The National Toxicology Program

ANNUAL PLAN

Fiscal Year 2001

U.S. Department of Health and Human Services
Public Health Service

NATIONAL TOXICOLOGY PROGRAM

ANNUAL PLAN

FOR FISCAL YEAR 2001

National Institute of Environmental Health Sciences/National Institutes of Health
National Center for Toxicological Research/Food and Drug Administration
National Institute for Occupational Safety and Health/Centers for Disease Control and Prevention

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National Toxicology Program
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PREFACE

The National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH), the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA) form the core agencies comprising the National Toxicology Program (NTP). The NTP is headquartered at the NIEHS/NIH and Dr. Kenneth Olden, Director NIEHS/NIH, also serves as the NTP Director.

The NTP is an interagency program whose mission is to evaluate agents of public health concern by developing and applying the tools of modern toxicology and molecular biology. This involves conducting toxicological evaluations of substances of public health concern, developing and validating improved (sensitive, specific, rapid) testing methods, developing approaches and generating data to strengthen the science base for risk assessment, and communicating with all stakeholders. The NTP has always drawn strength and direction from its commitment to open information exchange, adherence to impartiality, and rigorous scientific peer review. Its vision, leadership, and commitment to the concept of good science for good decisions create an atmosphere that allows flexibility in the NTP's approach toward addressing public health concerns. The NTP plays a critical role in providing needed scientific data, interpretations, and guidance concerning the appropriate uses of data to regulatory agencies and other groups involved with health-related research. Through its interactive relationship with regulatory agencies, the NTP plays an indirect, but important role in shaping public health policy.

The Program maintains an objective, science-based approach in dealing with critical issues in toxicology. The NTP conducts research and sponsors workshops through its primary agencies (NIEHS/NIH, NCTR/FDA, and NIOSH/CDC) and leverages resources through cooperative and/or collaborative agreements with other Federal agencies, academia, and industry. These interactions enhance opportunities to conduct toxicological evaluations of targeted agents, to strengthen the science base regarding mechanisms of disease etiology, and to promote the development of novel and alternative toxicology methods. Current initiatives and plans for NTP research and testing for FY 2001 are summarized herein.

The NTP welcomes comment and constructive criticism of its programs and policies. Comments may be directed to NTP Liaison and Scientific Review Office (NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709 or liaison@starbase.niehs.nih.gov).

Kenneth Olden, Ph.D.
Director

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OVERVIEW OF THE NATIONAL TOXICOLOGY PROGRAM

MISSION AND GOALS

Today more than 80,000 chemicals are registered for use in commerce in the United States. An estimated 2,000 new ones are introduced annually to be used in products we encounter in our daily lives such as food, personal care products, prescription drugs, household cleaners, and lawn care products. The effects of many of these chemicals on human health are unknown, yet people and our environment may be exposed to them during their manufacture, distribution, use, and disposal or as pollutants in our air, water, or soil. While relatively few such chemicals are thought to pose a significant risk to human health, safeguarding the public depends upon identifying the effects of these synthetics as well as certain naturally occurring chemicals or substances and the levels of exposure at which they may become potentially hazardous to humans.

The Department of Health and Human Services (DHHS) established the National Toxicology Program (NTP) in 1978 and charged the NTP with coordinating toxicological testing programs within the Public Health Service of the Department; strengthening the science base in toxicology; developing and validating improved testing methods, and providing information about potentially toxic chemicals to health regulatory and research agencies, scientific and medical communities, and the public. The NTP is an interagency program whose mission is to evaluate agents of public health concern by developing and applying the tools of modern toxicology and molecular biology. In carrying out its mission, the NTP has several goals:

- to provide evaluations of substances of public health concern,
- to develop and validate improved (sensitive, specific, rapid) testing methods,
- to develop approaches and generate data to strengthen the science base for risk assessment, and
- to communicate with all stakeholders including government, industry, academia, the environmental community, and the public.

ORGANIZATIONAL STRUCTURE AND OVERSIGHT

Three agencies, the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH), the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA) form the core for this program (Figure 1). The NTP is located administratively at the NIEHS/NIH and the Director of the NIEHS/NIH serves as the NTP Director. The National Cancer Institute of the National Institutes of Health (NCI/NIH) was a charter agency of the NTP and continues to participate on the NTP Executive Committee. Questions and inquiries about the NTP can be directed to the NTP Office of Liaison and Scientific Review (919-541-0530 or liaision@starbase.niehs.nih.gov, see Communication and Public Outreach).

NTP Management

Dr. Kenneth Olden, Director NIEHS/NIH serves as NTP Director

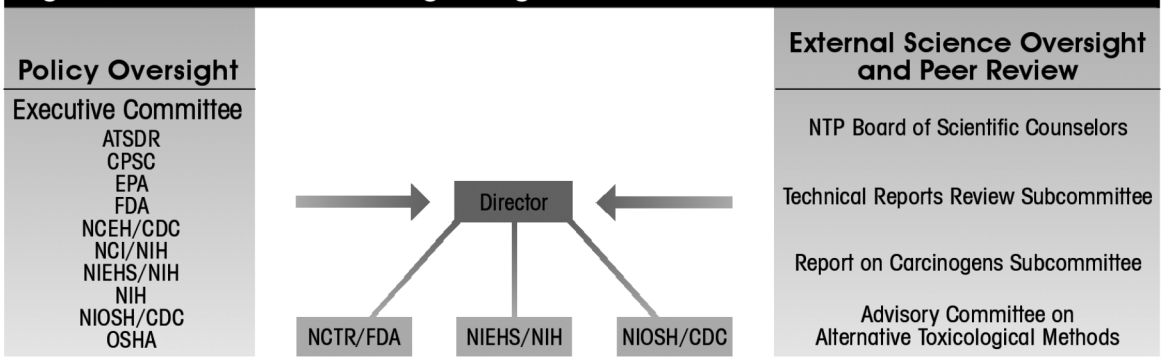
Dr. Christopher Portier, Director, Environmental Toxicology Program, NIEHS/NIH

Agency Program Management

NCTR/FDA: Dr. William Allaben, Associate Director for Scientific Coordination
NIEHS/NIH: Dr. Christopher Portier, Director, Environmental Toxicology Program
NIOSH/CDC: Dr. Albert Munson, Director, Health Effects Laboratory Division

Agency staffs involved with the Program and their contact information are provided in Appendix 1.

Figure 1. National Toxicology Program



ATSDR, Agency for Toxic Substances and Disease Registry; CPSC, U.S. Consumer Product Safety Commission; EPA, U.S. Environmental Protection Agency; FDA, Food and Drug Administration; NCEH/CDC, National Center for Environmental Health of the Centers for Disease Control and Prevention; NCI/NIH, National Cancer Institute of the National Institutes of Health; NCTR/FDA, National Center for Toxicological Research of the FDA; NIEHS/NIH, National Institute of Environmental Health Sciences of the National Institutes of Health; NIH, National Institutes of Health; NIOSH/CDC, National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention; OSHA, Occupational Safety and Health Administration.

Advisory Committees

NTP Board of Scientific Counselors

The NTP Board of Scientific Counselors (the Board) (Figure 1), composed of up to 35 scientists primarily from the public and private sectors, provides scientific oversight to the Program as well as to the NTP Center for the Evaluation of Risks to Human Reproduction. A list of the current membership (as of January 2001) is provided in Appendix 2. The Board's members serve terms of up to four years. Members of the Board are distributed among the parent committee and two subcommittees in order to provide the necessary scientific expertise to the Program. The Board's Technical Reports Review Subcommittee meets annually/semiannually and provides peer review of NTP long-term toxicology and carcinogenesis technical reports. This subcommittee also provides peer review by mail of NTP toxicity studies. The Report on Carcinogens Subcommittee of the Board provides external scientific evaluation and peer review of substances nominated for listing in or delisting from the *Report on Carcinogens* (see page 77). Information about the Board is available from the executive secretary, Dr. Mary S. Wolfe (NIEHS/NIH), and minutes from its meetings are accessible on the NTP web page (http://ntp-serve.niehs.nih.gov) or from Central Data Management (see page 5).

Advisory Committee on Alternative Toxicological Methods

The Advisory Committee on Alternative Toxicological Methods (ACATM) (Figure 1) meets biannually and provides oversight to the NTP on the Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Toxicological Methods (ICCVAM). It is composed of knowledgeable representatives from academia, industry, public interest and animal welfare organizations, other agencies, and the international community. The current roster (as of March 2001) is given in Appendix 3. The members serve rotating terms of up to four years. Summary minutes from these meetings are available through the NTP web page's link to NICEATM. In December 2000, under the ICCVAM Authorization Act of 2000 (PL106-545), a Scientific Advisory Committee was permanently established to provide guidance to NICEATM and ICCVAM (see page 74). The NTP will modify the composition and responsibilities of ACATM to comply with this law.

NTP Executive Committee

The NTP Executive Committee (Figure 1) provides oversight to the NTP for policy issues. This committee is composed of the heads of Federal health and regulatory agencies. Table 1 lists the current membership.

Table 1. NTP Executive Committee Membership Roster*

Member	Affiliation
Henry Falk, M.D., M.P.H., Assistant Administrator	Agency for Toxic Substances and Disease Registry
Bernard Schwetz, Ph.D., Acting Principal Deputy Commissioner	Food and Drug Administration
Richard D. Klausner, M.D., Director	National Cancer Institute of the National Institutes of Health
Richard Jackson, M.D., M.P.H., Director	National Center for Environmental Health of the Centers for Disease Control and Prevention
Lawrence Fine, M.D., Dr.P.H., Acting Director (through May 2001) Kathleen M. Rest, Ph.D., MPA, Acting Director (beginning June 2001)	National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention
Kenneth Olden, Ph.D., NTP Director	National Institute of Environmental Health Sciences of the National Institutes of Health
Christopher J. Portier, Ph.D., Director, Environmental Toxicology Program (Acting Director prior to June 2001)	National Institute of Environmental Health Sciences of the National Institutes of Health
Ruth Kirschstein, M.D., Acting Director	National Institutes of Health
Vacant, Assistant Secretary of Labor for Occupational Safety and Health	Occupational Health and Safety Administration of the U.S. Department of Labor
Ann Brown, Chairman	U.S. Consumer Product Safety Commission
Christine Todd Whitman, Administrator	U.S. Environmental Protection Agency

*Membership as of June1, 2001.

ADDRESSING SCIENTIFIC AND REGULATORY OPPORTUNITIES

The NTP uses its goals to set priorities as it moves forward to improve the nation's ability to evaluate human health effects from chemical exposures. Its vision, leadership, and commitment to the concept of good science for good decisions create an atmosphere that allows the Program to be flexible and innovative in its approach toward addressing public health concerns related to chemical exposures at home and work and in our environment. The NTP has expanded its scope beyond cancer to include examining the impact of chemicals on non-cancer toxicities such as those affecting reproduction and development, and the immune, respiratory, and nervous systems. As part of this effort, the NTP Center for Evaluation of Risks to Human Reproduction was created.

The NTP recognizes that initiatives addressing critical knowledge gaps in toxicological evaluations offer the best opportunities for preventing environmentally mediated diseases. Therefore, the Program's testing of chemicals is evolving to include more mechanism-based toxicology studies that focus on understanding the mode of actions of chemical agents. In recent years, the NTP has placed a greater emphasis on providing human context to the interpretation and understanding of toxicological information generated using animal or *in vitro* cell models. This is imperative in order to be at the forefront in studying and developing risk assessment methodologies for quantifying the sequence of events that starts with chemical exposure and ends with toxicity. Examples of activities it covers include:

- an increased effort to collect information on exposures, either environmental or occupational, and on substances or mixtures of concern;
- the increased application of mechanistic information and scientific judgement in the deliberations for listings in the *Report on Carcinogens*; and
- an enhanced effort to examine the merits of alternative testing methods that may give better information than current models using fewer animals, causing less pain or distress, and hopefully providing improved data to reduce uncertainties in risk assessments.

Nationally, the NTP rodent bioassay is recognized as the standard for identification of carcinogenic agents; however, the NTP continues to work to reduce the use of experimental animals and to develop and validate alternative testing methods. This effort has led to creation of the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods.

The strengthening of existing partnerships and the forging of new ones are important to achievement of the NTP's goals. Partnerships with sister Federal agencies are increasing and the NTP continues to collaborate with the private sector. Examples include co-sponsorship of numerous workshops, an interagency initiative in exposure assessment, establishment of an interagency committee (Interagency Coordinating Committee on the Validation of Alternative Methods) to oversee validation of alternative testing methods, and an interagency initiative to characterize occupational exposures. The NTP is supporting an effort to evaluate the phototoxicity of various compounds through establishment of the FDA-NIEHS Phototoxicology Research and Testing Laboratory and its designation as an NTP Center for Phototoxicology. In addition, the NTP is playing a role in providing toxicological assessments of water disinfection by-products and will provide this information to the U.S. Environmental Protection Agency (EPA) for its use in setting water standards.

Regulatory agencies make decisions for the protection of public health based on scientific information from multiple sources (e.g., toxicology, human studies, and basic research). The NTP plays a critical role in providing needed scientific data, interpretations, and guidance concerning the appropriate uses of these data to regulatory agencies as well as other groups involved in health-related research. The Program is committed to using the best science available in setting priorities for future studies and in designing, conducting, and interpreting the findings of those studies. The American people and government agencies at State and Federal levels rely on the science base provided by the NTP in making credible decisions that will protect public health without unnecessarily increasing the regulatory burden on industry. Over the past two decades, the NTP has developed an increasingly interactive relationship with regulatory agencies and through this relationship has played an important, although indirect, role in shaping public health policy. The Program maintains an objective, science-based approach in dealing with critical issues in toxicology. Recognition of this fact by all participants in the public debate over the regulation of chemicals has afforded the NTP the status of an "honest broker" in the continuing dialogue concerning the appropriate application of scientific advances to applied toxicology research and testing.

COMMUNICATION AND PUBLIC OUTREACH

Maintaining open communications and ensuring dialogue with Federal and State agencies, industry, stakeholders, academia, and the public are goals of the NTP. NTP advisory groups (see page 2) ensure regular scientific and public peer review and input. NTP conferences and workshops remain a priority and are designed to bring researchers, regulators, policy makers, and the public together to examine issues and achieve consensus on future directions in toxicology and risk assessment. Emphasis continues on ensuring broad dissemination of the results of NTP research and testing and communicating information about its evolving programs and priorities. The distribution of NTP testing and research results and its program plans, initiatives, announcements, press advisories, and publications occur through mailings, *Federal Register* announcements, and the world-wide-web that includes a subscription-based NTP List-Server. The NTP home page (http://ntp-server.niehs.nih.gov) offers access to information about the NTP, and links are available that detail and highlight ongoing and future initiatives, NTP centers, NTP documents, the *Report on Carcinogens*, and announcements.

On-line, searchable access and printed copies of NTP publications including the *Report on Carcinogens*, NTP Technical Reports, NTP Toxicology Reports, and other NTP documents, are available through the Environmental Health Information Service (EHIS) at http://ehis.niehs.nih.gov.

The NIEHS/NIH Central Data Management Office oversees distribution (upon request) of specific, chemical study information and printed NTP documents - the NTP Annual Plan, NTP Study Status Reports, pre-peer review copies of draft NTP Technical Reports, background documents for chemicals nominated to the NTP, and summaries of minutes from NTP Board meetings.

The NTP is interested in and welcomes stakeholder input into its programs and priorities. Nominations, inquiries, and comments from the public and other interested parties are welcome at any time. The NTP Liaison and Scientific Review Office at the NIEHS/NIH under the direction of Ms. Sandra Lange serves as the focal point for receiving input to the Program and for overseeing the distribution of information about programs, workshops, initiatives, etc.

Central Data Management
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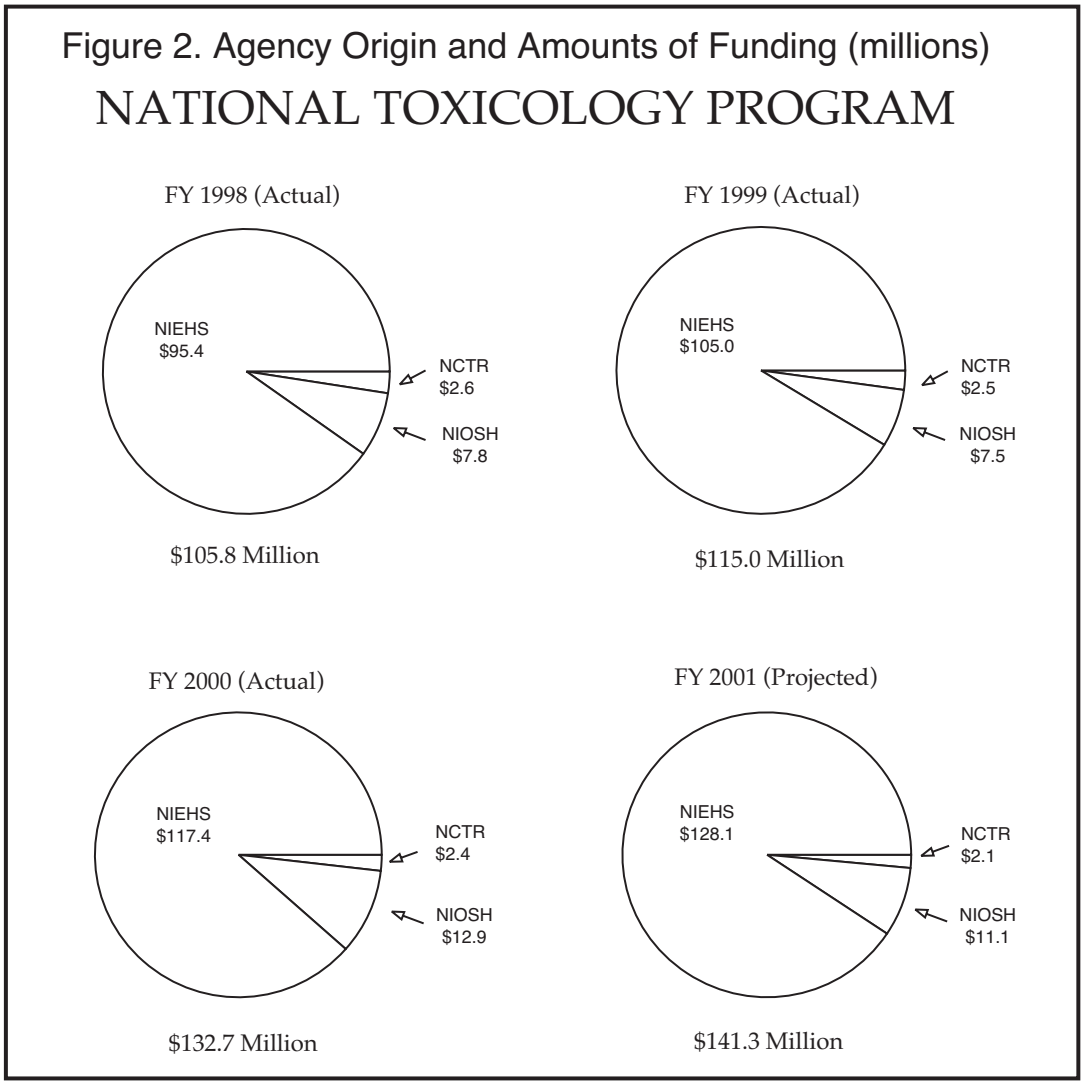
RESOURCES AND PLANNING

CURRENT AND PROJECTED RESEARCH CAPACITY

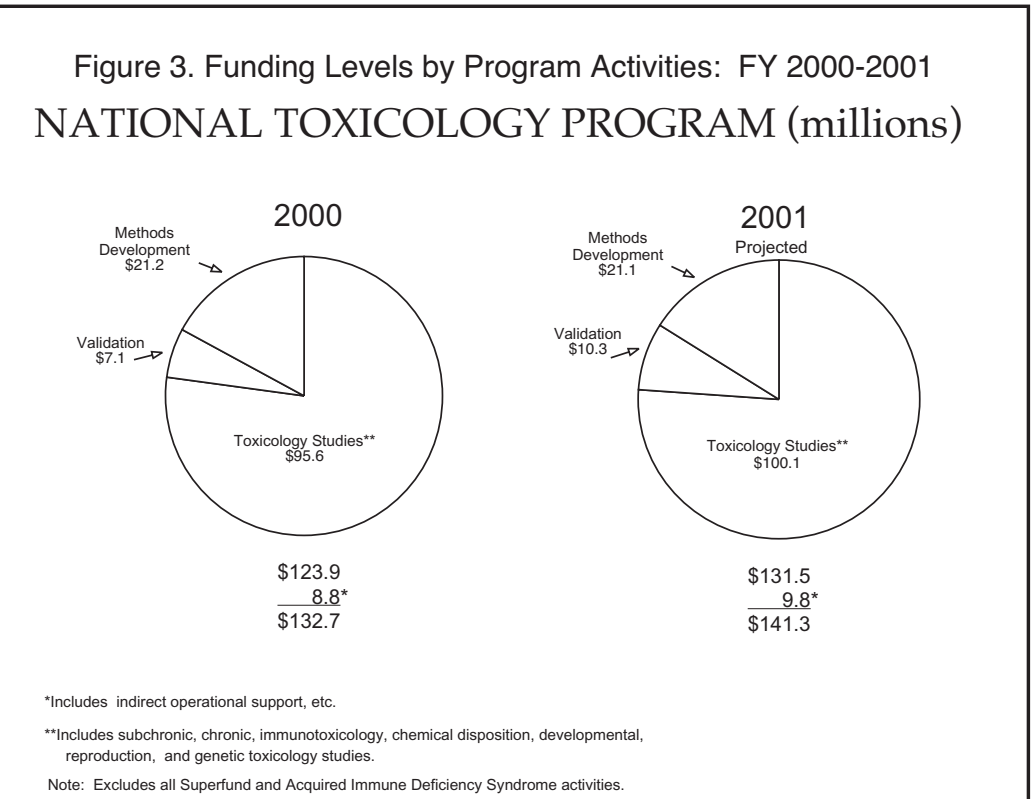
The NTP relies on voluntary allocations from the Program's three core agencies (NIEHS/NIH, NCTR/FDA, and NIOSH/CDC) for supporting its various programs and initiatives. These allocations are specified following the determination of yearly appropriations. As shown in Figure 2, the actual allocations from the principals toward the NTP have steadily increased over the past three years (1998-2000) and are projected to provide a total funding level of $141.3M (direct plus indirect) in FY 2001. The NTP primarily conducts its research studies in-house at the core agencies or through contact laboratories, but also supports cooperative and/or collaborative agreements and small extramural grants (R03) with other Federal agencies, academia and industry. Funds are also used to sponsor workshops and conferences and to produce and disseminate printed Program materials.

