogens, especially those that are defined as being nongenotoxic.

Background:

1. The in vitro DNA damage/repair (UDS) assay in rodent hepatocytes:
   sensitivity?; specificity?; predictivity?;

2. The in vivo-in vitro UDS assay in rodent hepatocytes:
   sensitivity?; specificity?; predictivity?;

3. Induction of scheduled DNA synthesis:
   Retrospective Study:
   a. 4/4 genotoxic hepatocarcinogens induce S-phase in the appropriate sex/species
      - bis(2-chloro-1-methylethyl)ether
      - (H.C. Blue 1 vs H.C. Blue 2)
   b. 7/8 nongenotoxic hepatocarcinogens induce S-phase in the appropriate sex/species
      - carbon tetrachloride; chloroform
      - (1,1,2,2-tetrachloroethane)

Priority Questions:

1. Specificity of the assay:

a) sex/species; re: hepatocarcinogenicity

-bis(2-chloro-1-methylethyl)ether

-, -, ; L+, +

-selenium sulfide L,- ; -,L

-1,2-dichloropropane -,E ; L,L

2. Sensitivity and specificity of the assay:
Carcinogens and noncarcinogens

a) genotoxins:

1) hepatocarcinogens

- 2,4-diaminotoluene vs (2,6-diaminotoluene)
- H.C. Blue 1 vs (H.C. Blue 2)
- furan

2) nonhepatocarcinogens

- o-anisidine
- 1,2,-dichloroethane
- dimethyl hydrogen phosphite

3) noncarcinogens - see part 1)

b) nongenotoxins:

1) hepatocarcinogens

- cinnamyl anthranilate
- dichloromethane
- 1,4-dioxane
- methyl carbamate

2) nonhepatocarcinogens

- phenesterin
- nitriloacetic acid
- reserpine
- N,N-diethylthiourea

3) noncarcinogens

- bisphenol A
- DL-menthol

4) hepatotoxic noncarcinogens

- acetaminophen
- lead nitrate

1. (Specificity of the assay: re: sex/species)

2. (Specificity and Sensitivity of the assay: Carcinogens and noncarcinogens)

3. Can the S-phase endpoint be developed into a short term in vivo assay that detects potential hepatocarcinogens?

4. Is the S-phase endpoint especially useful for detecting nongenotoxic hepatocarcinogens?

5. Is a repetitive dosing protocol more optimal for S-phase induction than a single acute dose?

The nongenotoxic chemicals selected for determining the sensitivity and specificity of the S-phase endpoint will also be included in the studies described by Dr. Langenbach in Part B of this proposal.

Dr. Robert Langenbach
Cellular and Genetic
Toxicology Branch
919/541-7558
May 2, 1988

CONCEPT REVIEW

TITLE: DNA adducts in rodent and human tissues

PERIOD AND TYPE OF AWARDS: 5 years - Research and Development Contract;
One award anticipated; $200,000/year

FUNDING MECHANISM: Competitive Contract Award

After establishing the carcinogenicity of a chemical in the rodent bioassay, a major challenge is the extrapolation of rodent carcinogenicity data to humans. The magnitude of the challenge of rodent to human extrapolation becomes more obvious when one considers the difficulties of even extrapolation from mouse to rat (or vice-versa) when carcinogenesis data for only one species is known. This extrapolation process could be aided by a knowledge of species differences in biological/biochemical effects induced by the chemicals. Some factors which contribute to species differences in response to chemical carcinogens are known (Langenbach et al, 1983), and it is generally agreed that differences in metabolism are a major factor. While total metabolism of a chemical is generally not predictive of tumorigenesis, an aspect of the metabolic process that is important in determining the carcinogenic effect of a genotoxic chemical is the amount of DNA reactive intermediates formed. DNA adducts therefore are a measure of a chemical DNA reactivity. Furthermore, DNA adducts can serve as a dosimeter (Perera, 1987) of the biologically effective dose of a chemical that a tissue/organ receives. The purpose of this study is to obtain DNA carcinogen adduct data that ultimately will be useful in human monitoring approaches and also for rodent to human carcinogenesis extrapolation.