Figure 2. Agency Origin and Amounts of Funding (millions)



The NTP maintains an objective, science-based approach to dealing with critical issues in toxicology. The Program continually, as resources permit, sets its priorities to improve the nation's ability to evaluate human health effects from environmental exposures and focuses its resources on three major program activities: toxicology studies, methods development, and validation. As shown in Figure 3, greater than three fourths (77%) of the NTP's allocations (direct only) in FY 2000 were directed toward program activities in basic and applied research (toxicology studies) and a similar level of effort is projected for FY 2001 (76%). The NTP also has ongoing activities for the development and validation of improved research tools for carrying out its research studies. Approximately 24% of the NTP allocations are budgeted in FY 2001 for methods development and validation. These include NTP initiatives such as transgenic mice, biomathematical modeling, and genomics. The directives in the 1993 NIH Revitalization Act regarding development of alternative methods that reduce, refine, or replace the use of animals in research provide legislative impetus for many of these efforts.

Figure 3. Funding Levels by Program Activities: FY 2000-2001



*Includes indirect operational support, etc.

**Includes subchronic, chronic, immunotoxicology, chemical disposition, developmental, reproduction, and genetic toxicology studies.

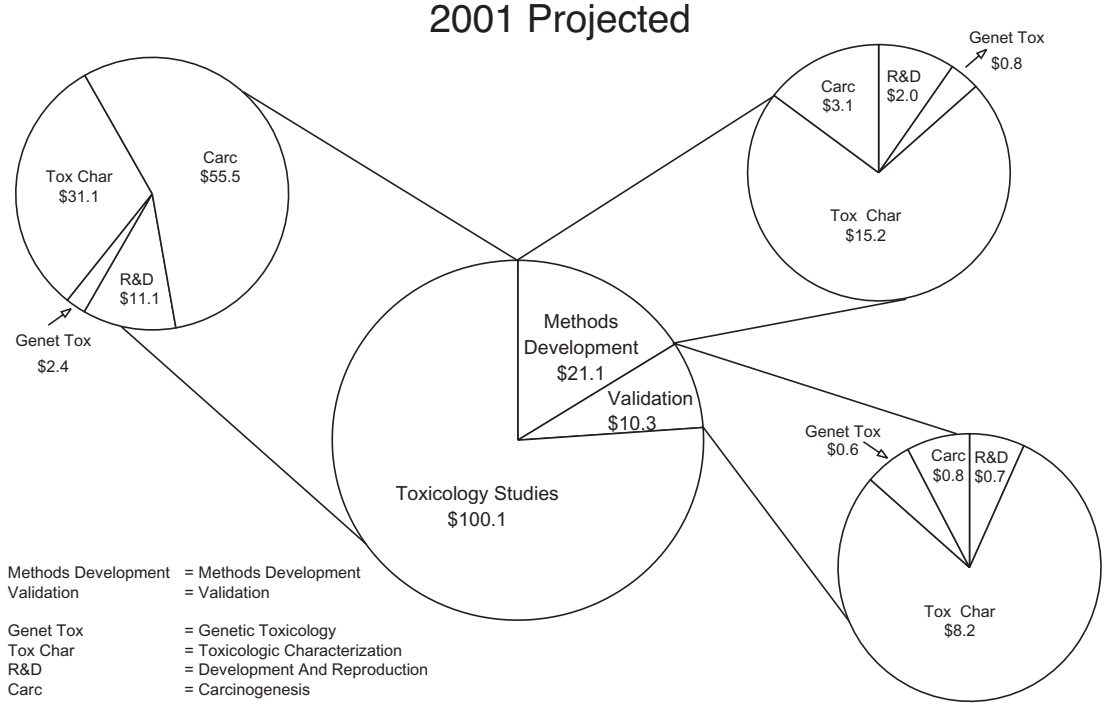
Note: Excludes all Superfund and Acquired Immune Deficiency Syndrome activities.

The actual funding level (in million dollars) for the NTP in FY 2000 is provided for the three program areas: toxicology studies, methods development, and validation. This includes direct and indirect costs. The funding levels for FY 2001 are projected. Funding represents combined allocations from the three core agencies.

Within each of the major program activities, the NTP targets multiple program areas broadly represented as genetic toxicology, toxicological characterizations (includes immunotoxicology, neurotoxicology, epidemiology, exposure assessment, and general toxicology), reproduction and development, and carcinogenesis. This involves conducting toxicological evaluations for cancer and non-cancer endpoints, generating data to strengthen the science base for risk assessment, developing and validating improved testing methods for targeted areas, and communicating with all stakeholders. Figure 4 shows the projected FY 2001 NTP allocations for each of these areas within the individual program activities. For toxicology studies, the primary single focus remains on carcinogenesis although total

projected funding for research on non-cancer endpoints (toxicological characterizations plus development and reproduction) studies is projected at 42%. Methods development and validation address strategies for both cancer and non-cancer endpoints. The majority of the funds for both methods development and validation ($15.2M or 72% and $8.2M or 80%, respectively) are allocated toward technologies that will improve the NTP's ability to characterize toxicities.

Figure 4. Funding Levels by Program Area



For each program activity (toxicology studies, methods development, and validation), the projected allocated funds for FY 2001 are given for each major program area genetic toxicology, (toxicological characterization, reproduction and development, and carcinogenesis).

In totality, the NTP is a comprehensive interagency research program whose core agencies are committed to providing resources for continuing the Program's research efforts and for communicating the knowledge learned to all stakeholders, public and private. The Program's efforts in toxicity testing and risk assessment are directed toward obtaining the best scientifically valid data that can be used by health, regulatory, and research agencies for making good decisions about potential human risk(s) from exposure to environmental toxicants. Toward that end, the NTP is continually evolving to remain at the cutting edge of scientific research and the development and application of technology.

EVOLVING PRIORITIES OF THE NATIONAL TOXICOLOGY PROGRAM

The NTP maintains a number of complex interrelated research and testing programs that provide unique data and knowledge used by health, regulatory, and research agencies to protect public health. These programs are well designed and are functioning successfully; although additional targeted resources are needed to take full advantage of opportunities afforded by advances in quantitative gene expression methods, transgenic and knockout models, exposure assessment, and other continuing and emerging scientific areas.

The NTP cancer bioassay program remains strong. In addition, the NTP has devoted programmatic and financial resources to two major efforts in the area of reproductive and developmental toxicology. One is the large study of endocrine disruptors underway at NCTR/FDA (see page 21), and the second is creation of the NTP Center for the Evaluation of Risks to Human Reproduction (see page 39). Children's health issues are of importance to the NTP and plans are underway to provide additional contract support to allow increased study of developmental immunotoxicology and developmental neurotoxicology. Efforts are of high priority that would strengthen intramural and extramural research activities in toxicogenomics, molecular biology, and molecular pharmacology. The NTP is a full partner in the newly established NIEHS National Center for Toxicogenomics and will coordinate activities in this area with NTP member agencies. Further linkage to the NIEHS/NIH Research Centers at universities throughout the United States is also envisioned.

Programmatic goals are constantly being updated and reevaluated. Ongoing efforts in FY 2001 will bring further clarity to the application of transgenic mouse models to augment and/or replace the traditional two-year cancer bioassay. New transgenic model development and selection will continue with closer ties established to similar efforts at the National Cancer Institute/NIH. Efforts to evaluate transgenic animals for non-cancer toxicity endpoints have started but may need greater resources in the future. Toxicogenomics and proteomics will become better established as routine technologies in literally all disciplines within the NTP, and additional resources being targeted to database development. These studies hold the promise of providing a true mechanistic basis for hazard identification through the use of short-term assays that can be practically applied over the broad range of agents to which humans are exposed. High priority research and testing programs *i.e.,* herbal medicines/dietary supplements, water disinfection by-products, phototoxicology, and DNA-based therapies will develop and expand, requiring an increase in dollars allotted to testing activities.

Finally, the NTP will continue to expand a collection of activities designed to place research and testing results in better human health perspective. This encompasses such efforts as human exposure assessment, toxicokinetics and physiologically based pharmacokinetic modeling of bioassay findings, and interpretation of results in molecular epidemiology for use in human hazard identification (e.g., *Report on Carcinogens*, NTP Center for the Evaluation of Risks to Human Reproduction). All of these initiatives need to be carried out in an arena of enhanced communication with all of our stakeholders. The NTP has always drawn strength and credibility from its commitment to open information exchange and strict adherence to impartiality and rigorous scientific peer review. This will remain a central priority of the program in FY 2001 and in the years to come.

TOXICOLOGY AND CARCINOGENESIS EVALUATIONS

TOXICOLOGY AND CARCINOGENESIS EVALUATION PROCESS

Nomination

The NTP seeks to maintain a balanced research and testing program that provides data addressing a wide variety of issues of importance to public health. Particular assistance is sought for the nomination of studies that permit the testing of hypotheses to enhance the predictive ability of future NTP studies, to address mechanisms of toxicity, or to fill significant gaps in the knowledge of the toxicity of chemicals or classes of chemicals. The NTP follows the principles for soliciting nominations listed in Table 2.

Table 2. Nomination Principles for NTP Studies

• Chemicals found in the environment not closely associated with a single commercial organization.
• Biological or physical agents that may not be adequately evaluated without Federal involvement.
• Commercial chemicals with significant exposure that were first marketed prior to current testing requirements or those that generate too little revenue to support further evaluations.
• Potential substitutes for existing chemicals or drugs that might not be developed without Federal involvement.
• Substances that occur as mixtures for which evaluations cannot be required of industry.
• Chemicals or agents that will aid the understanding of chemical toxicities or an understanding of the use of test systems to evaluate potential toxicities.
• Chemicals that should be evaluated to improve the scientific understanding of structure-activity relationships, and thereby help limit the number of chemicals requiring extensive evaluations.
• Emergencies or other events that warrant immediate government evaluation of a chemical or agent.

The nomination process is open to the public. The NTP routinely solicits nominations at conferences and workshops; through the NTP newsletter, *Federal Register* notices, and NTP homepage (http://ntp-server.niehs.nih.gov); and from academia, Federal and State regulatory and health agencies, industry, and labor unions, as well as from environmental groups and the general public (Masten, NIEHS/NIH). In addition, standing nomination committees within the NCI/NIH, FDA, NIOSH/CDC, and NIEHS/NIH routinely select and forward nominations to the NTP. The NTP also reviews toxic release inventories and exposure surveys [*e.g.*, National Health and Nutrition Examination Survey (NHANES) and National Human Exposure Assessment Survey (NHEXAS)] to identify chemicals of potential interest.

Selection

The nominations undergo a multi-step process of review [Figure 5, Interagency Coordinating Committee for Evaluating Chemicals (ICCEC), NTP Board of Scientific Counselors, and NTP Executive Committee]. During the entire process, the NTP works actively with regulatory agencies and interested parties to supplement information about nominated substances and to ensure that the nomination and selection process meets regulatory and public health needs. The ICCEC [1] plays a central role in recommending substances for NTP evaluation and coordinating NTP studies with other relevant agency activities. The ICCEC

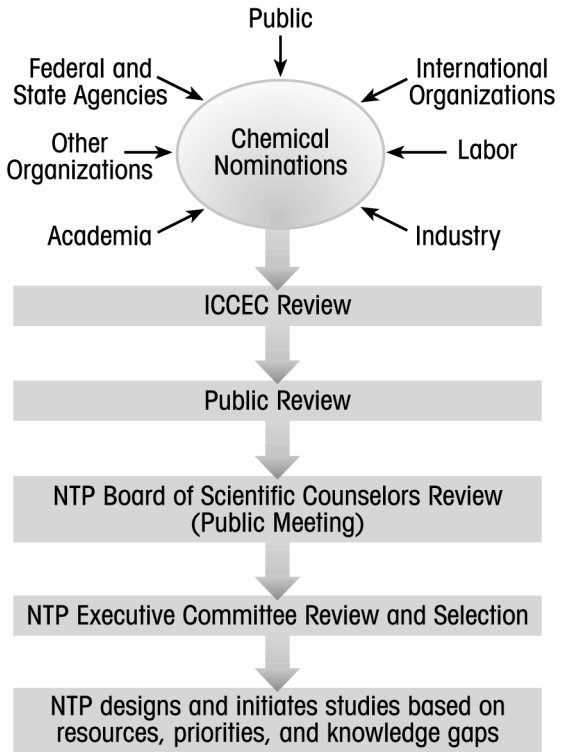
[1] Agencies represented on the ICCEC include: ATSDR, FDA, NCI/NIH, NIOSH/CDC, National Library of Medicine, Department of Defense, NIEHS/NIH, OSHA, EPA, and CPSC

reviewed nominations to the NTP for toxicological testing at its October 2000 meeting and its recommendations are given in Table 3. The ICCEC is scheduled to meet in spring 2001 to review the next set of nominations.

Public comments are solicited on nominated substances and those received are considered at all stages through study selection and design. At the final step of the formal process, the NTP Executive Committee reviews the substances and makes a final recommendation for acceptance of a nominated substance by the NTP. Table 4 lists substances approved by the Executive Committee at its June 2000 meeting.

The selection of a substance by the NTP Executive Committee does not automatically commit the NTP to its evaluation. The chemicals selected for study and the toxicology and cancer study designs are carefully considered to ensure that the dollars invested in NTP research are wisely spent. A chemical or study may be withdrawn if applicable research data or higher priority studies are identified or if a study proves impractical.

Figure 5. NTP Chemical Nomination and Selection Process



Evaluation

In carrying out its mission, the NTP provides toxicological evaluations on substances of public health concern. Unfortunately the NTP can initiate bioassays to characterize potential carcinogenicity of substances on only a small fraction of the thousands of chemicals for which there is little or no information. Many more chemicals are also studied to assess a variety of non-cancer health-related effects including but not limited to reproduction and development, immunotoxicity, neurotoxicity, and genotoxicity. Other biologic endpoints are often evaluated such as: quantifying the disposition and excretion of substances, identifying and correlating biochemical markers with exposure and metabolism, and examining genetic polymorphisms in human drug metabolizing enzymes to understand the susceptibility of individuals and populations to xenobiotic-induced toxicity.

An NIEHS/NTP project review committee reviews and evaluates a study's project plan (*e.g.*, design, methods, hypothesis, etc.) and proposes the vehicle for execution (*e.g.*, grant, contract, etc). The toxicological evaluation is generally conducted through repeated administration of a substance to groups of laboratory animals for variable periods of time up to two years. Many of the short-term studies are designed to provide dose-setting information for instigating chronic evaluations and to address specific deficiencies in the toxicology database. The adverse health effects from short- or long-term exposures of different dose levels of the substance are evaluated clinically, by histopathology, and by a variety of toxicology endpoints through comparison with groups of animals not administered the substance. Many substances are also studied using protocols specifically designed to address issues pertaining to the mechanism by which a substance causes a particular toxic outcome(s).

Table 3. Nominations for NTP Toxicological Testing Reviewed by the ICCEC[1] – October 2000

Substance (CAS No.)	Nominator	ICCEC Recommendation	Study Rationale; Other Information
Recommended by the ICCEC for testing			
Aluminum complexes found in drinking water • Aluminum fluoride • Aluminum citrate	EPA NIEHS/NIH	Long-term drinking water studies to address pharmacokinetics, neurotoxicity, bone development, and reproductive and developmental toxicity. Consideration for testing in transgenic models of neurodegenerative disease	A better understanding of pharmacokinetics and toxicity of aluminum species occurring in drinking water is needed.
Bilberry fruit extract	NCI/NIH	*In vitro* and *in vivo* genotoxicity testing.	Widespread human exposure through use as a dietary supplement.
Black cohosh	NCI/NIH NIEHS/NIH	Subchronic toxicity testing in young and female animals; two-generation reproductive and developmental toxicity testing.	Widespread human exposure as a dietary supplement; reported estrogenic activity.
Blue-green algae (dietary supplement and selected toxins)	NCI/NIH	Subchronic toxicity testing and neurotoxicity studies of commercial substances; consider follow-up studies of cyanobacterial toxins pending blue-green algae and microcystin-LR studies.	Widespread human exposure through drinking water and via contamination of algal dietary supplements.
Cefuroxine	FDA	Genotoxicity testing	Prescription drug with widespread and potentially long-term use.
Clarithromycin	FDA	Genotoxicity testing	Prescription drug with widespread and potentially long-term use.
D&C Red No. 27 and D&C Red No. 28	FDA	*In vitro* percutaneous absorption testing; photocarcinogenicity testing pending absorption study results.	Approved colorings for drugs and cosmetics that can lead to DNA damage.
N,N-Dimethyl-*p*-toluidine	NCI/NIH	Subchronic toxicity testing pending review of industry test plans and/or data developed under EPA's HPVC Challenge Program.	High production volume chemical with potential for widespread human exposure.
Lemon oil and Lime oil	FDA	Phototoxicity testing; Photocarcinogenicity testing pending phototoxicity testing study results.	Widespread consumer exposure as a fragrance component.
Local anesthetics that metabolize to 2,6-xylidine or *o*-toluidine • Bupivacaine • Prilocaine	Private individual NIEHS/NIH	Short-term *in vitro/in vivo* mechanistic studies for carcinogenic metabolite formation and genotoxicity of representative anesthetic compounds.	Widespread clinical use and human exposure; potentially metabolized to carcinogenic and neurotoxic intermediates.
Microcystin-LR	NIEHS/NIH	Toxicokinetic, subchronic, reproductive toxicity, chronic, toxicity and carcinogenicity studies; consider Medaka fish model studies.	Cyanobacteria and their toxins are drinking water contaminants with potentially widespread human exposure.
Organotins occurring in drinking water • Monomethyltin trichloride • Dimethyltin dichloride • Monobutyltin trichloride • Dibutyltin dichloride	EPA NIEHS/NIH	Long-term single chemical and binary mixture drinking water studies that address pharmacokinetics, neurotoxicity, immunotoxicity, and reproductive and developmental toxicity.	Drinking water contaminants with potentially widespread human exposure.
All-*trans*-retinyl palmitate	FDA	Phototoxicity and photocarcinogenicity testing.	Widespread use in cosmetic products.
S-Adenosylmethionine	NCI/NIH	*In vitro* genotoxicity testing; sub-chronic toxicity pending genotoxicity study results.	Widespread exogenous human exposure through use as a dietary supplement.

Substance (CAS No.)	Nominator	ICCEC Recommendation	Study Rationale; Other Information
Senna	FDA	Carcinogenicity testing in p53 transgenic model.	Data are needed to complete safety evaluation of stimulant laxatives.
Testing recommendation deferred pending receipt and consideration of additional information			
1,3,-Dichloropropane, 2,2,-Dichloropropane, and 1,1,-Dichloropropene	EPA NIEHS/NIH	Defer pending information about drinking water occurrence data, production volumes, and potential sources for contamination and anticipated regulatory value of additional toxicity data.	Drinking water contaminants with high health priority; known toxicity and carcinogenicity of structurally similar compounds.
Hydergine	NCI/NIH	Defer pending information about sales and use and information needed by regulatory agencies.	Ergot alkaloid prescription drug with recent increased used as a dietary supplement.
Yohimbe bark extract and Yohimbine	NCI/NIH	Defer pending information on use and patterns of use and on regulatory agency needs.	Significant human exposures through use as a dietary supplement; suspicion of carcinogenicity of yohimbine based on structural similarity to reserpine.

[1] ICCEC – Interagency Coordinating Committee for Evaluating Chemicals

Table 4. Recommendations for NTP Testing by the NTP Executive Committee – December 2000

Substance (CAS No.)	Nominator	ICCEC Recommendations	Study Rationale; Other information
Substances Recommended for Testing			
1-Bromopropane (106-94-5) and 2-Bromopropane (75-26-3)	OSHA NIOSH/CDC	1-Bromopropane: carcinogenicity, reproductive and developmental toxicity, toxicokinetics, mechanistic studies, neurotoxicity, genotoxicity, exposure studies in workers 2-Bromopropane: subchronic toxicity	Reported increasing production and use in many industrial applications as an alternative to ozone depleting substances; available data from limited repeat dose studies indicate toxicity to multiple organ systems 2-Bromopropane is a minor contaminant in reagent grade 1-Bromopropane with known reproductive toxicity
Chitosan (9012-76-4)	NCI/NIH	Mechanistic studies to evaluate vitamin E and mineral depletion	Significant human exposure through use as a dietary supplement and other commercial applications; potential toxicity from interference with dietary fat absorption
DNA-based products	FDA	Establish joint NIEHS/FDA program to evaluate long-term toxicity in anticipation of regulatory needs	Rapidly growing market for DNA-based therapeutic agents and a lack of adequate mechanisms and methodologies for evaluating safety
Juglone (481-39-0)	NCI/NIH	Mechanistic and metabolism studies, mouse lymphoma assay, mammalian mutagenicity, carcinogenicity testing pending results of preliminary studies	Potential human exposure resulting from use of walnut-based products as dietary supplements and natural dyes and stains; suspicion of carcinogenicity based on quinone structure
Potassium ferricyanide (13746-66-2)	NCI/NIH	Genotoxicity, subchronic toxicity	Potential consumer and worker exposure resulting from use in photographic processing; suspicion of toxicity based on potential for redox cycling; inadequate toxicity information available
Radiofrequency radiation emissions of wireless communication devices	FDA	Establish interagency program to design studies assessing cancer and non-cancer health effects to fulfill regulatory needs	Widespread consumer and worker exposure; available data is inadequate to properly assess safety

Support activities at the core agencies facilitate the conduct of these evaluations and include:

- animal production and care (Witt, NCTR/FDA; Rao, NIEHS/NIH)
- biological monitoring and health assessment (DeBord, NIOSH/CDC)
- chemistry/biochemistry (Turesky, NCTR/FDA: Smith, NIEHS/NIH; Teass & Frazer, NIOSH/CDC)
- clinical pathology (Travlos, NIEHS/NIH)
- microbiology (Cerniglia, NCTR/FDA; Rao, NIEHS/NIH)
- pathology (Hailey/Herbert, NIEHS/NIH; Salomon, NIOSH/CDC)
- quality assurance (Reed, NCTR/FDA; Bristol, NIEHS/NIH)
- statistical services (Kodell, NCTR/FDA; Haseman/Dunson, NIEHS/NIH; Krieg, NIOSH/CDC)
- study coordination/oversight (Jackson, NCTR/FDA; Bucher, NIEHS/NIH; Toraason, NIOSH/CDC)
- archives (Maronpot, NIEHS/NIH)
- information retrieval and analysis (Tatken, NIOSH/CDC; Wright, NIEHS/NIH)
- information systems and central files (Eastin, NIEHS/NIH), database management – CHEMTRACK, TDMS and LDAS (Rowley, NIEHS/NIH), NTP web page (Soward, NIEHS/NIH)
- technical report preparation (Alden, NIEHS/NIH)

In addition to toxicology research of compounds and exposures, the NTP supports the development of new techniques and methods for improving the ability to identify and assess potential environmental toxicants and the development and validation of novel and alternative testing methods that will reduce, replace, or refine animal use.

The NTP carries out toxicology and carcinogenicity research through two primary mechanisms: laboratory studies conducted in contract laboratories and in-house studies conducted at its core agencies: NIEHS/NIH, NCTR/FDA, and NIOSH/CDC. In addition, the NTP leverages resources through cooperative and/or collaborative agreements with other Federal agencies, academia, and industry.

The NIEHS/NIH Division of Extramural Research and Training in collaboration with the NTP supports investigator-initiated research to provide data to aid in defining the mechanisms of action of agents under study by the NTP. Currently research under the NIH R03 Small Grant mechanism is supporting investigator-initiated research on the mechanism of toxicity/carcinogenicity of water disinfection by-products (see page 16). A new initiative has been developed to encourage extramural investigations of animals/tissues/cells from animals undergoing NTP two-year cancer bioassays or shorter toxicological characterizations. This program will benefit the NTP by expanding its expertise base to the scientific community and obtaining novel toxicity of tested chemicals.

Review

The results of toxicology and carcinogenesis studies undergo rigorous peer review. These findings are published as NTP Technical Reports and may also be published in peer reviewed scientific journals. The NTP Technical Reports Review Subcommittee of the Board (see Advisory Committees, page 2) evaluates the technical reports in an open, public meeting. Candidates for peer review in 2001 are listed in Table 5. Abstracts of NTP Technical Reports are posted on the NTP web site and hardcopies and PDFs are available from the Environmental Health Information Service (see Communication and Public Outreach).

Table 5. Candidate Chemicals for Peer Review in 2001

Chemical	Technical Report No.	Information
May 3, 2001		
Acrylonitrile	TR506	Used widely in the production of acrylic fibers, elastomers, resins, and a variety of chemical intermediates; annual production is in the millions of tons
Citral	TR505	Used as a lemon flavoring in foods and beverages and as a lemon fragrance in detergents, perfumes, and toiletries
Methacrylonitrile	TR497	Used in the production of polymers, elastomers, and plastics including those used in beverage containers
o-Nitrotoluene	TR504	Nitrotoluene usually occurs as a mixture of three isomers, with 55% to 60% *o*-nitrotoluene, 35% to 40% *p*-nitrotoluene, and 3% to 4% *m*-toluene. The two major isomers were studied separately to compare their toxicologic profiles. The nitrotoluenes are widely used in synthesis of agricultural and rubber chemicals and of a variety of dyes
p-Nitrotoluene	TR498	
October 18, 2001		
2,4,-Hexadienal	TR509	A peroxidation by-product of animal, fish, and vegetable oils. It has a variety of commercial uses including as a flavoring agent and as a chemical intermediate in synthesis of pharmaceuticals, sorbitol, and dyes
Riddelliine	TR508	A pyrrolizidine alkaloid that occurs in a variety of plants in the rangelands of the southwestern United States. Residues of the chemical have been found in livestock and in certain herbal teas
Urethane/Ethanol	TR510	Urethane and ethanol occur as products of fermentation in a variety of alcoholic beverages
Vanadium Pentoxide	TR507	Used as the principal starting material from ores for the production of pure vanadium, which is used in a variety of metal alloys. Used as a catalyst in chemical syntheses, in catalytic converters, and in the manufacture of dyes and yellow glass. Vanadium pentoxide is also one of the major metallic components of fly ash; one main occasion for human exposure is during the cleaning of boilers and furnaces.

HIGHLIGHTED CURRENT NTP INITIATIVES

The NTP has a broad mandate to provide toxicological characterizations for chemicals and agents of public health concern and strives to balance the selection of chemicals for study. This has resulted in a diverse research program, but with emphasis on synthetic industrial chemicals, pesticides, various pharmaceuticals, metals, and food additives. The following section highlights some current NTP initiatives: several areas that have received inadequate attention in the past, *i.e.,* photoactive chemicals, contaminants of finished drinking water, endocrine disrupting agents, and certain occupational exposures; and research addressing potential safety issues associated with herbal medicines and dietary supplements or DNA-based therapies. In general, these initiatives are broad-based and include various health-related endpoints.

Phototoxicology

The exposure of U.S. citizens to UV radiation is increasing through more frequent use of tanning booths to augment skin coloration and the trend toward spending leisure/pleasure time in sunlight-oriented activities (*e.g.,* beach, swimming pools). The FDA has an ongoing interest in the phototoxicity and photocarcinogenicity of therapeutics, cosmetic, devices, and food supplements/additives. Recently the agency developed an inter-agency photobiology research program with the NIEHS/NIH resulting in establishment of the FDA-NIEHS Phototoxicology Research and Testing Laboratory and the new NTP Center for Phototoxicology (see page 37). This laboratory is designed to allow study of many types of photoactive and environmental agents (*e.g.,* cosmetics, tanning enhances, drugs, herbals, etc.)

for UV radiation- or simulated solar light-induced toxicity and cancers. Studies conducted in this facility should contribute to providing high quality data upon which to base public health decisions about the interactions of drugs or other compounds with sunlight. The key features of this new facility are the two six-inch zenon arc lamps that operate at 6,500 watts. The visible and UV radiation emitted from each lamp, when filtered through glass designed to simulate the earth's atmosphere, closely mimics the spectrum of solar light. About 5,000 mice can be exposed per day to the simulated solar light making this facility unique for handling the large number of animals required for carcinogenicity studies.

The α- and β-hydroxy acids are acidic compounds included in many over-the-counter cosmetics. The most widely used α-hydroxy acid is glycolic acid, while the most widely used β-hydroxy acid is salicylic acid. These compounds are keratolytic dermatological agents and their application to the skin results in solubilizing and restructuring of the epidermis (*i.e.* discohesion of corneocytes). The resulting cosmetic effect is an appearance of smoother and less wrinkled skin; however, the biologic effect is removal of the stratum corneum (outer most layer of skin) and increased cell proliferation of cells at the base of the epidermis. The combination of increased cell proliferation and altered light transmission properties of the skin (*i.e.* increased DNA damage by UV light in proliferating cells) has suggested that use of these acids in over-the-counter cosmetics could result in increased rates of skin cancer. The NCTR/FDA is studying the effects of dermatological chemoexfoliation using α– and β-hydroxy acids on cell proliferation and DNA damage in the SKH-1 hairless mouse exposed to simulated solar light. Experiments are in process to quantify the effects of continued exposure to these acids on the dose of light required for induction of skin edema, and future studies aim to examine the recovery rate for the epidermis following cessation of treatment with the skin creams. Protocols are being developed to study possible toxic effects of products derived from the aloe vera plant (Boudreau, NCTR/FDA). Both topical phototoxicity and oral administration dose-finding studies are presently included. Additional test animals (*e.g.*, transgenics models) may be included in subsequent proposals on these and other compounds/treatments (Howard, NCTR/FDA).

Occupational exposures in predominately outdoor professions may place workers at increased risk of skin damage and cancer. The molecular mechanisms involved in UV-induced toxicity and carcinogenesis are not fully understood. NIOSH/CDC is addressing potential health risks associated with occupational exposures to UV radiation. Specific aims include examining alterations in gene expression induced by UV radiation, understanding the involvement of signal transduction pathways in this gene alteration, studying the role of this gene alteration in cancer development, developing biomarkers for early detection of cancer, and elucidating the mechanisms for UV-induced skin cancer. Transgenic mouse epidermal cell lines as well as transgenic mouse models will be used (Ding, NIOSH/CDC).

Safe Drinking Water Program

Safe drinking water represents a balance between microbial and chemical risk and is of enormous public health concern, since it is estimated that more than 200 million Americans use treated drinking water. Chlorination of our water supply is a standard treatment technique that reduces mortality and morbidity from infectious disease; however, despite advances in expertise to purify and disinfect our water, chemical contaminants may still be found in finished water. These agents can be grouped into two broad categories, those that occur as a result of the disinfection process [disinfection by-products (DBPs)] and those that occur naturally or by contamination (candidate contaminants) in public water systems.

One of the most complex issues facing water utilities and the EPA is minimizing the potential for DBP-related health effects while still achieving effective control of waterborne microbial pathogens. The NTP is playing a critical role in providing data to assess the potential health

risks from human exposure to the major DBPs through a collaborative effort with the EPA. The research program includes a systematic, mechanism-based, toxicological evaluation of DBPs that can help provide data for policy makers in setting drinking water standards. The EPA is expected to set final water standards in 2002 or 2003.

A standard toxicological approach to evaluating DBPs is difficult, since different disinfection processes result in different DBPs; the source of water, time of year, and other factors can influence the presence and relative concentrations of different DBPs; and DBPs occur as complex mixtures in relatively low concentrations in drinking water. The selection of DBPs for study is based upon their presence in drinking water, their occurrence with different disinfection processes, their chemical structure, and their representation from among the different DBP families: trihalomethanes, haloacetic acids, and haloacetonitriles. Research focuses on reproductive toxicity, immunotoxicity, and neurotoxicity as well as carcinogenesis, and research approaches include investigations using transgenic mouse models, conventional rodent models, and studies in fish. A list of disinfection by-products under study by the NTP is given in Table 6.

Table 6. Water Disinfection By-Products under Evaluation

Chemical	NTP Studies (ongoing or planned)
2(5H)-Furanone (MX)	Reproductive testing
Bis(2-Chloro-ethyoxy)methane	Subchronic testing, chronic testing
Bromochloroacetic Acid	Subchronic testing, chronic testing
Bromodichloroacetic Acid	Subchronic testing, chronic testing
Bromodichloromethane	Chronic testing, transgenic models, Medaka fish model, neurotoxicity
Chloramine	Immunotoxicity
Chloroform	Immunotoxicity, Medaka fish model
Dibromoacetic Acid	Subchronic testing, chronic testing, immunotoxicity, neurotoxicity
Dibromoacetonitrile	Subchronic testing, chronic testing
Dibromochloroacetic Acid	Reproductional/developmental toxicity
Dichloroacetic Acid	Subchronic testing, chronic testing, transgenic models, immunotoxicity, Medaka fish model
Sodium Bromate	Transgenic models, immunotoxicity
Sodium Chlorate	Reproduction/development, chronic testing, Medaka fish model
Sodium Chlorite	Immunotoxicity

NTP research is being conducted at NIEHS/NIH and also through agreements with the U.S. Army for fish studies and EPA for immunotoxicity and neurotoxicity investigations. A collaborative government/industry partnership is in place where the NIEHS/NIH supplies transgenic animals and pathology expertise for industry inhalation studies on bromodichloromethane. The NTP is also involving the extramural research community through grant (R03) support of hypothesis-based mechanistic studies on DBPs and is working closely with the American Water Works Association Research Foundation (AWWARF) sharing protocols and research plans and making them aware of ongoing research activities. Some of the AWWARF's own research awards are being designed to complement activities of the NTP and EPA.

Besides the DBPs, a complex array of candidate contaminants can occur naturally (*e.g.,* arsenic, aluminum), as a result of contamination [*e.g.,* methyl tertiary butyl ether (MTBE), pesticides, organotins], or with environmental changes (*e.g.,* alga blooms resulting in mycotoxins and other toxins). The Safe Drinking Water Act amended in 1996 required the EPA to develop a list of unregulated contaminants. The EPA in consultation with the public, scientific community and a working group of the National Drinking Water Advisory Council

established the Contaminant Candidate List in 1998 comprised of microbial and chemical contaminants. The NIEHS/NIH in collaboration with the EPA is selecting the major contaminants for future study. Aluminum complexes, blue-green algal toxins, and organotins have been nominated to the NTP for toxicological testing (Melnick, NIEHS/NIH; see Nominations).

Occupational Mixtures and Exposures

The NTP is coordinating an interagency effort between NIEHS/NIH and NIOSH/CDC to better characterize worker exposures and to use this information both for worker education and to identify occupational health research gaps. This project involves NIOSH-wide participation and should impact the health agenda of both NIOSH/CDC and NTP by focusing NTP resources on obtaining "real world" information about worker practices, complex occupational exposures, and possibly related adverse health effects. Such information is needed to identify areas for research and to design better laboratory studies on the health effects of chemicals, complex mixtures, and exposure circumstances encountered in the workplace. NIOSH/CDC is working with the NTP in nominating agents for study and designing laboratory studies and is undertaking its own research projects under this agreement. Under this interagency agreement, current efforts are addressing worker exposure to 1-bromopropane and asphalt fumes (see below) and future initiatives are proposed for occupational mixtures such as welding fumes (Morgan, NIEHS/NIH; Sharpnack, NIOSH/CDC; Toraason, NIOSH/CDC).

A project currently underway is examining the physical and chemical characteristics of asphalt fumes generated in a laboratory setting under simulated road paving conditions. Asphalt fumes generated with road paving are associated with acute irritation of both mucous membranes and skin; cancer risk associated with this operation is not established. A new system has been designed and built and asphalt fumes equivalent to those produced by road paving crews can be reproducibly generated (Siegel, NIOSH/CDC). Using this system, methods have been developed for characterizing these fumes and for monitoring asphalt fume exposure in inhalation toxicity studies. A future goal is to apply these methods for evaluating health effects of occupationally exposed workers (Wang, NIOSH/CDC, see page 68). Laboratory studies are underway using these methods to evaluate the toxic effects of asphalt fume exposure *in vitro* and in rodents (Ma, NIOSH/CDC, see page 46: Munson, NIOSH/CDC, see page 33).

An industry consortium has petitioned the EPA to list 1-bromopropane as an alternative for ozone depleting solvents for general metals, precision and electronics cleaning, aerosols, and adhesives. If this occurs, there is the potential for a vast increase in the exposure of workers and the public. Currently, no appropriate occupational exposure limit for 1-bromopropane is available from NIOSH or OSHA. In order to obtain information on exposures to this chemical, NIOSH/CDC is conducting an industry-wide exposure assessment. The target population for this study is a variety of industrial sectors - chemical, aerosol, and adhesive manufacturers, adhesive users, metal degreasing and electronics industries. Sites will be selected based upon the quantity and manner of 1-brompropane use, the number of workers exposed, type of manufacturing process, and representativeness of the industry. Exposure at these sites will be characterized using inhalation, exhaled breath, and biological measures. Results from this study should facilitate 1) evaluating patterns of exposure and determining exposure associations with adverse health effects or health indices, 2) providing for the development and validation of biomonitoring methods, 3) developing intervention recommendations for reducing exposures and appropriate occupational exposure limits, and 4) evaluating the suitability of 1-bromopropane as an alternative for ozone depleting solvents by the EPA (Hanley, NIOSH/CDC).

Following identification of major sites for occupational exposure to 1-bromopropane, the NIOSH/CDC will undertake a multifaceted health and exposure evaluation of those workers. Endpoints to be assessed include biomarkers of internal dose, evaluations of reproductive dysfunction (both genders), neurotoxicity, genotoxicity, liver and kidney toxicity, and effects of exposure on hematologic endpoints. Biomarkers of exposure to both the parent compound and metabolites will be quantified along with workplace sampling. Data from this study may provide a basis for development of an occupational exposure limit for 1-bromopropane (Lynch, NIOSH/CDC).

NIOSH/CDC is currently planning to conduct a national, cross-sectional, on-site survey of establishments and workers analogous to the 1981-1983 National Occupational Exposure Survey (NOES) and the 1984-1989 National Occupational Health Survey of Mining (NOHSM). The new survey will include sequentially other industry sectors and will gather nationally representative data on chemical, physical, and biological agents to which workers are potentially exposed, as well as data on exposure controls and health and safety practices. This survey should provide information for targeting research in areas with the highest likelihood of reducing workplace illness and promoting occupational health and safety (Boiano, NIOSH/CDC).

The NIOSH/CDC is undertaking a new project to identify cohorts of workers exposed to reproductive toxicants and describe the demographic profiles of workers and companies. Data being collected includes the quantity of chemical in use, the manner of use, the number of workers exposed, types of manufacturing processes, and any company data on reported morbidity or health concerns. Chemicals initially targeted are dibutyl phthalate, boric acid, tricresyl phosphate and polyamides. In-depth evaluations of reproductive function are planned for exposed workers (Moorman, NIOSH/CDC).

Herbal Medicines and Dietary Supplements

Medicinal herbs are some of our oldest medicines and their increasing use in recent years is evidence of a public interest in having alternatives to conventional medicine. It is estimated that approximately one-third of the U.S. population uses some form of alternative medicine. The use of herbal medicines and other dietary supplements has increased substantially since passage of the 1994 Dietary Supplement Health and Education Act. Under this Act, proof of their safety is not required prior to herbal products being marketed. Dietary supplements currently account for one of the fastest growing markets in U.S. pharmacies and constitute a multi-billion dollar industry. Although approximately 1500 botanicals are sold as dietary supplements or ethnic traditional medicines, herbal formulations are not subject to FDA pre-market toxicity testing to assure their safety or efficacy.

The NTP has received numerous nominations for study of herbal medicines and other dietary supplements from both the public and Federal agencies. Based in part on input from a workshop held in September 1998, the NTP is currently planning and conducting research on several herbal medicines and other dietary supplements (Table 7). These studies focus on characterization of potential adverse health effects including reproductive toxicity, neurotoxicity, and immunotoxicity as well as those associated with acute high dose exposure and chronic exposure to lower doses (Burka, NIEHS/NIH).

Table 7. Herbal Medicines and Dietary Supplements under Evaluation

Herb or Ingredient	NTP Studies (on-going or planned)	Information
Aloe Vera Gel	Literature/evaluation stage. Proposed for oral and topical studies.	Seventh most widely used herb; used as both a dietary supplement and component of cosmetics. The gel has been used for centuries as a treatment for minor burns and is increasingly being used in products for internal consumption (*e.g.,* "health" drinks).
Androstenedione	Disposition and metabolism studies in progress. Subchronic and chronic studies to be awarded this year.	4-Androstene-3,17-dione is a steroidal precursor to testosterone and is marketed as a dietary supplement for increasing muscle mass.
Black Walnut Extract	Metabolism and disposition studies on juglone; design stages for pre-chronic and subchronic studies	Found in hair dye formulation and walnut oil stain. Used both internally and externally as a herbal remedy. Juglone is a major constituent.
Chromium Picolinate	Metabolism and disposition studies nearly complete. Subchronic and chronic studies will be awarded this year.	A dietary supplement promoted as a muscle builder and weight loss aid. Chromium appears to be more efficiently absorbed as a picolinate complex as compared to the uncomplexed metal.
Echinacea purpurea Extract	Immune function study in NZB/W mice, a model for human Systemic Lupus Erythmatosis	One of the most commonly used medicinal herb in the United States. Used as an immunostimulant to treat colds, sore throat, and flu.
Ginkgo biloba Extract	Subchronic testing	Among the five or six most frequently used medicinal herbs. Ginkgo fruits and seeds have been used medicinally for thousands for years. The extract of green-picked leaves has increasing popularity in the United States. Ginkgo biloba extract promotes vasodilatation and improved blood flow and appears beneficial, particularly for short-term memory loss, headache, and depression.
Ginseng and Ginsenosides	Design of pre-chronic, subchronic, and chronic studies is complete	Fourth most widely used medicinal herb; ginsenosides are thought to be the active ingredients. Ginseng has been used as a treatment for a variety of conditions: hypertension, diabetes, and depression, and been associated with various adverse health effects.
Goldenseal	Pre-chronic study will be conducted this year. Subchronic and chronic studies will be awarded this year.	Second or third most popular medicinal herb used in this country; traditionally used to treat wounds, digestive problems, and infections. Current uses include as a laxative, tonic, and diuretic.
Kava Kava	Design of pre-chronic, subchronic, and chronic studies is complete.	Reported to be the fifth most widely used medicinal herb, has psychoactive properties, and is sold as a calmative and antidepressant.
Milk Thistle Extract	Genetic toxicity testing of milk thistle extract, milk thistle tea, and individual components: silybin and silymarin. Subchronic and chronic studies will be awarded this year.	Used to treat depression and several liver conditions including cirrhosis and hepatitis and to increase breast milk production.
Pulegone	Design of pre-chronic, subchronic, and chronic studies complete; metabolism and disposition studies	A major terpenoid constituent of the herb, Pennyroyal, is found in lesser concentrations in other mints. Pennyroyal has been used as a carminative insect repellent, emmenagogue, and abortifacient. Pulegone has well-recognized toxicity to the liver, kidney, and central nervous system.
Thujone	Subchronic and chronic studies will be awarded this year.	Terpenoid found in a variety of herbs, including sage and tansy, and in high concentrations in wormwood. Suspected as the causative toxic agent associated with drinking absinthe, a liqueur flavored with wormwood extract

Endocrine Disrupting Agents

In response to a widespread public concern about potential effects of environmental estrogens on health, the NTP is examining the effects of endocrine disrupting chemicals. The NIEHS/NIH and NCEH/CDC are collaborating on a pilot project for quantifying approximately 70 chemicals found in either human blood or urine that are considered to be endocrine disruptors. The biological samples are from the National Health and Nutrition Examination Surveys (NHANES), which covers both men and women from a range of age, socio-economic, and ethnic groups. These data will be used to estimate human exposure to endocrine disrupting agents within the U.S. population and to identify the ones of greatest public health concern. Phytoestrogens and phthalate esters are two classes of compounds for which studies are being targeted. Results from the analysis of seven phthalate ester metabolites in urine have identified diethyl phthalate, dibutyl phthalate, and benzylbutyl phthalate used in detergents, lubricating oils, and solvents as well as cosmetics and wood finishes as being of the highest concentrations. This information complements the recently completed scientific peer review of phthalate esters by the NTP Center for the Evaluation of Risks to Human Reproduction (see page 39). Continued development of this interagency exposure initiative will focus on other NTP priority exposures, such as herbal medicines and drinking water disinfection by-products, to facilitate sound scientific evaluations of agents of priority for public health (Masten, NIEHS/NIH).

Endocrine disrupting chemicals are also of interest to the FDA and NIEHS/NIH and several laboratory studies are ongoing or planned (Table 8). The NCTR/FDA has an interagency agreement with the NIEHS/NIH to assess the effects of endocrine disrupting chemicals on reproduction, development of hormone-sensitive organs, and cancer endpoints in male and female rats over multiple generations; behavioral, neurological, and immunological endpoints are also included (Delclos, NCTR/FDA; behavioral – Ferguson, NCTR/FDA; neurotoxicity – Scallet, NCTR/FDA). The NCTR/FDA scientific staff is also interested in evaluating neuroanatomical and neurobehavioral endpoints associated with exposure to endocrine disrupting chemicals. Studies are underway to determine the neurohistological structure of sexually dimorphic regions of the hypothalamus with companion studies designed to assess male and female reproductive behaviors known to reflect neurotoxicological alterations, as related to endocrine active chemical insult. These are critical parallel studies to the multigeneration toxicology studies (Slikker, NCTR/FDA). Additional studies are also investigating the role of the p53 tumor suppressor gene in evaluating genistein (Morris, NCTR/FDA, see page 48).