The approaches will be divided into two major parts:

A. First, the nature and amount of DNA adducts in target organs in the rat and/or mouse will be determined. The DNA adducts in the target tissue will then be compared to adducts in a nontarget tissue and to those in circulating lymphocytes in the same species. A dose response study of the chemical(s) from the doses which gave a tumorigenic response to low doses which are not statistically detectable as tumorigenic in the bioassay will be conducted. Chemicals which are carcinogenic in both species as well as those that are carcinogenic in one species but less potent (or even non-carginogenic) in the other species will be utilized. Species differences in DNA adduct removal will also be determined. Liver and lung will be the major rodent target tissues studied. Chemicals studied in the NTP bioassay which induce tumors at these sites will be utilized and DNA adducts will be measured by ^{32}P-post labeling (Gupta, 1987). For chemicals of overlapping interest this project will be coordinated with Dr. Caspary's (see accompanying concept).

The following major outcomes are expected. First, DNA adduct dosimetry as it relates to carcinogenic potency in target rodent tissues will be obtained. Second, data relevant to low-dose extrapolation will be obtained. Third, DNA dosimetry in lymphocytes and target tissue will be compared and the results will be informative in understanding the validity of using circulating blood cells as a measure of target tissue exposure. Fourth a better understanding of the role of DNA adducts in determining target vs. nontarget tissues should be obtained.

B. In the second approach DNA adducts from both rodent and human tissue will be analyzed. Studies have indicated that for many carcinogens common tumor sites exist between test species and humans (Wilbourn et al. 1986). Liver will be the major tissue studied; however, when possible lymphocytes from the human liver tissue donors will be obtained and utilized. DNA adducts will be measured by ^{32}P-post labeling.

DNA adducts from target rodent tissues will be determined from in vivo exposures as described above. However, because DNA adducts from human tissues exposed in vivo would not be available, human hepatocytes would have to be exposed in vitro to the chemical. Therefore, before the approach could be utilized, the relationship of adducts formed by rodent liver in vivo to adducts formed by rodent hepatocytes in vitro would have to be established. After establishing the relationship of in vivo to in vitro adducts, human hepatocytes would be exposed to the chemical in vitro. The estimation of human liver adducts in vivo would then complete this parallelogram approach. Extensive dose response studies in vitro would be conducted to detect possible threshold differences between human and rodent hepatocytes. Human lymphocytes would also be exposed in vitro at various doses to determine possible differences between lymphocytes and hepatocytes from the same individual.

The following major outcomes are expected. First, differences (or similarities) in DNA adduct formation in rodent and human liver will be determined and these data will be useful for the quantitative extrapolation of rodent carcinogenesis data to humans. Second, the extent of interindividual variation in adduct formation in human hepatocytes and lymphocytes will be determined. Third, a better understanding of the relationship between adduct dosimetry in human lymphocytes and adduct dosimetry in human liver will be obtained.

For both parts A and B, animal to animal variation with the same sex/species in DNA adduct formation are expected to be low (Harris, 1985). However, interindividual variation with human tissue samples is expected to be large (Harris, 1985,1987; Rudo et al. 1987) and therefore it is estimated that at least 10 human specimens will be needed for each chemical studied. It is anticipated that an average of 5 chemicals per year over a 5 year period can be studied in part A. In part B only 1-2 chemicals per year will be studied.