Table 8. Endocrine Disrupting Agents under Study

Chemical	NTP Studies (ongoing or planned)
Diethylstilbestrol	Transgenic models
Ethinyl Estradiol	Reproduction/development, immunotoxicity, transgenic models
Genistein	Reproduction/development; transgenic models
Methoxychlor	Reproduction/development
Nonylphenol	Reproduction/development
Vinclozolin	Reproduction/development, immunotoxicity, neurotoxicity

The NIEHS/NIH is examining the cellular and molecular mechanisms through which chemicals with estrogenic activity or endocrine disrupting effects might interact with target tissues and cause permanent alterations that potentially could affect sexual differentiation or induction of hormone-sensitive cancers (*e.g.,* cervix, uterus, vagina, breast, testis, and prostate). Diethylstilbestrol is being used as the model compound for comparison of effects with naturally occurring substances (genistein and coumestrol), drugs (Tamoxifen), and

pesticides (methoxychlor). Other studies include identifying stem cells in target tissues, investigating the role of the estrogen receptor in induction and/or progression of neoplasms, identifying potentially altered growth factor pathways, and defining markers of pre-neoplasia (Newbold, NIEHS/NIH).

Studies at NIOSH/CDC are exploring *in vitro* how exposure to occupational chemicals impacts normal testicular function at different stages of maturation/development (fetal/neonatal, prepubertal, and adult). Primary cultures of rat Leydig (synthesize testosterone) and Sertolli (role in regulating spermatogenesis) cells are being used. These studies target the testing of chemicals (industrial plasticizers/surfactants - octylphenol and pesticides - methoxychlor and vinclozolin), which are reported to alter normal functioning of endogenous steroids (estrogens and androgens). Future studies will focus on establishing possible mechanism(s) of action (Murono, NIOSH/CDC). In another series of studies, the NIOSH/CDC is investigating the potential estrogenic activity of octamethylcyclotetrasiloxane. This involves assessing its estrogenicity in rats through the uterotrophic assay, elucidating the role of estrogen receptors α and β in its estrogenic actions using transgenic mice, and evaluating effects of octamethylcyclotetrasiloxane on gene expression (Munson, NIOSH/CDC).

Two NIEHS/NIH epidemiology studies are examining the potential effects of endocrine disrupting agents in human populations. One project is investigating the impact of exposure to hormonally active compounds on sexual differentiation in offspring (Rogan, NIEHS/NIH). Another project is focusing on the effects of early-life organochlorine exposure and includes examining the relationship between *in utero* exposure to polychlorinated biphenyls (PCBs) and neonatal thyroid function (Longnecker, NIEHS/NIH). NIOSH/CDC is examining the relationship between female worker exposure to polychlorinated biphenyls and breast cancer. These compounds are suspected carcinogens because of their estrogenic and lipophilic properties (Whelan, NIOSH/CDC).

Low-Dose Issues for Endocrine Disruptors

The EPA is implementing an Endocrine Disruptor Screening Program as required by the 1996 Food Quality Protection Act. As part of this program, the agency is in the process of choosing appropriate assays to use for screening those agents and developing standardized, validated protocols for those assays. A critical aspect of the protocol development is dose setting, especially since in this instance, hormonally active agents may cause effects at doses lower than normally selected for toxicological testing. On behalf of the EPA, the NTP established an independent, external, scientific peer review panel to review the evidence related to low-dose reproductive and developmental effects of endocrine disrupting agents and to consider their implications for the development, validation, and interpretation of test protocols. The meeting was held October 10-12, 2000 in Research Triangle Park, North Carolina. A peer review report is due in mid-2001 and will be posted on the NTP homepage (http://ntp-server.niehs.nih.gov) (Melnick, NIEH/NIH).

DNA-Based Products

DNA-based therapies are currently being developed for the treatment of a wide range of human diseases. Examples include plasmid DNA encoding one or more antigenic proteins for vaccines against viral and bacterial pathogens, triplex forming synthetic oligonucleotides to modulate gene expression, and viral vectors for gene therapy. Though DNA-based products show significant promise, by their very nature they all pose a risk of interacting with the host genome or disrupting normal cellular processes in unexpected and unpredictable ways and with potentially adverse consequences. It is essential to identify hazards and potential risks

associated with these therapies prior to widespread clinical application. Presently, the FDA has only limited authority to require evaluation of non-acute, long-term safety risk associated with these therapies. In addition, the majority of the manufacturers of DNA-based products are generally small biotechnology companies and academic sponsors that lack the resources to perform long-term, large-scale studies on their products. The NTP and FDA are collaborating on an initiative to study the safety of DNA-based products that will address three major safety issues:

- The intracellular persistence and potential for integration into the host genome. Since certain of these products are intended for use in children (*e.g.*, DNA vaccines), there is concern about life-long risk posed by integration.
- Their distribution to the gonads and the potential for integration and germ line transmission. DNA-based products may reach tissues in the body outside their presumably sequestered sites of administration (intradermal or intramuscular); therefore, there is concern about the potential for reproductive toxicity and/or transmission of altered genetic material to subsequent generations.
- Ectopic protein production and the potential for abnormal immune activation. Both viral vectors and DNA vaccines carry genes that stimulate host cells to secrete self and foreign proteins. There is concern about the potential for DNA-based products to promote development of autoimmune disease and disrupt immune homeostasis.

As this initiative gets underway, scientists from the Center for Biologic Evaluations and Research at the FDA and the NTP are working together to design studies that will address these important public health issues. While initial efforts are focusing on DNA-based therapies, the NTP is aware of public concern for other DNA-based products, such as bioengineered foods, and may consider future research in this area (Irwin, NIEHS).

GENERAL TOXICOLOGY

Current Research Initiatives

Pre-Chronic Phase of Study

Studies in the pre-chronic phase are carried out usually through contract mechanisms at several U.S. laboratories and involve exposures of rats and mice of both genders for periods of 14 to 90 days usually to chemicals, but sometimes to physical agents. Table 9 lists the agents currently in the pre-chronic phase in FY 2001; some of the studies target water disinfection by-products (see page 16) and herbal medicines and dietary supplements (see page 19). Studies for 12 chemicals are ongoing from FY 2000 and studies for eight chemicals are planned to start in FY 2001.

Table 9. Compounds in the Pre-Chronic Phase of NTP Study

Chemical Name	CAS No.	Project Leader[1]	Contract Project Officer[2]	Species: Strain	Route	Special Studies	Study Length[3]
Studies ongoing in FY 2000 as of 10/01/00							
Androstenedione	63-05-8	Eastin	Chhabra/ Vallant	Mice: B6C3F1 Rats: Fischer 344	Gavage		14 days
				Mice: B6C3F1 Rats: Fischer 344	Topical		14 days
3-Chloro-4-(dichloromethyl)-5-Hydroxy-2(5h)-Furanone (MX)	77439-76-0	Herbert	Herbert	Mice: B6C3F1 Rats: Fischer 344	Water	Mice: T3, T4, TSH (days 4,21); Rats: T3, T4, TSH, liver UDP-glucose (day 21)	14 days; 90 days
o-Chloropyridine	109-09-4	Chhabra	Chhabra/ Vallant	Mice: B6C3F1 Rats: Fischer 344	Topical		14 days; 90 days
Cumene	98-82-8	Chan	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Inhalation		14 days; 90 days
1,2-Dibromo-2,4-Dicyanobutane	35691-65-7	Dunnick	Chhabra/ Vallant	Mice: B6C3F1 Rats: Fischer 344	Topical		90 days
Dimethylaminopropyl Chloride, Hydrochloride	5407-04-5	Abdo	Chhabra/ Vallant	Mice: B6C3F1 Rats: Fischer 344	Gavage		90 days
α-Methylstyrene	98-83-9	Morgan	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Inhalation	Rats: Urinalysis, α2μ-globulin (optional)	90 days
Propargyl Alcohol	107-19-7	Hooth	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Inhalation	Rats: Urinalysis	14 days; 90 days
Water Disinfection By-Products							
Bromochloroacetic Acid	5589-96-8	Melnick	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Water	Acetyl-CoA-hydrolase, cell proliferation	14 days; 90 days
Bromodichloroacetic acid	71133-14-7	Melnick	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Water	Acetyl-CoA-hydrolase, cell proliferation	14 days; 90 days
Dibromoacetonitrile	3252-43-5	Melnick	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Water	Glutathione-S-transferase, liver cell proliferation	14 days; 90 days
Dichloroacetic Acid	79-43-6	Melnick	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Water	Cell proliferation	90 days
Studies proposed to start in FY 2000 as of 10/01/00; Others may be scheduled as protocols are finalized							
Bis(2-Chloro-ethoxy)methane	111-91-1	Dunnick	Orzech	Mice: B6C3F1 Rats: Fischer 344	Topical		14 days; 90 days
Estragole	140-69-0	Abdo	Orzech	Mice: B6C3F1 Rats: Fischer 344	Gavage	Rats: P450s, serum gastrin	90 days
Hexachlorobenzene	118-74-1	Hooth	Chhabra/ Vallant	Rats: Sprague-Dawley	Gavage	T3, T4, TSH; P450s	90 days
Isoeugenol	97-54-1	Abdo	Orzech	Mice: B6C3F1 Rats: Fischer 344	Gavage	Rats: P450s, serum gastrin	90 days
β-Myrcene	123-35-3	Chan	Orzech	Mice: B6C3F1 Rats: Fischer 344	Gavage	Rats: P450s	90 days

Chemical Name	CAS No.	Project Leader[1]	Contract Project Officer[2]	Species: Strain	Route	Special Studies	Study Length[3]
Herbal Medicines/Dietary Supplements							
Pulegone	89-82-7	Chan	Orzech	Mice: B6C3F1 Rats: Fischer 344	Gavage	Rats & mice: P450s; Glutathione-S-transferase	14 days; 90 days
Sodium Thioglycolate	367-51-1	Hooth	Chhabra/ Vallant	Mice: B6C3F1 Rats: Fischer 344	Topical		14 days; 90 days
3,3',4,4'-Tetrachloroazobenzene	14047-09-7	Hooth	Chhabra/ Vallant	Rats: Sprague-Dawley	Gavage	T3, T4, TSH; P450s	90 days

[1] Project Leader - NIEHS/NIH staff scientist (unless otherwise indicated) who oversees each chemical's evaluation

[2] Contract Project Officer - NIEHS/NIH staff scientist (unless otherwise indicated) who coordinates research activities with the contract laboratory

[3] Study Length:

14 days and 17 days: Repeated dose study generally 14 or 17 days of exposure to be used for determining the dose range for the subchronic study. The doses (usually five doses plus control) tested cover a wide dose range; 5 animals/group, 2 genders, 2 species; complete necropsies on all animals. Standard measurements: Organ weights (liver, thymus, right kidney, right testicle, heart, and lungs are taken at necropsy on all animals surviving at the end of the study. Histopathologic evaluations are done only on those organ/tissues showing gross evidence of treatment-related lesions plus corresponding control animals.

90 days: Subchronic Toxicity study generally 90 days of exposure to determine the toxic effects of the test chemical and to estimate the high dose for each gender of each strain and species to be tested in a chronic toxicity study. Five doses (plus control) are selected from the repeated dose study or based on other information; 10 animals/group, 2 genders, 2 species; complete necropsies on all animals. Standard measurements: Organ weights (liver, thymus, right kidney, right testicle, heart, and lungs are taken at necropsy on all animals surviving at the end of the study. Gross lesions are examined in all animals in all dose groups plus controls. Complete histopathologic evaluation is done on all control animals, all animals in highest dose group with at least 60% survivors at time of sacrifice, plus all animals in higher doses. Chemical-related lesions are identified and those organs plus gross lesions are examined in all lower doses to a no-effect level. A complete histopathologic evaluation is performed on all natural death/moribund sacrifice animals. Toxicological parameters evaluated include hematology, clinical chemistry, micronuclei determinations, and SMVCE (Sperm Morphology and Vaginal Cytology Evaluation): male organ toxicity is estimated by sperm motility, sperm count, and testicular spermatid head counts; female organ toxicity is evaluated by vaginal cytology and timing of the estrous cycle.

Mechanism-Based Toxicology

Chemical Disposition, Metabolism, and Toxicokinetics

Mechanistic information is obtained through evaluations of chemical disposition and metabolism. Those chemicals being evaluated in FY 2001 are listed in Table 10. Most studies are conducted in intact laboratory animals; some require incubations of human and rodent liver slices with the chemical. This information provides dosimetric data that can be integrated with other anatomical, biochemical, and physiological information into development of physiologically based pharmacokinetic/toxicokinetic models (see page 69). Such models are used increasingly in risk assessment to extrapolate between species, across dose ranges, and across different routes of exposure (Beland, NCTR/FDA; Burka, NIEHS/NIH).

Table 10. Chemical Disposition, Metabolism, and Toxicokinetic Studies

Chemical Name	CAS No.	Project Leader[1]	Test[2]
Studies ongoing in FY 2001 as of 10/01/00			
Allyl Acetate	591-87-7	Cunningham	Metabolism
Androstenedione	63-05-8	Cunningham	Chemical disposition
Bis(2-chloroethoxy)methane	111-91-1	Burka	Chemical disposition
Bromodichloromethane	75-27-4	Smith	Toxicokinetics
2-Butyne-1,4-diol	110-65-6	Burka	Chemical disposition
2-Chloropyridine	109-09-1	Burka	Chemical disposition
Chromium Picolinate	14639-25-9	Burka	Chemical disposition
Cumene Hydroperoxide	80-16-9	Burka	Chemical disposition
Dibromoacetic Acid	631-64-1	Smith	Toxicokinetics
Dibromoacetonitrile	3252-43-5	Burka	Chemical disposition
Divinylbenzene	1321-74-0	Collins	Chemical disposition
Estragole	140-67-0	Cunningham	Chemical disposition
Eugenol	97-53-0	Cunningham	Metabolism
Formamide	75-12-7	Cunningham	Metabolism
Isoeugenol	97-54-1	Cunningham	Metabolism
Methylene Blue Trihydrate	7220-79-3	Collins	Toxicokinetics
Myristicin	607-91-0	Cunningham	Metabolism
3,3,4,4,5-Pentachlorobiphenyl	57465-28-8	Smith	Toxicokinetics
2,3,4,7,8-Pentachlorodibenzofuran	57117-31-4	Smith	Toxicokinetics
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	1746-01-6	Collins	Toxicokinetics
Herbal Medicines/Dietary Supplements			
Pulegone	89-82-7	Burka	Chemical disposition
Studies proposed to start in FY 2001 as of 10/01/00; Others may be scheduled as protocols are finalized			
1-bromopropane	106-94-5	Burka	Chemical Disposition Toxicokinetics
Indol-3-carbinol	700-06-1	Cunningham	Chemical Disposition
2,4-Hexandienal	142-83-5	Cunningham	Mechanistic Study
Herbal Medicines/Dietary Supplements			
Kawain (associated with Kava kava, an herbal)	1635-33-2	Burka	Chemical Disposition

[1] Project Leader - NIEHS/NIH staff scientist who oversees each chemical's evaluation

[2] Chemical disposition: determines fate of radiolabeled chemical, its absorption, distribution, metabolism and excretion.
Metabolism: studies fate of chemical, usually done *in vitro*.
Toxicokinetics: finding the concentration of a single chemical or its metabolites in blood or other biological tissues.

Drug Metabolizing Enzymes

A major objective of the NTP is to identify and to characterize the acute and chronic toxicity of chemicals that humans encounter in their environment, work place, or as a result of food or drug consumption. Genetic polymorphisms in drug metabolizing enzymes affect the half-life, efficacy, and toxicity of clinically used drugs and influence susceptibility to environmentally caused diseases. Human cytochrome P450 enzymes (CYPs) metabolize foreign compounds including clinically used drugs, carcinogens, and other xenobiotics, and genetic polymorphisms in these enzymes appear to account for variability among humans in metabolism of xenobiotics. Studies are underway to identify the mechanism whereby cytochrome P450 genes are induced in response to xenobiotics (Negishi, NIEHS/NIH). Researchers at the NIEHS/NIH are working to identify new human CYP enzymes and any genetic polymorphisms, to develop genetic tests for these polymorphisms, to determine the amino acids important in substrate specificity of human CYP2Cs (CYP subfamily), and to identify the endogenous functional roles of CYP2C enzymes (Goldstein, NIEHS/NIH). A murine model is under development for studying the role of CYP2C enzymes in metabolism of endogenous substrates with long range plans to produce various P450 knockout mice. The mice would aid study of the physiological importance of CYPs in metabolism of environmental chemicals. An initiative focusing on the role of the cytochrome P450, CYP2E1 (thought responsible for oxidative metabolism of ethanol), in the toxicity of NTP chemicals has begun. Chemicals being studied include acrylonitrile, acrylamide, and 1,3-butadiene (Ghanayem, NIEHS/NIH).

In addition to drugs, cytochrome P450 enzymes metabolize fatty acids to biologically active mediators that can have potent effects on vascular tone, ion transport, and peptide hormone secretion. Studies are underway at the NIEHS/NIH to identify and characterize new P450 isoforms that metabolize arachidonic acid to epoxyeicosatrienoic acids and hydroxyeicosatetraenoic acids and to study their regulation and functional significance. This work should provide insight into basic pathogenic mechanisms of selected diseases including ischemic heart disease, hypertension, and atherosclerosis (Goldstein, NIEHS/NIH, Zeldin, NIEHS/NIH).

Oxidative Stress

Understanding the mechanisms by which environmental toxicants act may enable early interventions in disease and allow preventive measures to be taken. Many human diseases are associated with reactive oxygen including cancer, heart disease, and neurodegenerative diseases. This is one area under study at the NIEHS/NIH. The impact of dietary modulation on the expression of environmentally induced oxidative stress is being compared in normal and diseased states. In three animal models (ozone induced lung inflammation, dust-mite-induced allergy and oxazalone-induced delayed contact hypersensitivity) tested independently, caloric restriction appears to mitigate chemically induced oxidative stress (Kari, NIEHS/NIH).

Researchers at the NIEHS/NIH are also studying the consequences of mitochondrial DNA damage and its role in reactive oxygen-induced toxicity. The mitochondrion represents a target of reactive oxygen stress and mitochondrial DNA damage appears to be an early and sensitive marker of this stress. Hydrogen peroxide is produced through incomplete reduction of oxygen during oxidative phosphorylation and under certain conditions, such as inflammation, excessive amounts are produced. The impact of this excess hydrogen peroxide on subsequent adverse cellular events (*e.g.,* DNA damage, lipid peroxidation, glutathione depletion) is currently being addressed (Van Houten, NIEHS/NIH).

Free radical metabolites are possibly involved in the toxic effects of many drugs and environmental chemicals. Chemical reactions of free radical metabolites have known involvement in biochemical and toxicological consequences that cause cellular damage and death. Investigators at the NIEHS/NIH are using electron spin resonance and spin trapping to

detect and identify free radical metabolites of toxic chemicals, drugs, and biochemicals and to identify the role(s) and define the mechanisms associated with their radical-mediated toxicity (Mason, NIEHS/NIH). In addition, they are taking part in a multi-institutional initiative aimed at determining measurable, sensitive, and specific biomarkers for oxidative damage in animal models and humans. More than 25 putative markers measuring oxidative damage to lipids, proteins, DNA, and antioxidants have been evaluated using a rodent model (carbon tetrachloride poisoning). Several show significant promise --isoprostanes and aldehyde products of lipid peroxidation. Additional studies aimed at validating and comparing markers of oxidative stress are underway using independent models, *e.g.,* chronic iron overload, which causes oxidative stress without concomitant liver damage (Mason, NIEHSNIH; Kadiiska, NIEHS/NIH; Tomer, NIEHS/NIH). A spin trapping database (containing extensive biographic information) has been implemented at the NIEHS/NIH and is accessible to both extramural and intramural scientists via the Internet (http://epr.niehs.nih.gov/stdb1.html) (Chignell, NIEHS/NIH).

Xenobiotic Transport Mechanisms

Research at the NIEHS/NIH is focusing on understanding the basic cellular mechanisms that drive drug and toxicant transport in specialized excretory (kidney, liver, and choroid plexus) and barrier tissues (brain capillary endothelium) and how these processes interact to determine chemical toxicity and drug efficacy. The vertebrate renal proximal tubule excretes a large number of potentially toxic chemicals through multiple, specific, transport proteins that remove them from the blood and concentrate them in urine. Studies are underway to define the extracellular signals and intracellular pathways that are involved in renal xenobiotic transport, and as possible, will be extended to identifying specific xenobiotic transport mechanisms in the brain capillaries and choroid plexus (Miller, NIEHS/NIH). Other studies have examined the role of epithelial membrane transport in the elimination and toxicity of foreign chemicals and are applying that knowledge to study renal handling of toxic ions including herbicides and phenolphthalein. Future efforts will focus on examining sites that control the local concentration of toxicants within the brain, eye, and testis. The mechanisms, which are responsible for removal of potentially toxic xenobiotics, drugs, and even neurotransmitter metabolites from these sites, are largely unknown (Pritchard, NIEHS/NIH).

Atherosclerosis

Atherosclerosis is a leading cause of morbidity and mortality in the United States. Efforts continue for identifying agents that increase risk for this multi-factorial disease. A study at the NIEHS/NIH is examining whether environmental pollutants, such as carbon disulfide, might interact with other known risk factors, such as dietary fat, to exacerbate disease. The study includes examining macrophages' function because of their key role in lipoprotein processing and plaque development and characterizing the development of fatty streaks just below the aortic coronary sinus (Sills, NIEHS/NIH).

Electric and Magnetic Fields

Physical as well as chemical agents are of interest to the NTP. Under a Congressionally mandated program, the NIEHS/NIH in conjunction with the Department of Energy led an effort to determine what health effects, if any, arise from exposure to power-line frequency electric and magnetic fields (Boorman, NIEHS/NIH; Portier, NIEHS/NIH). Research is continuing at the NIOSH/CDC to try and reproduce effects reported in the literature and to determine what exposure metric(s) is effective in causing a biological response that might lead to an adverse health effect. In addition, the study of radiofrequency radiation associated with wireless communications is being included. Initial studies focus on RF exposure effects in cultured brain cells (Lotz, NIOSH/CDC; also see Carcinogenesis).

Toxicogenomics

National Center for Toxicogenomics

The NTP continually explores the use of new and improved test systems for improving its ability to evaluate potential toxicants. With the advent of novel molecular technologies, the NTP is moving into the arena of toxicogenomics - technology to apply the knowledge of genetics to the field of environmental medicine by studying the effect of toxicants on gene activity and the production of specific proteins by genes in response to toxicants. To oversee this effort and coordinate partners in academia, industry, and the public, the NIEHS/NIH is creating the National Center for Toxicogenomics (NCT). Plans for the NCT were announced on December 7, 2000. NCT will study the effect of toxins on thousands of genes and the production of specific proteins by these genes in response to toxins (Tennant, NIEHS/NIH). Genomic technologies, which are helping to move this field forward, include cDNA microarray, real time and quantitative PCR, and laser capture microdissection.

cDNA Microarray Technology

cDNA microarray technology has emerged as a gene expression tool by which scientists can detect genome-wide differential expression of thousands of genes and offers a methodological advancement for environmental health research. The application of a large number of genes or expressed sequence tags in a condensed array on a glass slide comprises a cDNA microarray. The NIEHS/NIH has a newly developed human cDNA chip, ToxChip, which contains copies of about 2,000 human genes, and a human Discovery Chip containing 12,000 clones. The Institute is testing known toxicants using these chips and building a database of expression information in order to determine the typical genetic changes or "signature" profiles that they produce. Microarrays have been made from common test animals and organisms including mice, rats, and yeast and are in use. There is also interest in developing a testis microarray for screening potential reproductive toxicants. (Afshari,NIEHS/NIH; Paules, NIEHS/NIH). At the NIOSH/CDC, a hepatic microarray, which is characterized for its xenobiotic metabolism, has been compiled and is currently under evaluation against a chemical battery including toluene, trichloroethylene, perchloroethylene, pipron metolachlor, and atrazine (Striley, NIOSH/CDC, see page 69).

Real Time and Quantitative PCR (RTAQ-PCR) Technology

Quantitative RTAQ-PCR is used to measure specific levels of gene products within a sample obtained from either cells or tissues. Real-time, fluorescence detection of PCR products is a recent advancement in this technology that allows for quantitative analyses to be conducted more rapidly and on more samples at a time thereby making this method favorable for large studies on gene expression. Efforts are ongoing at the NIEHS/NIH and NTP to develop the use of RTAQ-PCR for the analysis of 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD)-inducible, peroxisome proliferator-inducible, and DNA damage-inducible genes. This method is planned for future use in gene expression analysis of tissues obtained from animals treated with dioxin-like compounds (Walker, NIEHS/NIH).

Laser Capture Microdissection Technology

Under a cooperative research and development agreement between the NIH and Arcturus Engineering, laser capture microdissection (LCM) technology is being developed at the NIEHS/NIH. LCM enables accurate procurement of groups of specific cells or single cells of interest for molecular analysis. This technology can be used to microdissect pre-invasive lesions with subsequent molecular analysis in order to provide a genetic "fingerprint" of early changes and can be performed on stained (routine and immunohistochemical) or unstained, frozen or fixed specimens. In addition to DNA and RNA analysis of microdissected samples, the analysis of protein is being investigated. This technique will offer the NTP the ability to expand its use of molecular biology tools to characterize interactions of chemicals with critical target genes (Maronpot, NIEHS/NIH).

Areas for Future Initiatives and Resources

Development and Application of Genomic Technologies

The NTP is currently faced with being able to study only a limited number of the chemicals, substances, or exposure circumstances to which individuals are exposed environmentally and occupationally. The NTP needs to continue to focus resources on the development of genomic technologies. These technologies have potential for multiple applications: identification of toxicities for individual substances or mixtures, determination of dose-response relationships, identification of susceptible tissues and cell types, identification of expression patterns for specific cellular signals and processes at the molecular level, and cross-species extrapolation.

Both cDNA microarray and RTAQ-PCR has potential applicability for laboratory and epidemiology studies that would allow inclusion of mechanism-based endpoints by permitting the evaluation of potential molecular biomarkers of exposure and/or response. cDNA microarray technology could be used to characterize exposures and screen potential toxicants through direct comparison of the expression of thousands of genes simultaneously in control and exposed samples. Such arrays could be constructed targeting populations-at-large or to simulate populations-at-risk. By comparing gene expression patterns from various exposures, one could potentially identify "signature" patterns that might serve as biomarkers of potential exposure or potential disease. This would afford the opportunity to determine which gene expression patterns are most likely linked to environmental causes of human disease and greatly enhance initiatives such as the NIEHS Environmental Genome Project (see page 63) as well as serve as screening tools for identifying potential occupational and environmental toxicants. In addition, RTAQ-PCR affords a high throughput method for the analysis of DNA polymorphisms, such as those being identified in the NIEHS Environmental Genome Project.

IMMUNOTOXICOLOGY

Current Research Initiatives

NTP immunotoxicity studies address adverse effects on the immune system that may result from occupational, accidental, or therapeutic exposure to environmental chemical, biological materials, or therapeutic agents. The identification of chemicals, which have potential to cause injury to the immune system, is of considerable public health significance as alterations in immune function can lead to increased incidence of hypersensitivity disorders, autoimmune or infectious disease, or neoplasias. Table 11 lists the substances under consideration for potential effects on the immune system. Testing for sixteen chemicals is ongoing from FY 2000 and testing for three more is planned to begin in FY 2001. Several of these agents are water disinfection by-products and are being tested as apart of the NIEHS/NIH interagency agreement with the EPA for the Safe Drinking Water Program (see page 16). The NCTR/FDA is evaluating the effects of endocrine disrupting chemical effects on the immune system in multigenerational studies (see page 21). Patulin is a mycotoxin and children potentially have high exposure due to increased consumption of apple juice, a common vehicle. In response to contradictory findings about its potential immunosuppressive activity in mice, studies on Patulin were initiated in rats.

Table 11. Substances Being Tested for Immune System Toxicity[1]

Chemical	CAS No.	Species: Strain	Route	Testing Battery[2]
Studies ongoing in FY 2001 as of 10/01/00				
Cadmium Chloride	10108-64-2	Rats: Brown Norway	Gavage/Water	AI
Echinacea	90028-20-9	Mice: B6C3F1	Gavage	IM
Hexachlorobenzene	118-74-1	Rats: Sprague-Dawley	*in utero*, Gavage	MG
Itraconazole	84625-61-6	Mice: B6C3F1	Gavage	RF
Patulin	149-29-1	Rats: Fischer 344	Gavage	IM
Rifamycin	14897-39-3	Mice: Balb/c	Topical	HY
Saquinavir Mesylate (AIDS initiative)	149845-06-7	Mice: B6C3F1	Gavage	IM
Sodium Metasilicate	13517-24-3	Mice: Balb/c	Topical	HY
Trichloroethylene	79-01-6	Rats: Brown Norway	Gavage	AI
Endocrine Disrupting Agents				
Ethinyl Estradiol	57-63-6	Rats: Sprague-Dawley	*in utero*, Feed	IM, MG
Vinclozolin	50471-44-8	Rats: Sprague-Dawley	*in utero*, Feed	IM, MG
Water Disinfection By-Products				
Chloramine	10599-90-3	Mice: B6C3F1	Water	IM
Chloroform	67-66-3	Mice: B6C3F1	Water	IM
Dibromoacetic Acid	631-64-1	Mice: B6C3F1	Water	IM
Dichloroacetic Acid	79-43-6	Mice: B6C3F1	Water	IM
Sodium Chlorite	7758-19-2	Mice: B6C3F1	Water	IM
Studies proposed to start in FY 2000 as of 10/01/00; Others may be scheduled as protocols are finalized				
Nelfinavir			TBD	IM
Pyrogallol			TBD	HY
Genistein			TBD	AI

[1] Project Leader - Dr. Germolec, NIEHS/NIH serves as the staff scientist who oversees each chemical's evaluation.

[2] Testing Battery

AI = Automimmunity studies

HY = Hypersensitivity, evaluated by the mouse ear swelling test and the local lymph node assay

IM = Immunomodulation, a two-tiered panel to evaluate the potential of agents to induce immunosuppression

MG = Multiple generations, assess effects on immunologic function and cancer endpoints for multiple generations

RF = Range finding, evaluation over a range of doses to identify a response

Dermal Hypersensitivity

The local lymph node assay is being used in hypersensitivity testing at the NIEHS/NIH. This method was deemed as an acceptable alternative method to the traditional guinea pig assay for assessing contact dermatitis by an expert panel convened by the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods. Since its review by the Panel, the local lymph node assay has received regulatory acceptance (see page75) (Stokes, NIEHS/NIH).

A major area of research at the NIOSH/CDC focuses on dermal exposure including identifying potential occupational allergens, developing screening tools, understanding the mechanisms for dermal hypersensitivities, and evaluating intervention strategies. A three-part project is addressing the molecular mechanisms of irritant contact dermatitis and allergic contact dermatitis. The first part addresses the role of irritancy in development of contact hypersensitivity and the relationship between chronic low dose workplace exposures and dermatitis. The second part will focus on the effect of stress for development of contact dermatitis, and the third part will evaluate on the ability of particles (*e.g.,* workplace dust) to penetrate the skin and initiate a dermal response. These studies should advance knowledge about the chemical-cellular interaction of dermal response to workplace toxicants (Tinkle, NIOSH/CDC).

Several NTP studies are focusing on natural latex rubber hypersensitivity. One study is investigating the role of dextran glove powder in development of latex allergy by determining the mitogenic activity of dextran *in vitro* using murine and human models and evaluating the potential of naïve dextran glove powder to elicit an IgE response in a murine inhalation model. This research requires establishing a method for inhalation exposure to powdered latex proteins (Meade, NIOSH/CDC). Another study is focusing on understanding the relative contributions of skin versus respiratory exposures on the development of latex sensitization including characterizing the allergen specific IgE response and identifying the primary allergen(s) responsible; this information would be directly applicable to the workplace setting (Meade, NIOSH/CDC). This latter project and others are addressing the development of animal models of latex allergy and evaluating their utility. Such models could be useful for studying the mechanisms underlying latex allergy and for testing intervention strategies (Meade, NIOSH/CDCD; Germolec, NIEHS/NIH). The potential for latex proteins to penetrate into and through intact or abraded human skin is being evaluated *in vitro* using hairless guinea pig skin as a surrogate for human skin. Sensitization of hairless guinea pigs via topical exposure is also being assessed *in vivo as* a model of natural rubber latex protein-induced Type I hypersensitivity. Future studies will focus on penetration of the individual latex proteins and on the effect of co-exposure to other occupational irritating and sensitizing chemicals for development of latex allergy (Meade, NIOSH/CDC).

Through complementary studies, dermal penetration of occupational chemicals is being addressed with the development and validation of a new mathematical/computational model of transdermal chemical penetration. Penetration is being modeled using skin structures recreated from microscopic images that were taken from different animal species. The long-term utility of this model is to permit rational extrapolation of chemical penetration studies from animal models to humans. Such predictions are needed as a screening tool to detect chemicals for which skin exposures may cause systemic effects (Frasch, NIOSH/CDC). Health and dermatitis absorption models are also being developed to guide NIOSH/CDC in making improved dermal policy recommendations (Qiao, NIOSH/CDC).

A method of screening chemicals for their potential to induce irritation or IgE-mediated or T cell-mediated hypersensitivity responses is under development at the NIOSH/CDC. Such a method would be beneficial for identifying candidate chemicals and controlling their workplace levels or release into the environment. The method includes irritancy/phenotypic analysis using flow cytometric analysis. An elevation in B220+cells serves as an indicator of a potential sensitizer and an elevation in IgE+B220+cells as an indicator of the potential for induction of an IgE-mediated response. The method appears to provide quick and cost effective means to begin differentiation between IgE-mediated and T cell-mediated sensitizers. Validation studies using a broad panel of chemicals and comparative analysis with other methods are ongoing (Meade, NIOSH/CDC).

Developing intervention strategies and determining their effectiveness are important efforts at the NIOSH/CDC relative to trying to minimize occupational exposures. Laboratory studies using Yorkshire pigs are currently ongoing to evaluate the efficacy of various decontamination procedures for reducing dermal exposure to occupational toxicants (Qiao, NIOSH/CDC). This project complements a current field study evaluating dermal pesticide exposure of agricultural workers (see Exposure Assessment, page 67).

Hepatotoxicity and Regeneration

An interest at the NIEHS/NIH is to identify the factors that can modify host resistance to endotoxin, such as hepatic damage or dysfunction, in order to characterize potential adverse interactions of toxic chemicals and bacterial products and to predict potential toxicity for humans. This is being addressed by focusing on *in vivo* mouse models of endotoxin

hypersensitivity and the relationship between tumor necrosis factor (TNF)-α signaling and oxidative stress (Germolec, NIEHS/NIH).

Immune Cell Depletion and Host Resistence

The NIEHS/NIH in collaboration with FDA is studying the effect of monoclonal antibody-induced immune cell reduction on host resistance, as measured by flow cytometry. The goal is to examine the relationship between decrements in circulating immune cell phenotypes and susceptibility to infection or tumors. These results will be directly applicable to the interpretation of immune cell phenotype determinations in clinical medicine and for risk assessment in human populations exposed to potential toxicants. Initial dose-response and kinetic studies in C57B16 mice are underway. This study will also validate the usefulness of flow cytometry in non-clinical immunotoxicology studies (Germolec, NIEHS/NIH).

Immunotoxicity Testing Panel Evaluation

Over the past 15 years, experimental animal data collected using standardized testing panels has provided a database from which to evaluate the sensitivity and predictability of a variety of tests commonly used for screening chemicals for immunotoxicity. Both international organizations and industry favor harmonization of testing guidelines. Upon review of the Organization for Economic Cooperation and Development (OECD) 407 testing guidelines by an expert panel, a recommendation was made to supplement the standard 28-day toxicity testing protocol with histopathologic evaluations (spleen, thymus, lymph node, GALT, and bone marrow). Disagreement has ensued about whether this histopathologic battery is sufficient to detect potential immunotoxicants or whether functional tests are needed. A 1998 workshop at the NIEHS/NIH was held to establish criteria for tissue and histological evaluations; immunotoxicology on 11 chemicals was subsequently completed. Studies are currently underway using statistical modeling to determine the sensitivity and predictability overall and for individual tissues of extended histopathology as compared to functional testing (Germolec, NIEHS/NIH; Portier, NIEHS/NIH).

Respiratory Allergens

The respiratory system maintains an effective antimicrobial environment to prevent colonization by airborne microbes. Recent evidence suggests a role of neutrophils and natural killer (NK) cells in sustaining early stages of protective immunity. Researchers at NIOSH/CDC are studying the influence of airborne particulates on the function and dynamics of the macrophage-NK cell axis using *in vitro* and *in vivo* models (Lewis, NIOSH/CDC).

Occupational allergies are increasingly recognized as an important hazard in certain work environments and may play a role in the etiology of many workplace-related diseases. One area of interest is the effect of exposure to asphalt fumes. NIOSH/CDC has been working to characterize and reproduce field conditions experimentally (see page18). Reproducibility of the asphalt fumes within the NIOSH/CDC inhalation facility (see page 45) has been validated and studies are targeting effects on the immune system with the goal of identifying the active chemical component (Munson, NIOSH/CDC).

Workshop – Assessment of the Allergic Potential of Genetically Modified Foods

Both the general public and scientific community have a growing concern regarding the potential toxicity of genetically modified (GM) foods. Of specific interest is the ability of GM proteins to elicit potentially harmful immunologic responses including hypersensitivity and/or autoimmunity. The lack of information on the potential toxicity of these products has created a considerable backlash against the producers and users of these crops. To address

these issues, the NTP, along with the EPA and FDA is sponsoring a workshop September 24-26, 2001 in Research Triangle Park, NC. Participates will include experts in food allergy, GM crops, and the regulatory aspects of these products, along with bench scientists and clinicians. The specific aims of this workshop are to examine the current state of knowledge in the area, identify the critical issues regarding these materials, and develop testing strategies to examine the toxicity of these compounds. (Germolec, NIEHS/NIH).

Areas for Future Initiatives and Resources

Studies of Specific Areas

Very little is known about the potential for chemicals to affect the immune system, as well as an understanding about the basic cellular mechanisms of such effects. Continued initiatives are needed that focus on expanding that data base especially for areas of high public or regulatory concern (*e.g.,* drinking water contaminants, DNA-based products, natural products, and therapeutic drugs) to provide information for use in risk assessment and for the accurate estimation of safe levels of chemical exposures. As part of these efforts, the NTP will begin assessments of the validity of previous analyses as to the sensitivity and predictivity of specific immune function tests. The Program will continue its studies of the relationship between impairment of immune function and the organism's ability to resist infections or kill tumor cells through the NIEHS/NIH-FDA collaboration (see page 33) examining the effect of monoclonal antibody-induced immune cell reduction on host resistance. Future efforts will be directed toward host resistance studies to determine the relationship between lowering particular immune cell phenotypes and susceptibility to particular infections or tumors. Studies are proposed targeting the effect of individual AIDS therapeutics and combination therapies on flow cytometric parameters and, in tandem, host resistance in the C57Bl6 mouse.

The public is continually concerned about *in utero* and postnatal chemical exposures resulting in adverse health outcomes in children and adults. The NTP has been a leader in the evaluation of developmental effects on the immune system through laboratory studies of perinatal exposure to pesticides, HIV therapeutics, and endocrine disrupting compounds. The NTP will continue this effort with future initiatives targeted at perinatal exposure to environmental agents in experimental models of autoimmune disease. In addition, priority will be given to a comprehensive assessment of the functional and structural development of targeted tissues, such as the nervous and immune system, following prenatal and perinatal exposure to test agent(s). These laboratory studies could be used in the future for either "stand-alone" assessment of developmental effects during the childhood and early adulthood periods or as the first level of a tiered approach for evaluations of children's health. The NTP will address this issue, in part, through expansion of assessments for immunotoxicity in rodent bioassays.

Animal Model Development

Animal experimental studies of autoimmune phenomena have enhanced insights into the underlying biology and plausibility of environmental factors as causative agents. However, there are no validated and generally applicable, predictive animal models for evaluating the potential of environmental agents to induce or exacerbate autoimmune disease. Such models, in combination with current knowledge about human immune system biology, would facilitate the design of better-formulated studies for investigating disease etiology. The NTP will support collaborative, targeted laboratory animal and epidemiology studies specifically designed to elucidate how exposure to particular environmental agents (*e.g.,* silica and hexachlorobenzene) impact the frequency and severity of autoimmune disease.

NEUROTOXICOLOGY

Behavioral and neurologic alterations in response to deleterious environmental agents often represent the earliest observable manifestation of toxicity. Neurotoxicology screening of NTP compounds often employs the EPA Neurobehavioral Screening Battery, Functional Observational Battery (FOB), with addition of locomotor activity measurements or the NIEHS Neurobehavioral Test Battery. The testing batteries examine the various neurobehavioral systems: sensory, motor, autonomic, and peripheral nervous system. The FOB employs observational screening while the NIEHS test battery uses automated test systems to evaluate the various nervous system components. Neurotoxicology testing is generally done in association with subchronic 90-day testing and Table 12 lists substances being evaluated for possible neurotoxicity. Seven studies are ongoing from FY 2000 and five are proposed to start in FY 2001. Carbonyl sulfide was nominated for NTP study because it is a high production hazardous air pollutant and health data is needed under the Toxic Substances Control Act (TSCA). Neurobehavioral batteries are also being included in assessments of occupational cohorts (see Exposure Assessment, page 67).

Table 12. Substances Undergoing Neurotoxicity Assessment

Chemical	CAS No.	Project Leader[1]	Species: Strain	Route
Studies ongoing in FY 2001 as of 10/01/00				
Carbonyl Sulfide	463-58-1	Morgan	Rats: Fischer 344	Inhalation
Dibromoacetic Acid	631-64-1	Harry	Rats: Fischer 344	Water
Molinate	2212-67-1	Harry	Rats: Fischer 344	Feed
Tellurium	13494-80-9	Harry	Rats: Long Evans Hooded	Feed
Endocrine Disruptoring Agents				
Ethinyl Estradiol	57-63-6	Delclos (NCTR)	Rats: Sprague-Dawley	Feed
Methoxychlor	72-43-5	Delclos (NCTR)	Rats: Sprague-Dawley	Feed
Vinclozolin	50471-44-8	Delclos (NCTR)	Rats: Sprague Dawley	Feed
Studies proposed to start in FY 2001 as of 10/01/00; Others may be scheduled as protocols are finalized				
Cyanazine	21725-46-2	Harry	Frog: *Xenopus*	Bath fluid
Metolachlor	51218-45-2	Harry	TBD[2]	TBD
Pesticide/Fertilizer Contamination - Mixture 3		Harry	Frog: *Xenopus*	Bath fluid
6-Propyl-2-Thiouracil	51-52-5	Harry	TBD	Water
Water Disinfection By-Products				
Dibromoacetic Acid	631-64-1	Harry	Rats: Fischer 344	Water

[1] Project Leader - NIEHS/NIH staff scientist who oversees each chemical's evaluation, unless otherwise indicated.
[2] TBD: to be determined

Current Research Initiatives

Biomarkers of Neurotoxicity

Broadly applicable biomarkers of neurotoxicity, both for use in humans and animals, are not available for screening the thousands of potentially neurotoxic compounds found in the environment and workplace. Workers who use vibrating tools are at increased risk for developing hand-arm vibration syndrome (HAVS). Vasospasms in response to cold temperatures; numbness, parasthesia, and a loss of grip strength; and changes in nerve and blood vessel morphology characterize HAVS. Studies at NIOSH/CDC are investigating HAVS etiology and its progression using animal models in order to try and identify possible biomarkers (Lindsley, NIOSH/CDC).

Developmental Neurotoxicology

The developing nervous system can be vulnerable to the effects of chemical exposures that may cause subtle alterations in the brain's organization and lead to dysfunction in the mature organism in the absence of gross neuropathology or neurochemical changes. During development, gene expression is an active process and is required for normal brain development and function such as learning and memory, repair and regeneration, and modulation of individual responses to stress. Various environmental agents (chemical and physical) are being tested for their ability to alter the spatio-temporal expression of mRNA for various developmentally regulated proteins. A future objective is to develop a battery of probes, which is linked to identification of long-term adverse effects on the nervous system's function and plasticity, that could be used to assess the maturation of neural cells in the developing animal. This battery could be used in conjunction with current batteries for reproductive and developmental toxicity testing (Harry, NIEHS/NIH).

Another NIEHS/NIH study is investigating the potential relationship between chemical exposure-induced deficiency in thyroid hormone levels and alterations in the structure of the developing nervous system. Through an interagency agreement between NIEHS/NIH and the EPA, the neurotoxic effect on development from *in utero* exposure to mercury vapor is being investigated in rodents. The project's objectives are to model mercury concentration in the brain of newborn animals, to determine the dose-response for any neurotoxic effects in the newborn induced by material exposure, and to develop a pharmacokinetic simulation model for mercury distribution in the pregnant rat with future expansion to humans. These studies should provide data useful to regulatory agencies for risk assessment purposes (Morgan, NIEHS/NIH).

NCTR/FDA staff is interested in examining neuroanatomical and neurobehavioral endpoints associated with exposure to endocrine disrupting chemicals. See page 21 for details. Within the excitatory amino acid/mediators of neuroanatomical susceptibility to neurotoxicants area, NCTR/FDA scientists are studying neonatal hormonal conditions that can change adult reproductive behaviors in addition to the biochemical events occurring during the apopotic and proliferate sexual differentiation stages of the developing and maturing brain (Scallet, NCTR/FDA; Ferguson NCTR/FDA).