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Dr. William Caspary
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Toxicology Branch
919/541-2150
May 2, 1988

TITLE: Chemically Induced DNA Modifications in vivo and in vitro

PERIOD AND TYPE OF AWARDS: 5 years - Research and Development Contract;
Four awards are anticipated; $300,000/yr.
Initiation Date: Summer 1989

FUNDING MECHANISM: Competitive Contract Award

OBJECTIVES:

1. The Cellular and Genetic Toxicology Branch has been examining the mutagenic activity of compounds that have been tested in the NTP rodent carcinogenicity assay. At present, there are carcinogenicity and mutagenicity results (Salmonella reversion, mouse lymphoma forward mutations, CHO aberration and SCE results) for over 260 chemicals. From this group of chemicals, a number have been tentatively identified as nonmutagenic carcinogens and mutagenic noncarcinogens. This concept will emphasize, though not be restricted to, studies to be performed with this discordant group of chemicals.

DNA adducts are measures of genotoxicity of a chemical. They have been implicated as primary lesions leading to mutagenesis and carcinogenesis. They can be measured in vivo and in vitro. An objective of this effort is to measure adducts in target and non-target tissue of rodents and in cells used for mutagenicity studies. This will provide further information on the validity of such terms as nongenotoxic carcinogens and genotoxic noncarcinogens. As a secondary result of this study, chemicals will be categorized into classes defined by their responses to adduction and its repair, and to carcinogenic and mutagenic activity of chemicals. This categorization will be useful in designing future experiments to understand the host response to these chemicals. For example, compounds that do not adduct to DNA and are not mutagenic might be candidates for studies on their ability to modulate CpG methylation patterns in the genome.

2. Much work has been performed on the relationship between adducts in bulk DNA and carcinogenesis and mutagenesis. While relationships are often found, they are generally not universal. Lack of concordance between DNA adduction and tumor formation may be due to our inability to examine adduction at the appropriate target. There is evidence that results of bulk DNA adduction and CpG methylation studies can differ significantly from those on specific regions of DNA. Therefore, another objective of this effort is to study the induction of DNA lesions and the repair of these lesions at specific functional and structural regions of the chromatin and to examine CpG methylation patterns at these sites. Cells used in mutation studies and target and nontarget tissue in rodents will be studied. Three areas will be emphasized: 1. Genomic regions associated with the nuclear matrix are actively transcribed and will be examined for adducts and CpG methylation patterns. 2. DNA sequencing techniques will be

used to examine specific sequences that are especially susceptible to adduction. Methylation patterns at these sites will be examined since atleast some carcinogens seem to preferentially bind to guanine rich regions of the genome and these adducts can inhibit methyl transferase. 3. Adducts at specific genes such as various oncogenes or selectable genes will be monitored for adduction and methylation. These endpoints will be compared within a cell system to see if DNA modification at one gene is representative of modifications at another gene and between rodent tissue and cells in culture.

Note: This branch is developing a transgenic mouse model to be used in mutagenesis studies. A long term goal of this second objective would be to link techniques that will be developed for measuring DNA adducts at specific genes with the transgenic mouse model for mutagenesis.

BACKGROUND:

In Vivo and In Vitro Tests Used to Identify Mutagens and Carcinogens:

Carcinogens do not always induce tumors uniformly in both sexes and/or in different rodent species. In a recent analysis of 73 chemicals tested by the NTP (Tennant et al, 1987), the concordance in the carcinogenic responses between the rat and mouse was 67%. This was not significantly different from the historical concordance of 74% for rat and mouse (198 of 266) for all chemicals studied by the NTP (Huff et al., 1986).

For the 73 NTP chemicals, the concordance between carcinogenicity results in the rodent bioassay and mutagenicity results in each of four commonly used in vitro assays was approximately 60%. In these studies, a number of carcinogens have been identified that were not able to induce a mutagenic response in any of the mutagenicity assays under the protocols used. These chemicals are tentatively identified as non-mutagenic carcinogens and it is presumed, until evidence shows otherwise, that the mechanism of tumor formation does not involve DNA damage. Similarly, compounds have been identified that are mutagenic noncarcinogens.