NCTR/FDA scientists develop and validate quantitative biomarkers and immediate precursor events of neurotoxicity and elucidate toxic modes of action. Unique features of these research efforts include the capability to determine target tissue concentrations and cellular interactions of suspected neurotoxicants. Focal areas of interest are 1) excitatory amino acids as mediators of development, aging and neuroanatomical susceptibility to neurotoxicants; 2) the role of aromatic monoamines in neurotoxicity; 3) disruptors of energy metabolism and axonal transport; 4) oxidative-stress-induced neurotoxicity; 5) interspecies extrapolation and validation of animal models; and 6) development, validation, and application of novel neurohistochemical tracers (Slikker, NCTR/FDA).

Neurodegeneration

Inflammatory processes and initiation of the cytokine cascade within the nervous system have been hypothesized as critical in the development of various neurodegenerative diseases. A cytokine response has been identified in neurodegenerative disorders targeting either neurons (Alzheimer's Disease), axons (peripheral nerve axonopathy), or the myelin sheath (multiple sclerosis). Using pharmacological interventions and specific transgenic mouse models, ongoing studies at the NIEHS/NIH are evaluating the role of specific cells, cytokines, and growth factors in the inflammatory response within the brain and its relevance to neurodegeneration. These studies may help determine whether there is a contributing role for an early pro-inflammatory response in subsequent degeneration. Future efforts will focus on

whether the microglia's response and its associated cytokine or toxic product formation and release are critical for astrocyte reactivity and neurodegeneration (Harry, NIEHS/NIH).

Through an interagency agreement with the Veteran's Administration Medical Center (Durham, NC) another NIEHS/NIH study is addressing whether apolipoprotein E (apo E), a cholesterol transporter, might be a signaling factor in the process of neurodegeneration. Using apoE knockout mice with transgenes for human apoE alleles 2, 3, and 4, studies are testing whether apoE plays a role in the critical immune response of the brain to injury as measured by regulation of cytokines in the central nervous system. If it does, future studies will target the ability of environmental agents and diet (*i.e.* copper deficiency or dietary fat) to modulate the level of susceptibility and provide a testable link between environmental exposure and neurodegeneration (Harry, NIEHS/NIH).

Areas for Future Initiatives and Resources

Studies of Specific Areas

Neurodegenerative diseases such as Alzheimer's Disease, Parkinson's Disease, and multiple sclerosis are proposed to have environmental origins. Continued NTP research is needed on the mechanisms related to how toxicant exposure may contribute to disease etiology and on developing models for studying neurotoxicity and susceptibility. Such information would greatly facilitate the development of therapeutic intervention approaches addressing these diseases.

Another area for future initiatives focuses on understanding the impact of exposure to environmental and workplace agents on the nervous system during maturation and development. The developing nervous system can be selectively vulnerable to insult by environmental and workplace toxicants resulting in long-term alterations in overall neurological function. Research would address the interdependency between processes such as learning and memory, repair and regeneration, and modulation of individual responses to stress induced by environmental agents, and as possible, use this information to identify biomarkers useful for neurotoxicity screening. The NTP will also address this issue, in part, through expansion of assessments for neurotoxicity in rodent bioassays.

PHOTOTOXICOLOGY

NTP Center for Phototoxicology

The FDA has an ongoing interest in phototoxicity and photocarcinogenicity of therapeutics, cosmetic, devices and food supplements/additives, and the NTP has reviewed nominations of drugs/chemicals that require phototoxicology testing. Interest in developing a jointly (FDA and NIEHS/NIH) funded and operated NTP Center for Phototoxicology was heightened by an interagency agreement for joint research between the two agencies and the nomination of the α- and β-hydroxy acids to the NTP for photocarcinogenicity testing. The FDA-NIEHS Phototoxicology Research and Testing Laboratory is now operational at the National Center for Toxicological Research in Jefferson, Arkansas and has been designated an NTP Center for Phototoxicology. Dr. Paul Howard, NCTR/FDA, serves as the Center Director. The Center's primary purpose is to conduct mechanistic-based research and photocarcinogenesis studies on compounds of regulatory importance to the FDA. Mechanistic-based studies are concurrently conducted, as needed, to facilitate interpretation of the photocarcinogenesis studies.

The Phototoxicology Laboratory is designed to allow study of many types of compounds including cosmetic chemicals and additives, sun block additives, tanning enhancers, skin colorants, and tattoo inks with regard to their effects on UV radiation or simulated solar light-induced toxicity and cancer. Two 6.5 kWatt xenon-arc lights provide the light sources for solar light simulation. The light is filtered through quartz-glass filters and closely matches the spectrum of terrestrial solar light. This emulates the conditions to which humans are exposed and models the effects of the various spectral regions (*e.g.*, UV-A, UV-C, visible) on the skin. Changing the quartz filters, thereby allowing simulation of most latitude, altitude, and atmospheric ozone levels, can vary the spectrum of light. The facility also has a portable fluorescent lamp assembly that can be equipped with most of the available fluorescent lamps (*e.g.,* UV-B lamps, tanning lamps, and germicidal lamps) for use in studying the biological effects of these light sources on animals.

The SKH-1 hairless mouse is the animal model being used for phototoxicology testing. Currently there is one animal room and expansion of additional rooms is planned for April 2001 and June 2003. Upon completion of this expansion, the Laboratory will have the capacity for the simultaneous photocarcinogenicity testing of up to four chemicals.

Chemicals are nominated for testing by each of the FDA Centers and Offices within the FDA Commissioner's Office. A FDA committee (Phototoxicology Chemical Selection Working Group) prioritizes the nominations and forwards them to the ICCEC for entry into the NTP nomination and selection process (see page 10). A standing committee (Toxicology Study Selection and Review Committee), composed of scientists with expertise in this area from FDA, NTP, NIEHS/NIH, other Federal agencies, and academia, reviews the design of protocols and progress on studies. Such studies should generate critically important scientific data for use in determining potential human health risks from the effects of therapeutic agents, chemicals used in cosmetics, device materials, food additives and supplements, tanning enhancers, etc. on light-induced skin toxicity and skin cancer.

For specific questions about the Phototoxicology Center and the FDA-NIEHS Phototoxicology Research and Testing Laboratory methods contact Dr. Paul C. Howard, NCTR, HFT-110, 3900 NCTR Road, Jefferson, Arkansas, 72079; T: (870) 543-7137; phoward@nctr.fda.gov.

Current Research Initiatives

Dermatological Chemoexfoliation

A study at the NCTR/FDA proposes to investigate the effects of dermatological chemoexfoliation using α- and β-hydroxy acids on cell proliferation and DNA damage in the hairless mouse exposed to simulated solar light. For details of this initiative see page 15 (Howard, NCTR/FDA).

Photosensitization

Photosensitization can result when light interacts with endogenous or exogenous chemical agents in the skin and eyes. This process can produce undesirable clinical consequences, such as exaggerated sunburn, allergic reactions, or skin cancer, or can have beneficial effects as in tumor photodynamic therapy or psoralen therapy for psoriasis. NIEHS/NIH scientists are studying the photochemical mechanisms whereby photosensitizers exert their toxic or therapeutic effects. Agents being examined include fluoroquinolones, a relatively new class of antibacterials useful in the treatment of gram-negative bacterial infections. In another

study, the photochemical properties of water samples from selected ponds in Minnesota and Vermont in which malformed frogs are found are under study (Chignell, NIEHS/NIH).

UV-Induced Carcinogenesis

Researchers at NIOSH/CDC are studying the mechanisms of toxicity and carcinogenicity of UV radiation in skin diseases (Ding, NIOSH/CDC; see page 57).

Phototoxicology Methods Development

In order to understand the photochemistry and photophysics of environmental chemicals it is necessary to use modern chemical analysis and spectroscopic techniques of many kinds. The following spectrometers are being built and tested at the NIEHS/NIH: steady state and lifetime spectrophotofluorometer, steady state and lifetime singlet oxygen luminescence spectrometers, and a laser flash photolysis spectrometer (Chignell, NIEHS/NIH).

Areas for Future Initiatives and Resources

Laboratory Expansion

Exposure of the U.S. population to UV radiation and the use of topical skin agents are increasing annually. The new NTP Center for Phototoxicology offers a unique opportunity for the NTP to become a leader in the evaluation of these types of substances for their phototoxic and photocarcinogenic potentials. The Laboratory has expanded its animal facility to handle simultaneous photocarcinogenicity testing of up to three chemicals and hopes to undergo additional expansion in the future. This expansion would enable testing of alternate animal models (*e.g.* transgenic) for their suitability as replacements for the SKH-1 albino mouse and to address phototoxic effects for endpoints other than cancer, such as photoimmunotoxicity and ocular toxicity.

Studies of Specific Diseases

The SKH-1 mice get cataracts when exposed to simulated sunlight. With expansion of the Laboratory, the NTP plans to initiate studies to characterize this effect and investigate its etiologic mechanism.

REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

NTP Center for the Evaluation of Risks to Human Reproduction

The NTP has become increasingly responsive to a growing concern both public and scientific that the ability of humans to conceive offspring and the normal healthy development of children during pregnancy and childhood may be adversely affected by environmental exposures. Reports of increased numbers of infertile couples, reduced sperm counts, increases in abnormal sexual development and cancers of the reproductive system, and occupational exposures leading to adverse pregnancy outcomes have all contributed to higher levels of public concern about the contribution of environmental exposures to reproductive and children's health issues. In response to these concerns, the NTP and the NIEHS/NIH established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in

June 1998. The Center is located at the NIEHS/NIH and Dr. Michael Shelby serves as the Center Director. The primary purpose of the Center is to provide scientifically based, uniform assessments of human and experimental evidence for adverse effects on reproduction, including development, caused by agents to which humans may be exposed. The goals of individual chemical assessments are to:

- interpret for and provide information to the general public about the strength of the scientific evidence that a given exposure or exposure circumstance poses a hazard to reproduction and the health or welfare of children;
- to provide regulatory agencies with objective and scientifically credibly assessment of data related to the reproductive/developmental health effects associated with exposure to specific chemicals or classes of chemicals, including descriptions of any uncertainties associated with the risk assessments; and
- identify knowledge gaps to help establish research and testing priorities.

The NTP Board of Scientific Counselors (see page 2) advises the Center on its processes, priorities, and direction. Information about the CERHR and the nomination process is available on its web page (http://cerhr.niehs.nih.gov) or by contacting Dr. Shelby, NTP-CERHR, NIEHS, P.O. Box 12233 EC-32, Research Triangle Park, NC 27709; T: (919) 541-3455; F: (919) 316-4511; shelby@niehs.nih.gov. The Center's website also provides information covering common questions and concerns regarding healthy pregnancy and the potential of various exposures to affect adversely the development of children.

Expert Panel Peer Reviews

Seven Phthalate Esters

The Center's first Expert Panel was formed in the summer of 1999 to evaluate the scientific evidence that seven selected phthalate esters may pose a reproductive and/or developmental risk to exposed humans. The seven phthalates are butyl benzyl phthalate, di(2-ethylhexyl)phthalate, di-isodecyl phthalate, di-isononyl phthalate, di-*n*-butyl phthalate, di-*n*-hexyl phthalate, and di-*n*-octyl phthalate. The Phthalates Expert Panel met three times and completed its review in 2000 and copies of the expert panel reports' are available on the CERHR website or by contacting the Center. The NTP will solicit public comment on this report in 2001 and then based upon the total body of information (*i.e.*, new information, public comments, and Expert Panel Report), the NTP will prepare its Center Report for transmittal to federal agencies, interested stakeholders, the public, and others later this year.

Methanol

The next chemical planned for evaluation by the Center is methanol (CAS No. 67-56-1). This meeting is scheduled for summer/fall 2001 in Alexandria, VA. A large toxicity database exists on the reproductive and developmental effects of methanol. Methanol is a commercially important, high production volume chemical with potential for occupational, consumer and environmental exposure.

Current Research Initiatives

The NTP has developed a wide range of techniques and testing regimes (Table 13, Column 5) for evaluating potential toxic effects of chemical exposure on the reproductive system of rodent models and/or the developing embryo of rodent models. A variety of agents are being evaluated by the NTP for their potential reproductive or developmental toxicity. The study of twenty-nine compounds is on-going from FY 2000 and six studies are proposed to start in FY 2001. Some of these substances are identified as possible drinking water contaminants (see

page16) or endocrine disrupting agents (see page 21); others are herbal medicines or dietary supplements (see page 19). Pesticides (*e.g.,* carbaryl, chlorpyrifos, heptachlor, methoxychlor, and tebuconazole) are under study for determining whether developmental exposures have lasting effects on functioning of the nervous, immune, or reproductive system in adult rats (juvenile pesticide assessment; Bishop, NIEHS/NIH). Many of the *in vivo* and *in vitro* laboratory activities complement human reproductive and developmental initiatives in environmental epidemiology and exposure assessment (see Epidemiology, page 62 and Exposure Assessment page 67).

Table 13. Substances under Consideration for Reproductive and Developmental Toxicity

Chemical	CAS No.	Project Leader[1]	Species: Strain	Route	Testing Battery[2]
Studies ongoing in FY 2001 as of 10/01/00					
3'-Azido-3'-Deoxythymidine (AIDS initiative)	30516-87-1	Bishop	Mice: Swiss (CD-1)	Gavage	RACB
3'-Azido-3'-Deoxythymidine/ 2',3'-Dideoxycytidine (AIDS initiative)	AZTDDCCOMB	Jahnke	Mice: Swiss (CD-1)	Gavage	TER
3'-Azido-3'-Deoxythymidine + 2',3'-Dideoxycytidine (AIDS initiative)	AZTDDICOMB	Bishop	Mice: C57BL/6	Gavage	RACB
Benzophenone	119-61-9	Jahnke	Rats: Sprague-Dawley	Gavage	TER
			Rabbit: New Zealand White	Gavage	TER
					TRP
Berberine Chloride Dihydrate	5956-60-5	Jahnke	Mice: Swiss (CD-1)	Feed	TER
			Rats: Sprague-Dawley	Feed	TRP
			Mice: Swiss (CD-1)	Feed	TRP
Boric Acid	10043-35-3	Bishop	Rats: Sprague-Dawley	Gavage	RACB
Chlorpyrifos (Dursban)	2921-88-2	TBD[4]	Rats: Sprague-Dawley	Gavage	JPA
Dibutyl Phthalate	84-74-2	Bishop	Rats: Sprague-Dawley	Feed	RACB
cis-Dichlorodiamine Platinum	15663-27-1	Harris	Rats: Sprague-Dawley	IP/IJ[3]	TER
cis & trans 1,2-Dichloroethylene	540-59-0	Heindel	Rats: Sprague-Dawley	Gavage	TRP
Di(2-ethylhexyl) Phthalate	117-81-7	Bishop	Rats: Sprague-Dawley	Feed	RACB
Emodin	518-82-1	Jahnke	Mice: Swiss CD-1	Feed	TER
			Rats: Sprague-Dawley	Feed	TER
					TRP
Formamide	75-12-7	Jahnke	Rabbit: New Zealand White	Gavage	TER
Heptachlor	76-44-8	TBD	Rats: Sprague-Dawley	Gavage	JPA
Hexachlorobenzene	118-74-1	Bishop	Rats: Sprague-Dawley	Gavage	RACB
Isoeugenol	97-54-1	Bishop	Rats: Sprague-Dawley		RACB
		Hunter	Rabbit: New Zealand White	Gavage	TRP
Methyleugenol	93-15-2	Jahnke	Rats: Sprague-Dawley	Gavage	TER
Sodium Thioglycolate	367-51-1	Jahnke	Rats: Sprague-Dawley	Topical	TER
			Rabbit: New Zealand White	Topical	TER
					TRP
Tebuconazole	80443-41-0	TBD[3]	Rats: Sprague-Dawley	Gavage	JPA
Endocrine Disrupting Agents					
Ethinyl Estradiol	57-63-6	Delclos (NCTR)	Rats: Sprague-Dawley	Feed	MG
Genistein	446-72-0	Delclos (NCTR)	Rats: Sprague-Dawley	Feed	MG
Methoxychlor	72-43-5	Delclos (NCTR)	Rats: Sprague-Dawley	Feed	MG
Nonylphenol	104-40-5	Delclos (NCTR)	Rats: Sprague-Dawley	Feed	MG

Chemical	CAS No.	Project Leader[1]	Species: Strain	Route	Testing Battery[2]
Vinclozolin	50471-44-8	Delclos (NCTR)	Rats: Sprague-Dawley	Feed	MG
Pesticides in Children Project					
Carbaryl	63-25-2	Harris	Rats: Sprague-Dawley	Gavage	JPA
Methoxychlor	72-43-5	Harris	Rats: Sprague-Dawley	Gavage	JPA
Water Disinfection By-Products					
DBP Mixture	DWDBPMIXTURE	Bishop	Rats: Sprague-Dawley	Water	RDGT
Sodium Bromate	7789-38-0	Bishop	Rats: Sprague-Dawley	Water	RACB
Sodium Chlorate	7775-09-9	Jahnke	Rabbit: New Zealand White	Gavage	TER
Studies proposed to start in FY 2001 as of 10/01/00; Others may be scheduled as protocols are finalized					
1-Bromopropane	106-94-5	Bishop	Rats: TBD	Gavage	RACB
2-Bromopropane	75-26-3	Bishop	Rats: TBD	Gavage	RACB
2'3'-Dideoxyinosine +D4T (AIDS initiative)	DDI/D4TCOMB	Jahnke	Mice: Swiss (CD-1)	Gavage	TRP
Silver Acetate	563-63-3	Jahnke	Rats: Sprague-Dawley	Feed	TER
					TRP
α-Solanine	20562-02-1	Jahnke	Mice: Swiss Albino	Gavage	TER
					TRP
Herbal Medicines/Dietary Supplements					
Powdered Root of Goldenseal	GOLDENSEALRT	Jahnke	Rats: Sprague-Dawley	Feed	TER
					TRP
			Mice: Swiss (CD-1)	Feed	TER
					TRP

[1] Project Leader - NIEHS/NIH staff scientist (unless otherwise indicated) who oversees each chemical's evaluation

[2] Testing Battery

PA = Juvenile Pesticide Assessment; exposure during development with assessment of central nervous, immune, and reproductive systems in the adult animal.

MG = Multigenerational Endocrine Disruptor Studies; assess effects on reproduction and cancer endpoints over multiple generations.

PZE = Preimplantation Zygote Effects; a test in mice used to examine effects during the earliest stages of embryo development (equivalent to the first trimester in humans) and to identify those effects that may cause birth defects or other developmental toxicities if the embryo is exposed during these stages of development.

RACB = Reproductive Assessment by Continuous Breeding; two-generation study design for identifying long-term effects on male and/or female reproduction, characterizing toxicity, and examining dose-response relationships of the test compound.

RDGT = Reproductive/Developmental & General Toxicity; identify the physiologic processes (development; female reproduction; male reproduction; various somatic organs/processes) that are the most sensitive to exposure.

TER = Teratology; examines both maternal factors of the pregnant dam as well as the health and well being of the developing fetus including body weight, visceral and skeletal examination, and as possible, LOAEL (lowest observed adverse effect level) and NOAEL (no observed adverse effect level) are determined for maternal and fetal toxicities.

TRC = Total Reproductive Capacity; detection of infertility, spontaneous abortion, fetal and neonatal death, birth defects, and genetic susceptibility.

TRP = Teratology Pilot Study; dose screening study designed to help set doses for the definitive teratology study. Study design considers maternal toxicity, fetal toxicity, fetal body weight, and external malformations, but does not include visceral or skeletal examination of the fetus.

[3] IP/IJ = Intraperitoneal Injection

[4] TBD = to be determined

Children's Health

The public is concerned about the potential effects of environmental and occupational toxicants on the developing young and through both the CERHR and research studies, the NTP is addressing this issue. The Juvenile Pesticide Assessment (see Table 12) is evaluating issues related to children's health and functional deficiencies previously identified in human adults who received perinatal and/or prepubertal exposures to pesticides. In these evaluations, relatively high chemical exposure doses are being included in order to obtain information useful to regulatory agencies for making determinations about human safety indices and for comparing the data with currently acceptable levels of allowable pesticide residues in the food supply. Studies are also ongoing to evaluate the impact of environmental agents on the developing immune (see page 30) and nervous systems (see page 35) as well as future efforts to design testing batteries and identify biomarkers for use in assessment of children's health. Many epidemiology studies (see page 62) are evaluating environmental agents as potential risk factors because of concerns for potential effects on reproduction and development and childhood illnesses. The NTP is interested in developing models that allow better estimations of human risk from environmental toxicants in the young (see page 70).

Female Reproductive System

Researchers at the NCTR/FDA are actively involved in experiments designed to define the normal and estrogen-altered reproductive tract developmental profile using the rodent model. This information is being used to create and validate a computerized knowledge base. Using this information several Quantitative Structure Activity Relationship models for chemical binding to estrogen receptors are under development (Sheehan, NCTR/FDA).

Male Reproductive System

Establishing the mechanism(s) of how toxicants adversely affect the reproductive system is an area of ongoing research at both NIOSH/CDC and NIEHS/NIH. The NIEHS/NIH has a project aimed at understanding the molecular responses of the testis to toxicants in rodents and learning how exposed testicular cells interact to produce a toxic response. Future plans target application of a newly developed and refined co-precipitation technique to explore the functional control of sperm release; inhibited release is the most common testicular lesion and is produced by nearly every testicular toxicant (Bishop, NIEHS/NIH). Studies at the NIOSH/CDC are exploring how exposure to environmental/occupational chemicals that mimic or antagonize the effects of endogenous hormones (especially estrogens or androgens) can adversely affect normal developmental/reproductive functions regulated by these hormones (Murono, NIOSH/CDC, see page 22).

Through an interagency agreement with the Lawrence Livermore National Laboratory/DOE, the NIEHS/NIH is sponsoring development of an assay that employs fluorescence *in situ* hybridization (FISH) to detect structural and numerical chromosome damage in sperm and early embryonic cells of rats and mice. Currently, FISH is being used to evaluate the aneugenic and clastogenic potential of environmental chemicals tested in NTP bioassays, as well as to address questions about dose response, differential stage sensitivity, and the relationship between defects found in sperm and those transmitted to the embryo. Efforts will continue to validate the use of the rodent assays (Bishop, NIEHS/NIH).

Mammalian Mutagenesis

Through an interagency agreement with Oak Ridge National Laboratory, the NIEHS/NIH is sponsoring an evaluation of chemicals for assessing their hazard potential as germ cell mutagens using an *in vivo* mouse model and investigating the mutation process. These studies

should provide data on environmental agents that present risk of heritable genetic damage, data for assessing their potential genetic risk, information about the specific gender and germ cell stage affected, and details about the molecular nature of the induced genetic damage. This type of information will enhance understanding the effects of environmental exposures on infertility, pregnancy loss, birth defects, and genetic disease (Bishop, NIEHS/NIH).

Somatic and germ cell mutations can have a severe impact on the fitness of multicellular organisms and their offspring, respectively. The NIEHS/NIH has developed a procedure using the well-characterized *am3* mutation of PhiX 174 to differentiate mutations that are fixed within C57BL6/J mice from those that arise from *ex vivo* events that damage DNA. This procedure potentially has multiple applications: 1) studying mutations induction by environmental toxicants during development, 2) investigating homeostasis of mutations during the aging process, 3) identifying characteristics of tumor progression from preneoplastic to neoplastic growth in relation to genomic stability, and 4) evaluating systemic effects of somatic mutations in development of age-related degenerative diseases (Malling, NIEHS/NIH).

Teratology

Field studies have confirmed that there is a significant elevation in the frequency of malformed amphibians at several study sites in Minnesota and Vermont. Studies are continuing at the NIEHS/NIH to identify the etiology of these effects. Water and sediment samples are being tested for the capacity to cause malformation of frogs in the laboratory. Studies continue about whether pathways associated with thyroid metabolism may be involved, and future efforts will target gene markers for frog embryogenesis, retinoid/thyroid receptor binding, endocrine response, and transgenic fish mutagenesis assays. It is proposed that a complex mixture of man-made chemical degradation products and natural compounds may be acting synergistically to cause these effects; this hypothesis is being tested (Burkhart, NIEHS/NIH).

Areas for Future Initiatives and Resources

Areas for Specific Studies

The NTP will continue its efforts to develop methods and strategies for identifying the effects of environmental and occupational toxicants on reproductive and developmental endpoints and recognizes that this area is one of increasing public interest. Future initiatives will address the unique sensitivities of developing humans to toxic insults and the potential for consequential exposure-related adverse health effects that may manifest themselves anytime from conception through adulthood. In addition, research efforts will be directed toward the sites and mechanisms of action of reproductive and developmental toxicants and on developing a wide range of techniques for evaluating those effects. To facilitate this research effort, the NTP is modifying the concept for toxicology and carcinogenesis studies that is used to employ contract mechanisms for these studies using animals. The expanded concept includes the potential for studying *in utero* and postnatal exposures and for expanding the range of assessments to include developmental immunotoxicity, neurotoxicity, and reproductive and developmental effects. This broadened scope will enhance the NTP's opportunities for gaining knowledge about exposure-related non-cancer disease etiologies and dysfunction(s) and enrich the science base available to regulatory agencies for assessment of human risk.

Resources for the Center

The NTP Center for the Evaluation of Risks to Human Reproduction provides a unique service to the public through its evaluation of the scientific evidence for potential reproductive and developmental effects of chemicals and its communication of that assessment. The Center is still in its infancy, but is receiving accolades for its efforts from other agencies, industry, and the public. The number of chemicals for evaluation is vast, and efforts must be directed toward streamlining this system to maximize output, but without compromising the integrity of the evaluation process. The Center also serves as a resource for identifying research gaps that can be addressed through basic reproductive research and additional resources may be needed by the NTP to meet those research needs.

Development of Registries

Understanding the impact of environmental exposures on reproduction and development requires that efforts be directed toward assessing both exposures and effects in humans. More extensive birth registries are needed for determining the types, frequencies, and geographic distribution of birth defects, and efforts are needed for gathering reliable information about children's exposures to environmental agents. Such information would greatly facilitate future research initiatives and provide important and useful human exposure information for the Center's use in selecting chemicals for evaluation and in assessing the potential adverse effects on reproduction and development from those chemical exposures.

RESPIRATORY TOXICOLOGY

Current Research Initiatives

Inhalation exposure to environmental and occupational toxicants is a major contributing factor to human health problems. Chemicals being studied or planned for laboratory study by inhalation routes include carbonyl sulfide, cumene, decalin, divinylbenzene, α-methylstyrene, mercury vapor, methyl isobutyl ketone, propargyl alcohol, propylene glycol mono-tertiary-butyl ether, stoddard solvent (type IIc), and vanadium pentoxide (see Tables 8, 12 and 14); others may be studied as time and resources permit. Several agents are also under consideration as causative for adult or childhood respiratory diseases through epidemiology studies. These include studies on air pollution, beryllium, and pesticides and are outlined in greater detail in the section, Epidemiology (see page 62).

Inhalation Facilities and Methods Development

Many NTP research efforts are directed toward understanding the biochemical and molecular mechanisms of toxicity of inhaled chemicals. The activities within the inhalation facilities at NIEHS/NIH and NIOSH/CDC provide support for NTP-sponsored inhalation studies by conducting special studies on inhalation dosimetry and mechanisms of toxicity. Additional activities include development of inhalation exposure technology and models for investigating pulmonary disease (Goldsmith, NIOSH/CDC; Moorman, NIEHS/NIH).

Exposure of laboratory animals by inhalation closely duplicates the way that humans are exposed to airborne toxicants and is essential for studying the role of chemicals in respiratory disease; however, inhalation studies are technologically difficult to perform and require unique equipment and resources. A prototype whole-body vibration system for small laboratory animals is now developed at the NIOSH/CDC and currently is being tested for use in studying whether whole-body vibration affects aerosol deposition and gas absorption in the

lungs. Future plans include modifying the system to accommodate multiple animals and to integrate existing inhalation exposure methods (Frazer, NIOSH/CDC).

Inhaled xenobiotics often affect the ventilation mechanics of test animals, and physiological modeling and dosimetry rely heavily on an estimate of the rate of alveolar ventilation. A whole body plethysmograph has been developed at the NIEHS/NIH to use with the nose only exposure systems for measuring the minute alveolar volume in test animals under exposure conditions. This information is being used in conjunction with disposition and toxicity data in a physiological simulation to build a physiologically realistic description of the toxicokinetics of compounds under study; a toxicokinetic model to describe inhalation of styrene in rats and mice has been developed (Moorman, NIEHS/NIH).

Magnetic resonance imaging (MRI) provides an opportunity to visualize internal organs at microscopic resolution in live animals and to conduct specialized studies on fixed blocks of pathologic specimens. Techniques at the NIEHS/NIH have been refined for use of hyperpolarized helium in imaging fine structures of the lung in live animals. Using the elastase rat lung model for emphysema, data are currently being collected using hyperpolarized helium as well as standard proton images for assessment of pulmonary damage and pulmonary function (Maronpot, NIEHS/NIH).

Asthma

The morbidity and mortality of asthma is increasing and occupational asthma from workplace exposures comprises a significant risk associated with hospital admissions. The airway epithelium is a primary point of contact of inhaled substances and is intimately involved in early events triggered by inhaled occupational asthmagens. Using animal models, investigators at NIOSH/CDC are modeling occupational asthma with selected asthmagens and agents (*e.g.,* ozone, toluene diisocyanatae, bacterial lipopolysaccharide, hard metals, ovalbumin) known to be associated with occupational asthma and obstructive disease in workers. *In vitro* studies will investigate the mechanistic actions and pathophysiological effects of these agents on the epithelium (Fedan, NIOSH/CDC). Another study in rodents is addressing whether female reproductive status (pregnancy and lactation) alters pulmonary immune responses to hazardous agents that might be inhaled in the workplace (Huffman, NIOSH/CDC).

Lung Disease Susceptibility

Several efforts at NIOSH/CDC are working to understand how inhalation of particulates can affect susceptibility of workers to lung disease. One study is evaluating the physical and chemical characteristics of respirable particles (nonfibrogenic silicate mineral, quartz, diesel exhaust, and hard metal process materials) and testing their interactions with pulmonary surfactant and surfactant components. Particle properties will be related to toxicological properties (Keane, NIOSH/CDC). Another project is addressing how different occupational dusts and fibers affect susceptibility to pulmonary infections. A variety of metal-containing particles (e.g., residual oil fly ash or welding fumes) differing in their inflammatory and fibrotic characteristics are being evaluated for toxic and immune effects in the lungs (Antonini, NIOSH/CDC). Using *in vitro* and *in vivo* models, NIOSH/CDC is investigating the role of diisocyanates-thiol reaction products in mediating diseases commonly seen in polyurethane user industries as a result of inhalation exposure to isocyanates (Siegel, NIOSH/CDC).

Asphalt fume exposure has been associated with airway irritation and hyperactivity in some pavers. The effects of asphalt fumes on airway irritation, pulmonary inflammation, airway reactivity, and lung damage in a rodent model is currently being investigated. Using knowledge gained from direct evaluation of asphalt paving conditions with the design and

construction of a generator, this study is able to recreate exposures similar to those generated during road paving. Preliminary results from this inhalation study suggest that exposure does not cause significant inflammation or lung injury, but may affect metabolic function in the lung as measured by pulmonary P450 metabolism (Ma, NIOSH/CDC).

Fibrosis

Occupational toxicants, such as asbestos and crystalline silica, are identified and associated with lung disease; therefore, there is great interest in identifying synthetic vitreous fibers as suitable replacements to these substances. However, to ascertain the safety of such replacements, knowledge is needed about the mechanisms associated with fibrotic processes. A NIOSH/CDC study is underway to evaluate the cytotoxicity of abrasive substitutes for silica, to determine the importance of fiber length to the cytotoxicity of fibers, and to elucidate mechanisms for initiation and progression of fibrosis. A dielectrophoresis system, which separates fibers according to length, is now developed and will allow an evaluation of the role of physical dimensions versus chemical properties in the development of pulmonary disease (Castranova, NIOSH/CDC). The methods for crystalline silica analysis currently require extensive sample preparation. Validation studies are ongoing for testing (sensitivity, variability, interference effects, particle-size effects, and matrix effects) whether Photoacoustic Fourier transform (PA-FTIR) infrared spectroscopy can be a valid measurement or field technique for crystalline silica analysis. After this evaluation, field samples will be analyzed (Orr, NIOSH/CDC).

A key feature of environmental fibroproliferative diseases is fibroblast hyperplasia. Researchers at the NIEHS/NIH are investigating the mechanisms for fibrosis caused by the fibrogenic metal, vanadium pentoxide. Vanadium stimulates a wide spectrum of inflammatory mediators including cytokines, growth factor receptors, and intracellular signaling intermediates. Future work will focus on elucidation of molecular events and the development of intervention strategies (Bonner, NIEHS/NIH).

Positron emission tomography (PET) is being tested for use in *in vivo* pulmonary fibrosis detection and evaluation. PET imaging of silicosis in a rabbit model is being compared with histopathologic characterization of lung tissue one to five months following dust challenge. A future study is planned that will detail the contributions of fibrosis versus inflammatory response to tracer uptake and toxicity testing of the fluoroproline probe (Wallace, NIOSH/CDC).

Modeling of Occupational Settings

Under an interagency agreement between NIEHS/NIH and NIOSH/CDC, efforts are underway to expand the ability to model occupational settings and especially to evaluate the way workers are potentially exposed to toxicants. Initiatives under this agreement target respiratory toxicology and are described on page 18.

Areas for Future Initiatives and Resources

The NTP through its NIEHS/NIH – NIOSH/CDC interagency agreement is making a major effort toward addressing the impact of occupational exposures on worker health. The initial efforts under this initiative are successful (*i.e.* worker exposure to asphalt fumes and cellulose inhalation) and studies are moving forward. The NTP needs to continue the evaluation of other occupational settings (*e.g.,* metal working fluids and welding fumes). The NTP also needs to address how it will use knowledge learned from these studies to communicate its findings and to improve worker education.

CARCINOGENESIS

Current Research Initiatives

Chronic Phase of Study

Two-year studies in rodents remain the primary laboratory method by which chemicals or physical agents are identified as having the potential to be hazardous to man. Identifying substances with the potential to cause cancer in animals provides valuable guidance for identifying potential hazards to humans and serves as a basis for the hazard identification step of risk assessment and the risk management process undertaken by regulatory agencies. These long-term toxicology and carcinogenesis studies (bioassays) in rodents generally employ both genders of rats (Fisher 344) and mice (B6C3F1 hybrid) with three exposure levels plus untreated controls in groups of 50 animals for two years; fish models are also occasionally used. If adequate data exists in the literature for one rodent species, then typically only the remaining species is studied. The NTP interfaces its testing with regulatory agencies and the private sector in order to minimize duplication of effort. Table 14 lists the chemicals currently in the chronic phase of NTP study. Testing for 34 compounds is ongoing from FY 2000 of which 10 studies are scheduled to end with preparation of Technical Reports for review in May and October 2001 (see page 14). Studies for eight chemicals are scheduled to start in FY 2001 and awards are anticipated for future study of 8-10 additional chemicals per year. Several studies including urethane and ethanol are being conducted at NCTR/FDA.

Radiofrequency Emissions Associated with Wireless Technologies

In response to a nomination from the FDA (see page 13), the NTP is currently evaluating the feasibility of performing rodent studies on the radiofrequencies used in cellular telephone transmissions for potential carcinogenic effects. Such studies will be extremely complex. NTP staff is currently working with radiofrequency experts at the National Institute of Standards and Technology to evaluate studies being planned or underway by a consortium of European investigators under the auspices of the European Union and by investigators at the Cancer Research Center of the European Ramazzini Foundation of Oncology and Environmental Sciences Commission. Following this careful and in-depth assessment, the NTP will determine whether a need exists to perform an independent evaluation (Melnick, & Roycroft, NIEHS/NIH).

Mechanism-Based Carcinogenesis

DNA Repair

DNA damage and mutations are important in the etiology of some human diseases. The process by which repair proteins find damaged bases within DNA represents an important type of protein-DNA interaction that is not well understood. Work is underway at the NIEHS/NIH to investigate the structure and function of DNA repair proteins, UvrA, UvrB, and UvrC (Van Houten, NIEHS/NIH).

Cell Cycle Control

Exposure to environmental carcinogens can result in loss of normal cellular growth control and eventual tumor formation. A variety of activated oncogene (*e.g., ras, mos,* MEK) products and tumor suppressor gene products (*e.g.*, ataxia telangiectasia, RB and p53) interact directly or indirectly with vital cell cycle control signal transduction pathways. Researchers at the NIEHS/NIH are investigating molecular mechanisms of cell cycle control and how

Table 14. Compounds in the Chronic Phase of NTP Study

Chemical Name	CAS No.	Project Leader[1]	Contract Project Officer[2]	Species: Strain	Route	Special Studies[3]	Study Length[4]
Studies to be completed in FY 2001 as of 10/01/00 for NTP Technical Report Review							
Acrylonitrile	107-13-1	Ghanayem	Orzech	Mice: B6C3F1	Gavage	Toxicokinetics: 2 weeks & 3, 12, and 18 months	2 years
Citral	5392-40-5	Chhabra	Orzech	Mice: B6C3F1 Rats: Fischer 344	Micro-encapsulation in feed		2 years
Ethanol	64-17-5	Beland (NCTR)	Bucher	Mice: B6C3F1	Water	DNA adducts; cell proliferation/apoptosis	2 years
Methacrylonitrile	126-98-7	Ghanayem	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Gavage	Toxicokinetics: 2 weeks; 3, 12, and 18 months	2 years
o-Nitrotoluene	88-72-2	Dunnick	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Feed	Stop study in male rats - 2000 & 5000 ppm for 3 months; Oncogene; Toxicokinetics: 2 weeks; 3, 12, and 18 months	2 years
p-Nitrotoluene	99-99-0	Dunnick	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Feed	Toxicokinetics: 2 weeks; 3, 12, and 18 months	2 years
Riddelliine	23246-96-0	Chan & Chou (NCTR)	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Gavage	DNA adduct analysis of female rat livers; oncogene	2 years
Urethane (ethyl carbamate)	51-79-6	Beland (NCTR)	Bucher	Mice: B6C3F1	Water	DNA adducts; cell proliferation/apoptosis at same dose levels	2 years
Urethane + Ethanol (Combination)	URETHCOMB	Beland (NCTR)	Bucher	Mice: B6C3F1	Water	DNA adducts; cell proliferation/apoptosis at same dose levels	2 years
Vanadium Pentoxide	1314-62-1	Roycroft	Roycroft	Mice: B6C3F1Rats:Fischer 344	Inhalation	Toxicokinetics: 3, 6, 12, and 18 months; special female rat and mouse study (40 mice or rats/treated group)	2 years
Studies ongoing in FY 2001 as of 10/01/00							
AZT Transplacental Carcinogenesis Study	30516-87-1	Rao	Rao	Mice: Swiss CD-1	*In utero*		19 months
Benzophenone	119-61-9	Chhabra	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Feed	Toxicokinetics: 2 weeks; 3, 12, and 18 months	2 years
trans-Cinnamaldehyde	14371-10-9	Bucher	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Micro-encapsulated in Feed	Toxicokinetics: rats at 2 weeks, 3, 12, and 18 months	2 years
Decalin	91-17-8	Chan	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Inhalation		2 years

Chemical Name	CAS No.	Project Leader[1]	Contract Project Officer[2]	Species: Strain	Route	Special Studies[3]	Study Length[4]
Diisopropylcarbodiimide	693-13-0	Chhabra	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Topical		2 years
Dipropylene Glycol	25265-71-8	Chhabra	Orzech	Mice: B6C3F1 Rats: Fischer 344	Water		2 years
Divinylbenzene	1321-74-0	Morgan	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Inhalation		2 years
Elmiron (Sodium Pentosanpolysulfate)	37319-17-8	Abdo	Orzech	Mice: B6C3F1 Rats: Fischer 344	Gavage		2 years
2,4-Hexadienal	142-83-6	Chan	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Gavage	DNA adduct analysis of forestomach and liver in males	2 years
α-Hydroxy Acid (lactic)	50-21-5	Howard (NCTR)	Bucher	Mice: SKH-1	Topical		1 year
β-Hydroxy Acid (glycolic)	79-14-1	Howard (NCTR)	Bucher	Mice: SKH-1	Topical		1 year
Leucomalachite Green	129-73-7	Culp (NCTR)	Bucher	Mice: B6C3F1 Rats: Fischer 344	Feed		2 years
Malachite Green	569-64-2	Culp (NCTR)	Bucher	Mice: B6C3F1 Rats: Fischer 344	Feed		2 years
Methylene Blue Trihydrate	7220-79-3	Chhabra	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Gavage	Toxicokinetics: 4 and 13 weeks and 19 months	2 years
2-Methylimidazole	693-98-1	Chan	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Feed	Thyroid hormones; Liver P450s and UDP-glucose; 20/sex/species/group at 8 days and 13 weeks	2 years
4-Methylimidazole	822-36-6	Chan	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Feed		2 years
Methyl Isobutyl Ketone	108-10-1	Chhabra	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Inhalation		2 years
Propylene Glycol Mono-*t*-butyl Ether	57018-52-7	Herbert	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Inhalation	Toxicokinetics: 2 and 6 weeks; 3, 6, and 12 months for urine; 2, 6, and 13 weeks for blood	2 years
Simazine	122-34-9	Trnovec	Boorman	Mice: B6C3F1	Feed		2 years
Stoddard Solvent (Type IIC)	64742-88-7	Chhabra	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Inhalation	Rats: 10/sex group kidney cell proliferation and α2μ-globulin	2 years
Triethanolamine	102-71-6	Bucher	Orzech	Mice: B6C3F1	Topical		2 years
Fish Project 1							
Nitromethane	75-52-5	Boorman	Bernheim	Fish: Medaka (*Oryrias Latipes)* Fish: Guppy (*Poecilia Reticulata)*	Aqueous Exposure		9 months; 16 months
2,2-Bis(Bromormethyl)-1,3-	3296-90-0	Boorman	Bernheim	Fish: Medaka (*Oryrias Latipes)*	Aqueous		9 months;

Chemical Name	CAS No.	Project Leader[1]	Contract Project Officer[2]	Species: Strain	Route	Special Studies[3]	Study Length[4]
propanediol				Fish: Guppy (*Poecilia Reticulata)*	Exposure		16 months
1,2,3-Trichloropropane	96-18-4	Boorman	Bernheim	Fish: Medaka (*Oryrias Latipes)* Fish: Guppy (*Poecilia Reticulata)*	Aqueous Exposure		9 months; 16 months
Toxic Equivalency Factor Evaluation							
Binary Mixture	TEFBINARY MIX	Chhabra	Orzech	Rats: Sprague Dawley	Gavage	T3, T4, TSH, P450s; Toxicokinetics	2 years
Dioxin Mixture	TEFDIOXIN MIX	Chhabra	Orzech	Rats: Sprague Dawley	Gavage	T3, T4, TSH, P450s; Toxicokinetics	2 years
PCB 153: 2,2'-4,4',5,5'-Hexachlorobiphenyl	35065-27-1	Chhabra	Orzech	Rats: Sprague Dawley	Gavage	T3, T4, TSH, P450s; Toxicokinetics; stop study	2 years
PCB-118: 2,3',4,4',5-Pentachlorobiphenyl	31508-00-6	Chhabra	Orzech	Rats: Sprague Dawley	Gavage	T3, T4, TSH, P450s; Toxicokinetics; stop study	2 years
PCB-126: 3,3',4,4',5-Pentachlorobiphenyl	57465-28-8	Chhabra	Orzech	Rats: Sprague Dawley	Gavage	T3, T4, TSH, P450s; Toxicokinetics; stop study	2 years
PECDF: Pentachlorodibenzofuran	57117-31-4	Chhabra	Orzech	Rats: Sprague Dawley	Gavage	T3, T4, TSH, P450s; Toxicokinetics; stop study	2 years
TCDD: 2,3,7,8-Tetrachloro-dibenzo-*p*-dioxin	1746-01-6	Chhabra	Orzech	Rats: Sprague Dawley	Gavage	T3, T4, TSH, P450s; Toxicokinetics; stop study	2 years
Water Disinfection Byproducts							
Bromodichloromethane	75-27-4	Melnick	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Water		2 years
Dibromoacetic Acid	631-64-1	Melnick	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Water		2 years
Sodium Chlorate	7775-09-9	Melnick	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Water	At days 4 & 21, and at 13 weeks thyroid hormones/ pathology; 10/sex/species	2 years
Studies proposed to start in FY 2001 as of 10/01/00; Others may be scheduled as protocols are finalized							
Water Disinfection By-Products							
Cumene	98-82-8	Chan	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Inhalation		2 years
Formamide	75-12-7	Irwin	Orzech	Mice: B6C3F1 Rats: Fischer 344	Gavage		2 years
5-Hydroxymethyl-furfural	67-47-0	Irwin	Orzech	Mice: B6C3F1 Rats: Fischer 344	Gavage		2 years
α-Methylstyrene	98-83-9	Morgan	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Inhalation		2 years
β-Myrcene	123-35-3	Chan	Orzech	Mice: B6C3F1 Rats: Fischer 344	Gavage		2 years

Chemical Name	CAS No.	Project Leader[1]	Contract Project Officer[2]	Species: Strain	Route	Special Studies[3]	Study Length[4]
Water Disinfection By-Products							
Bromochloroacetic Acid	5589-96-8	Melnick	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Water		2 years
Dibromoacetonitrile	3252-43-5	Melnick	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Water		2 years
Dichloroacetic Acid	79-43-6	Melnick	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Water		2 years

[1] Project Leader - NIEHS/NIH staff scientist (unless otherwise indicated) who oversees each chemical's evaluation

[2] Contract Project Officer - NIEHS/NIH staff scientist (unless otherwise indicated) who coordinates research activities with the contract laboratory

[3] Special Studies:
T3 = Thyroxine 3 and T4 = Thyroxine 4
P450 = Cytochrome P450 enzymes
TSH = Thyroid stimulating hormone
TK = Tyrosine kinase

[4] Standard evaluations include necropsy evaluation and histopathologic examination of tissues.

environmental agents might impact those pathways, particularly focusing on the potentially vulnerable cell cycle checkpoints regulating G1/S and G2/M transitions (Paules, NIEHS/NIH).