The discrepancies in tumorigenic responses between different species and opposite sexes of the same species and the discrepancies between in vitro and in vivo results provide an opportunity to examine these systems further in an attempt to understand the mechanisms leading to the lack of agreement.

DNA Adducts

The factors that influence the extent of adduction and the identity of the adduct produced by a particular chemical are not completely understood. For alkylating agents that favor SN2 chemistry, reactions tend to occur at sites with relatively high nucleophilic strength such as N7 of guanine. As the reaction mechanism shows more SN1 character, exocyclic sites such as O6 of guanine will attract more adduction. While O6 and N7 guanine reactions appear to dominate the reactions of alkylating agents with DNA, a different pattern appears with larger carcinogens and mutagens. Polycyclic aromatic hydrocarbons prefer N2 of guanine and many aromatic amines react at C-8 of guanine. Thus, it

is difficult to discern any relationship between site specific reactivity and mutagenesis and carcinogenesis. However, for chemical analogs that modify similar sites, correlations between levels of binding and carcinogenicity and mutagenicity are often found.

Gene Specificity for Induction and Repair of DNA Damage

In most of the studies attempting to relate DNA damage and biological endpoints such as carcinogenesis, mutagenesis, and survival, it has been assumed that induction and repair of DNA damage is uniform throughout the genome. It has also been assumed that the genome is uniformly mutable. Most DNA adduction and repair studies have been performed on bulk DNA. The genome is, however, functionally and structurally heterogeneous. It would not be surprising if the effects of the induction and repair of critical DNA lesions depended on the accessibility of particular genomic regions to the chemical and to repair enzymes and could not be inferred from studies on bulk DNA.

A number of compounds have been found to preferentially bind to regions of chromatin that are digestible by DNase I (Bailey et al, 1980; Cox, 1979; Pegg and Hui 1978; Baranyi-Furlone and Goodman, 1984). Other workers have exploited the ease of isolation of repetitive sequences in genomic DNA by the use of restriction enzymes (Gupta, 1984; Leadon and Hanawalt, 1984; Zolan et al, 1984). While these studies show that the induction and repair of DNA lesions can be specific for certain regions of the genome, the significance of these studies is not clear because of the uncertainty in the function of these regions. However, more relevant regions of the genome have been studied. Perhaps the most significant are those which have shown that DNA associated with specific genes, with specific sequences or with the nuclear scaffold show responses to the induction and repair of chemically induced DNA lesions different from bulk DNA.

Actively transcribed genes show different adduction patterns than non-transcribed DNA. For example, Gupta et al (1985) fractionated DNA loops into three regions and found non-random persistence of the deacetylated AAF-guanine adduct with repair being markedly less efficient at lesion sites close to the loop's attachment to the scaffold. Benzo(a)pyrene adducts also showed site specific adduction with DNA at the region of the DNA loop's association with the matrix (Hemminki and Vainio, 1979; Ueyama et al, 1981; Mironov et al, 1983). While these and other investigators have isolated DNA in association with the nuclear matrix, the transcriptional activity of genomic material in each of the isolated fractions has only been assumed and not shown in concurrent studies. Obi et al (1986) and Ryan et al (1986) have attempted to deal with this criticism by measuring the distribution of transcribed and non-transcribed DNA sequences in four liver subnuclear fractions by dot-blot hybridization. Actively transcribed genes had different adduction patterns than the remainder of the DNA in the genome.

In 1985, Bohr et al introduced a new approach to identify adducts at specific sequences of DNA. They quantified the repair of thymidine dimers after uv irradiation in the active dihydrofolate reductase (DHFR) gene that was amplified

50-fold. Restricted genomic DNA was treated with T4 endonuclease V, electrophoresed and hybridized to an DHFR probe. The T4 endonuclease generates strand breaks at pyrimidine dimer sites and this technique is limited to examining compounds that induce pyrimidine dimers. The proportion of fragments free of endonuclease sensitive sites in each sample was determined from the difference in the amount of probe hybridized at the position of full length fragments for enzyme treated and untreated samples.

Using these techniques, Bohr et al (1985, 1986) found that more than two thirds of the dimers were removed from a 14.1 kb restriction fragment of the gene 26 hours after irradiation (20 J/sq m) while little removal was detected in upstream fragments and 15% removed from the bulk genome. These authors also investigated the paradoxical phenomenon that rodent cell lines typically exhibit uv resistance similar to that of repair proficient human cell lines even though they repair such damage with much less efficiency. Examining DNA repair at the DHFR gene in human and rodent cells, they found similar repair capacity, even though there were differences at the bulk DNA level.

The most general method to date that measures adduction at specific genes involves the use of ABC excinuclease (Thomas et al, 1985). This method (Thomas et al, in press) is similar to the method using T4 endonuclease but has greatly expanded the spectrum of adducts that can be monitored. Using this method, they have studied adducts induced by benzo(a)pyene, mitomycin C, psoralens, 4-nitroquinoline, platinum drugs, adriamycin, nitrogen and sulfur mustards, nitrous acid, carbodimide, and uv.

Chemical DNA interactions appear to be DNA sequence specific. Using DNA sequencing gels, Hartley et al (1986) have shown that, when plasmid DNA is treated with a series of three antitumor chloroethylating agents, the one with the most electrophilic character preferentially produced N7-guanine adducts in guanine rich regions of the genome while those with less electrophilic character showed no sequence specificity. Similar results were obtained with mustard analogs (Mattes et al, 1986). Benzo(a)pyrene diol-epoxide has also been shown to preferentially bind to guanine rich sequences (Lobanenkov et al, 1986).

These data suggest that DNA damage control can vary according to function or activity of the affected sequences and that bulk DNA damage may not provide an accurate picture of the role of induction of DNA damage and repair in mutagenesis and carcinogenesis. It also emphasizes the need to develop methods to examine DNA modifications at specific regions of the genome. If the location of the lesion on DNA is important in the initiation of mutagenesis and carcinogenesis, then many of our ideas about the mechanism of action of chemicals may have to be revised since previous data on bulk DNA may not be relevant.

Methylation

There is a form of adduction whose effects are considered to be epigenetic rather than genotoxic. The changes associated with this type of adduction are heritable from parent to daughter cell and can masquerade as mutations. For mutations, the heritable lesion is a change in base sequence (e.g., base pair

substitution, deletion, etc.). For the epigenetic effect, the heritable lesion is a methylated base. The methylation is specific for one base - the 5 position of cytidine located at the 5' end of a CG palindrome. A number of investigators have shown a correlation between hypomethylation at this position and gene expression (for review, see Doerfler, 1983).

The vertebrate genome is deficient in cytidine and guanine bases. There are about 30,000 clusters of CpG sites at the 5' end of many "housekeeping" genes, but these clusters or "islands" are not found in most tissue specific genes (Bird, 1985; Bird, 1987). The islands, unlike most of the DNA from vertebrates, are not deficient in CpG and are not methylated. It appears that these islands tend to extend downstream into the transcribed region, usually including the first one or two exons. CpG islands are likely to contain the gene's promoters. There is evidence suggesting that the degree of methylation of these islands is associated with the transcriptional state of the gene (Keshet et al, 1985). Since these islands apparently are not associated with tissue specific genes such as the g-globin gene, methylation may not affect the ability of these genes to transcribe.

More recent evidence (Melton et al, 1986; McGrogan et al, 1985; Sazer et al, 1986; Ishii et al, 1985a; Ishii et al, 1985b) suggests that the promoter regions for housekeeping genes do not contain the TATA box sequence found in the concensus promoter sequences of tissue specific genes. For example, the 5' flanking regions of HPRT and DHFR genes are CG rich. Interestingly, the epidermal growth factor gene and the Harvey ras oncogene also contain neither a TATA nor a CAAT box and are CG rich in the 5' flanking region. Tissue specific genes such as those for histones, immunoglobins, globins, etc, produce large amounts of RNA during certain phases of development whereas housekeeping genes direct RNA synthesis in all cells with less regulation and at relatively low levels. The CG rich regions are potential sites of binding of the Sp1 transcription factor. Bird (1985) has suggested that Sp1 may hinder methylation of cytosines in this region leading to a hypomethylated state. As mentioned previously, CG rich sites are also preferential sites for DNA adduction.

Not only do chemical carcinogens bind at CG rich sites in the genome, a number of them have also been shown to inhibit DNA-methyltransferase. In experiments performed by Wilson and Jones (1983) and Wojciechowski and Meehan (1984), DNA-methyltransferase was examined for its ability to transfer a methyl group from S-adenosylmethionine to hemimethylated DNA. Ten carcinogens were examined and all inhibited the methyl transfer reaction. Wilson and Jones (1983) examined the bulk DNA 5-methylcytosine content of CG palindromes in C3H/10T1/2 and BALB/3T3 cell lines after treatment with transformation inducing doses of benzo(a)pyrene and found that the 5-methylcytosine content was reduced in BALB/3T3 cell line. Methylation patterns at specific genes were not examined.

Wilson and Jones (1983) showed that alkylation of DNA by benzo(a)pyrene diolepoxide inhibited methyltransferase activity. However, adduct formation with this compound did not have to occur at the methylation sites to inhibit the enzyme. This suggested that the bulky adduct prevented accessibility of the substrate to the enzyme or inhibited the processive scanning function of the enzyme.

When compared with methylation patterns of adjacent normal tissue, DNA from benign colon polyps and malignant carcinomas from 23 tumors was substantially hypomethylated (Goelz et al, 1983). This suggested that hypomethylation is a consistent biochemical characteristic of human colonic tumors. Since these investigators also found that the hypomethylation patterns in benign and malignant tissue were similar, hypomethylation appeared to be an alteration that preceded malignancy. These authors also demonstrated that hypomethylation was not random, that different genes manifested different levels of hypomethylation and that hypomethylation was not as apparent in bulk DNA as in certain genomic sequences. In another study, Feinberg and Vogelstein (1983) examined the degree of methylation of two cellular oncogenes, c-Ha-ras and c-Ki-ras, in primary human carcinomas. The c-Ha-ras gene was hypomethylated in six of eight carcinomas, including five colonic adenocarcinomas and one small lung carcinoma when compared to adjacent normal tissue. For the c-Ki-ras gene two of the colon carcinomas were hypomethylated while six other tumor samples remained fully methylated when compared to controls.

FINAL COMMENTS

The themes presented in this review mirror the frustrations felt in the attempts to identify the initial lesions leading to cancer induction. It is believed that a tumor arises from a single cell, implying that the defect is heritable. Yet compounds have been identified that appear to be non-mutagenic (an hypothesis that will be challenged in this effort) and previous correlations linking tumorigenesis with DNA adduction have had only limited success. Along with the frustrations, however, new avenues have been suggested. Two of them, adduction at specific regions and CG methylation patterns are the basis for this concept.

The proposals that will be generated from this concept will consider the heterogeneity of the genome both in heritable methylation patterns and in the induction and repair of DNA lesions as two promising areas for further study. Heritability need not involve mutation and damage at one part of the genome need not represent damage at another. Initially, the work will involve compounds that are considered the "classical" carcinogens but will quickly expand to NTP chemicals with emphasis on the discordant class. Because the work will be directed from one source, it will be coordinated with the same chemicals examined in the different protocols and among the various laboratories that will be chosen to do the work. Meetings among the various personnel from the different laboratories and among an ad hoc committee to advise the project officer in overseeing these contracts will help in their coordination.

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