Inactivation of the p53 tumor suppressor gene is the most common genetic abnormality of human cancers. As a transcription factor, p53 is important for maintaining genomic integrity and regulating cell growth and death. Alterations of the p53 gene may result from spontaneous mutation or from various environmental factors such as radiation or chemical exposures. Post-translational modification is the major mechanism for regulating p53 induction and activation, primarily by phosphorylation and acetylation. Efforts are ongoing at the NIEHS/NIH to study how changes in p53 phosphorylation relate to its function (Merrick, NIEHS/NIH). Additional studies in the p53 transgenic mouse are investigating the role of the p53 tumor suppressor gene in the evaluation of genistein, an estrogenic compound of soy products (Morris, NCTR/FDA).

Oxidative Stress

Normal organismal homeostasis is maintained through a careful balance of cell proliferation and cell death processes. Cancer cells, while rapidly undergoing division and expansion, may also undergo cell death. Because the susceptibility of cells to undergo apoptosis differs at various stages of the neoplastic process, researchers at the NIEHS/NIH are studying the apoptotic pathway in response to oxidative damage in multiple cell types. Current studies are investigating early gene responses to a variety of agents that induce oxygen free radicals, and there are plans to use cDNA microarray technology (see page 29) to establish a molecular "signature" of the cellular response to this class of agents (Barrett, NIEHS/NIH).

Researchers at NIOSH/CDC are studying carcinogenic mechanisms of occupational exposures. Several transition metals such as chromium(VI), nickel(II), vanadium(V) and cobalt(II) are established carcinogens and each is able to generate reactive oxygen species upon reaction with cells; therefore, the role of free radical reactions leading to carcinogenesis is currently being investigated *in vitro* and will be expanded to *in vivo* studies in the future (Shi, NIOSH/CDC).

Peroxisome proliferators have been shown to produce hepatocarcinomas in rodents. Preliminary studies using the peroxisome proliferator, Wyeth-14643 (Wy), implicate the production of fatty acid metabolites by cyclooxygenase isoform, COX-1 in non-parenchymal cells with the subsequent hepatocyte metabolism of these products in the formation of mitogenic metabolites. Additional experiments are in progress to confirm these findings and further characterize the roles of hepatocytes and non-parenchymal cells in Wy-induced liver tumors (Ghanayem, NIEHS/NIH).

Genetic Toxicology and Mutagenesis

Genetic toxicity test results are used in making decisions about whether a substance should be tested for carcinogenicity in rodents; to aid in the interpretation of toxicity, carcinogenicity, or other *in vivo* test results; and to provide a data base for use in structure-activity analyses. Testing is conducted at contract laboratories. Testing using the *in vitro Salmonella typhimurium* assay for 42 chemicals is ongoing from FY 2000 (Table 15); testing capacity is set at 50 chemicals.

Gene mutations and DNA damage are examined in most tissues; cytogenetic effects, measured as the induction of micronuclei, are generally examined in bone marrow cells or in peripheral erythrocytes. The erythrocyte studies are integrated with other toxicity evaluations to minimize the use of animals and to expand the toxicology information for the chemical in the same animals. The testing of micronuclei in peripheral blood erythrocytes is ongoing from FY 2000 for 20 chemicals (Table 15) (Caspary, NIEHS/NIH). Genetic toxicity information obtained on chemicals tested by the NTP is forwarded to the Human Genome and

Table 15. Compounds Being Evaluated for Genetic Toxicity

Chemical	CAS No.	Species: Strain[1]: Gender[2]	Route	Testing Battery[3]
Studies ongoing in FY 2001 as of 10/01/00				
Ammonium molybdate	13106-76-8			SLM
t-Amyl methyl ether	994-05-8			SLM
Aspartame	22839-47-0			SLM
5,6-Benzoflavone	6051-87-2			SLM
Bis(2-chloroethoxy)methane	111-91-1			SLM
Bromodichloromethane	75-27-4			SLM
1-Bromopropane	106-94-5			SLM
2-Bromopropane	75-26-3			SLM
Butene	25167-67-3			SLM
2-Butene-1,4-diol	110-64-5			SLM
Chlorine Dioxide	10049-04-4			SLM
Chromium Picolinate	14639-25-9			SLM
Diadzein (4-,7-dihydroxyisoflavone)	486-66-8			SLM
o-Dinitrobenzene	528-29-0			SLM
p-Dinitrobenzene	100-25-4			SLM
1,3-Dichloro-2-butene	926-57-8			SLM
FD&C Green No. 3 (Fast green FCF; C.I. 42053)	2353-45-9			SLM
5-(Hydroxymethyl)-2-furfural	67-47-0			SLM
Hexahydro-1,3,5-tris-(hydroxyethyl)triazine	4719-04-4			SLM
Hexylamine	111-26-2			SLM
5-(Hydroxymethyl)-2-furfural	67-47-0			SLM
Indole-3-carbinol	700-06-1			SLM
3-Methoxypropionitrile	110-67-8			SLM
4-Methyl thiazole	693-95-8			SLM
Nonylic vanillylnonamide	2444-46-4			SLM
Paraldehyde (2,4,6-Trimethyl-1,3,5-trioxane)	123-63-7			SLM
Picolinic Acid	98-98-6			SLM
Potassium ferrixyanide	13746-66-2			SLM
1-Propyne	74-99-7			SLM
Thiophenol (Benzenethiol)	108-98-5			SLM
Thiouracil	141-90-2			SLM
Triacetin (1,2,3-Propanetril triacetate)	102-76-1			SLM
1,3,5-Trioxane	110-88-3			SLM
Vanillyl-*n*-nonolyamide	2444-46-4			SLM
Vinyl pyridine	100-69-6			SLM
Endocrine Disrupting Agents				
Genistein (4',5,7-Trihydroxyisoflavone)	446-72-0			SLM
Nonylphenol	25154-52-3			SLM
Herbal Meidicines/Dietary Supplements				
Aloe vera gel	8001-97-6			SLM
Ginseng and ginsenosides	50647-08-0			SLM
Juglone (5-Hydroxy-1,4-naphthoquinone)	481-39-0			SLM
Kava Kava Extract	9000-38-8			SLM
Milk Thistle Extract (aqueous)	84604-20-6			SLM
Acrylonitrile	107-13-1	Mice: B6C3F1: M/F	Gavage	Erythrocytes: MN
Allyl bromide	106-95-6	Mice: FVB/N: M/F	Gavage	Erythrocytes: MN
Aspartame	22839-47-0	Mice: p53(+/-) (C57BL/6): M/F	Feed	Erythrocytes: MN
		Mice: Tg.AC/(FVB/N) Hmz	Feed	Erythrocytes: MN
		Mice: p16(INK4A) (+/-) (C57BL/6)	Feed	Erythrocytes: MN
Citral	5392-40-5	Mice: B6C3F1: M/F	Feed	Erythrocytes: MN

Chemical	CAS No.	Species: Strain[1]: Gender[2]	Route	Testing Battery[3]
2,4-Decadienal	25152-84-5	Mice: B6C3F1: M/F	Gavage	Erythrocytes: MN
1,2-Dibromo-2,4-dicanobutane	35691-65-7	Mice: B6C3F1: M/F	Topical	Erythrocytes: MN
Di(2-ethylhexyl)phthalate	117-81-7	Mice: Tg.AC/(FVB/N) Hom: M/F	Topical	Erythrocytes: MN
Dimethylaminopropyl Chloride	5407-04-5	B6C3F1: M/F	Gavage	Erythrocytes: MN
Dipropylene Glycol (mixture)	25265-71-8	Mice: B6C3F1: M/F	Water	Erythrocytes: MN
Ethinylestradiol	57-63-6	Mice: FVB: M/F	Gavage	Erythrocytes: MN
		Mice: Tg.AC/(FVB/N): M/F	Topical	Erythrocytes: MN
		Mice: Tg.AC/(FVB/N): M/F	Gavage	Erythrocytes: MN
Glycidol	556-52-5	Mice: p16(INK 4a)(+/-): M/F	Gavage	Erythrocytes: MN
Indium Phosphide	22398-80-7	Mice: B6C3F1: M/F	Inhalation	Erythrocytes: MN
Methylphenidate HCl	298-59-9	Mice: Tg.AC/(FVB/N) Hmz: M/F	Feed	Erythrocytes: MN
		Mice: p53 (+/-) (C57BL/6): M/F	Feed	Erythrocytes: MN
Pentaerythritol Triacrylate	3524-68-3	Mice: Tg.AC/(FVB/N) Hmz: M/F	Topical	Erythrocytes: MN
Trimethylolpropane Triacrylate	15625-89-5	Mice: Tg.AC/(FVB/N) Hmz: M/F	Topical	Erythrocytes: MN
WY-14643	50892-23-4	Mice: Tg.AC/(FVB/N) Hom: M/F	Topical	Erythrocytes: MN
Water Disinfection By-Products				
Bromodichloromethane	75-27-4	Mice: B6C3F1: M/F	Water	Erythrocytes: MN
Dibromoacetic Acid	631-64-1	Mice: B6C3F1: M/F	Water	Erythrocytes: MN
Dichloroacetic acid	79-43-6	Mice: Tg.AC/(FVB/N): M/F	Topical	Erythrocytes: MN
		Mice: Tg.AC/(FVB/N): M/F	Water	Erythrocytes: MN
		Mice: p53(+/-) (C57BL/6): M/F	Water	Erythrocytes: MN
Sodium Bromate	77889-38-0	Mice: Tg.AC/(FVB/N): M/F	Topical	Erythrocytes: MN
		Mice: Tg.AC/(FVB/N): M/F	Water	Erythrocytes: MN
		Mice: p53 (+/-) (C57BL/6): M/F	Water	Erythrocytes: MN

[1] Studies conducted in B6C3F1 mice are for 90 days. Studies in p53(+/-) (C57BL/6), Tg.AC/(FVB/N), and P16(INK4A)(+/-) (C57BL/6) are for 26 weeks.
Hmz: hemizygous; Hom: homozygous

[2] M: male and F: female

[3] Testing Battery:
SLM: Salmonella Mutagenicity Test. Up to eight Salmonella tester strains (TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, and TA1538) are used in a pre-incubation modification of the Ames Salmonella/microsome test. Volatile chemicals and gasses are tested in a desiccator. Exogenous metabolic activation is provided by liver homogenates from Aroclor 1254-induced male Sprague-Dawley rats and Syrian hamsters. Testing is performed so that if a chemical is mutagenic in strains TA98 or TA100, it will not be tested in other strains, unless specific questions concerning the test chemical's spectrum of activity are to be addressed. If the chemical is not mutagenic in strains TA98 or TA100, it is tested in strains TA97 and TA1535. If the chemical is negative or equivocal in any of these strains or depending on the chemical's structure and presumed active products, it may also be tested in one or more of the other tester strains.
Erythrocytes: MN - Induction of micronuclei (MN) in peripheral blood erythrocytes of mice. Mice were treated with chemical for 90 days or 26 weeks depending upon the strain being tested. When a chemical is tested in short term (>14 day) toxicity studies in mice, blood smears are made at the time of animal sacrifice. These smears are coded and sent to a contract laboratory to be scored for the presence of micronuclei in the peripheral blood erythrocytes. Blood smears from males and females are scored.

Toxicology Group at Oak Ridge National Laboratory, Oak Ridge, Tennessee for inclusion in the Environmental Mutagen Information Center's computerized database. Information within this database is a part of National Library of Medicine's TOXNET and is available to the scientific community through the Library's TOXLINE.

Knowledge about the spectrum of genetic alterations in chemically induced rodent tumors, their temporal appearance in progression from pre-neoplastic lesions to neoplasms, and the way in which these factors differ among tissues and among chemicals may provide a molecular basis for distinguishing between spontaneously and chemically induced neoplasms. A systematic effort is being made at the NIEHS/NIH to identify alterations in oncogenes and tumor suppressor genes in the most frequent sites of spontaneous and chemically induced neoplasms from F334 rats and B6C3F1 mice used in chronic bioassays. Point mutations in *K-ras* were found in lung neoplasms from B6C3F1 mice treated with 2,2-bis(bromomethyl)-1,3,-propanediol (BMP). Preliminary studies examined lung tumors for loss of heterozygosity on distal chromosome 6 (the site of a major lung susceptibility locus in the region of the *K-ras* gene) and allelic imbalances were detected in BMP-induced lung neoplasms. These studies are being extended to include evaluation of ozone-induced neoplasms and BMP-induced Harderian gland neoplasms. Forestomach tumors induced by a class of carcinogens (chloroprene, isoprene, and 1,3-butadiene) are being examined for activating mutations in the *ras* gene. Mutational spectra from other sites will be compared to that of the forestomach to determine whether a similar mechanism is involved (Sills, NIEHS/NIH).

It is hypothesized that epoxide intermediates play a role in the pathogenesis of hemangiosarcomas by causing genetic alterations in tumor suppressor genes and proto-oncogenes. In the B6C3F1 mouse, chemicals such as chloroprene, butadiene and tetrafluoroethylene cause hemangiosarcomas in the liver and other organ systems. Researchers at the NIEHS/NIH are looking at genetic alterations in the mouse model to determine whether those in cancer-related genes are similar to genetic alterations that occur in humans following environmental exposures (Sills, NIEHS/NIH).

Researchers at NCTR/FDA are examining genetic polymorphisms as markers for potential genotoxicity. Current efforts are directed toward characterizing DNA adducts from tamoxifen metabolites and analogues and determining if tamoxifen or its derivatives increases the frequency of mutations at the *Hprt* gene and if these mutations can be biomarkers for potential genotoxicity of anti-estrogens. Other studies have found that abnormal folate metabolism is associated with polymorphisms in the methylene tetrahydrofolate reductase and methionine synthase reductase genes in mothers of children with Down's syndrome. Follow-up studies are underway to determine if DNA hypomethylation secondary to inadequate maternal folate might contribute to abnormal chromosomal segregation and non-disjunction of chromosome 21 (Beland, NCTR/FDA).

NCTR/FDA scientists conduct fundamental research aimed at defining the pathways from initial DNA damage to mutation and such research centers on the development and validation of new *in vitro* and *in vivo* methodologies by which to assess genetic risk. The understanding of mutational mechanisms, combined with test systems with an increased capability to detect genetic damage, provides the regulatory process with the most current knowledge on which to base regulatory decisions (Moore, NCTR/FDA).

At NCTR/FDA, studies are underway investigating the mutagenicity of malachite green, and its primary metabolite, leucomalachite green, in the Big Blue rat model, where the lacl gene is integrated into the genome of every cell in the animal. The gene can be retrieved as a reporter gene for mutational analyses (Manjanatha, NCTR/FDA).

Breast Cancer

Scientists at the NCTR/FDA are developing methods to assay hydroxylation of endogenous estrogens for possible use in screening for breast cancer risk. This effort is an outgrowth of clinical and experimental data that have shown differences in 2- and 4-hydroxylation of endogenous estrogen relative to risk for developing breast cancer (Beland, NCTR/FDA).

Lung Cancer

Lung cancer is the most common malignancy in the United States and is ranked second only to bladder cancer in the proportion of cases thought to be due to environmental exposures. Evidence indicates that there is a strong genetic component in susceptibility to adenocarcinoma of the lung, which is rapidly becoming the most common lung cancer type. Researchers at NIOSH/CDC are studying inbred strains of mice, which are highly susceptible or highly resistant to the development of lung adenocarcinoma, in order to identify and map susceptibility/resistance genes for this disease. Other studies will include individuals from families having a high incidence of lung cancer (Reynolds, NIOSH/CDC). Researchers at the NIEHS/NIH are continuing their efforts to identify murine lung tumor susceptibility genes and are concentrating on the *Par2* locus on chromosome 18. The goal is to determine if human homologues of these genes play a role in lung cancer (Devereaux, NIEHS/NIH).

A comparative pathology project at NIOSH/CDC is examining the relationship between coal dust deposition in the lungs of miners and alterations in the biochemical pathways involved in the bio-activation of carcinogens in cigarette smoke. The lungs of non-smoking and smoking miners are being evaluated for the interactions of smoking and coal dust exposure in the occurrence of alveolar epithelial cell hypertrophy and hyperplasia, pulmonary fibrosis, lung cancer, and cytochrome P4501A1 activity. Biochemical and immunohistochemical studies are also being carried out in rodents (Hubbs, NIOSH/CDC).

Research at NIOSH/CDC is also focusing on understanding the mechanism(s) by which occupational chemicals, such as cadmium, beryllium, tetrachloroethylene, and metal working fluids induce carcinogenesis, specifically the activation and/or inactivation of certain cancer related genes. This project is testing whether proto-oncogene activation and tumor suppressor gene inactivation are associated with cell transformation induced by chemical exposure and examining what role methylation and /or genetic alteration may play in occupational carcinogenesis (Ong, NIOSH/CDC).

Skin Cancer

Investigators at both NIOSH/CDC and NCTR/FDA are interested in learning how environmental factors and exposures affect susceptibility and development of skin cancer (Howard, NCTR/FDA; Ding, NIOSH/CDC; see page 15).

Transgenic Animal Evaluations

In addition to standard rodent models, transgenic animals are being used increasingly in NTP studies. Their development (see page 73) is part of the NTP's expanding efforts to understand the mechanism(s) of environmental toxicant induction of cancer and associated molecular genetics. Such information is critical for developing risk assessment procedures and strategies for intervention and prevention of environmental disease. Twenty-one chemicals are currently being studied for their carcinogenic potential using transgenic mouse models and one is currently scheduled to begin in 2001 (Table 16). Chemicals being tested include water disinfection by-products (see page 16), herbal medicines and dietary supplements (see page19), and endocrine disruptors (see page 21).

Table 16. Compounds Being Tested for Carcinogenic Potential using Transgenic Models

Chemical Name	CAS No.	Project Leader[1]	Contract Project Officer[2]	Species: Strain[3]	Route	Special Studies[4]	Study Length[5]
Studies ongoing in FY 2001 as of 10/01/00							
Allyl Bromide	106-95-6	Dunnick	Chhabra/ Vallant	Mice: FVB/N	Topical	Genetic analysis and molecular biology of frozen tissues and tumors	17 days
Prevention 2							
Melatonin	73-31-4	Rao	Ney	Mice: MMTV/NEU (Tg.NK)	Feed		26 weeks
Silymarin	65666-07-1	Rao	Ney	Mice: MMTV/NEU (Tg.NK)	Feed		26 weeks
Silymarin + Melatonin	SILYMARN +MEL	Rao	Ney	Mice: MMTV/NEU (Tg.NK)	Feed		26 weeks
Prevention 3							
Melatonin	73-31-4	Rao	Ney	Mice: MMTV/NEU (Tg.NK)	Feed		26 weeks
Prevention 4							
Curcumin	458-37-7	Rao	Ney	Mice: MMTV/NEU (Tg.NK)	Feed		26 weeks
Indole-3-Carbinol	700-06-41	Rao	Ney	Mice: MMTV/NEU (Tg.NK)	Feed		26 weeks
Melatonin	73-31-4	Rao	Ney	Mice: MMTV/NEU (Tg.NK)	Feed		26 weeks
Melatonin + Curcumin	MEL+CUR CUMIN	Rao	Ney	Mice: MMTV/NEU (Tg.NK)	Feed		26 weeks
Melatonin + Indole-3-Carbinol	MEL+INDO LCAR	Rao	Ney	Mice: MMTV/NEU (Tg.NK)	Feed		26 weeks
Transgenic Model Evaluation							
Diethylstilbestrol (endocrine disrupting agent)	56-53-1	Eastin	Chhabra/ Vallant	Mice: FVB/N Mice: Tg.AC (FVB/N) Hom	Topical	Micronuclei	14 days; 26 weeks
Ethinyl Estradiol (endocrine disrupting agent)	57-63-6	Eastin	Chhabra/ Vallant	Mice: FVB/N Mice: Tg.AC (FVB/N) Hom	Topical	Micronuclei	14 days; 26 weeks
				Mice: FVB/N Mice: Tg.AC (FVB/N) Hom	Gavage	Micronuclei	14 days; 26 weeks
Melphalan	148-82-3	Eastin	Chhabra/ Vallant\	Mice: FVB/N	Gavage		28 days
Transgenic Model Evaluation II							
Acesulfame Potassium	55589-62-3	Irwin	Chhabra/ Vallant	Mice: p53 +/- (C57BL/6) Mice: Tg.AC (FVB/N) Hmz	Feed	Micronuclei	39 weeks
Aspartame	22839-47-0	Dunnick	Chhabra/ Vallant	Mice: p53 +/- (C57BL/6) Mice: Tg.AC (FVB/N) Hmz	Feed	Micronuclei	39 weeks
				Mice: p16(INK4A)/(+/-) (C57BL/6)	Feed		39 weeks
Benzene	71-43-2	Dunnick	Chhabra/ Vallant	Mice: p16(INK4A)/(+/-) (C57BL/6)	Gavage		26 weeks
Glycidol	556-52-5	Dunnick	Chhabra/ Vallant	Mice: p16(INK4A)/(+/-)	Gavage		39 weeks

Chemical Name	CAS No.	Project Leader[1]	Contract Project Officer[2]	Species: Strain[3]	Route	Special Studies[4]	Study Length[5]
				(C57BL/6)			
Phenolphthalein	77-09-8	Dunnick	Chhabra/ Vallant	Mice: p16(INK4A)/(+/-) (C57BL/6)	Feed		26 weeks
Water Disinfection By-Products							
Bromodichloromethane	75-27-4	Melnick	Chhabra/ Vallant	Mice: p53 +/- (C57BL/6) Mice: Tg.AC (FVB/N) Hmz	Water		26 weeks
				Mice: Tg.AC (FVB/N) Hmz Mice: FVB/N	Dermal	15 mice receive TPA as a positive control	14 days & 26 weeks; 39 weeks
				Mice: p53 +/- (C57BL/6) Mice: Tg.AC (FVB/N) Hmz	Gavage		26 weeks
				Mice: p53 +/- (C57BL/6) Mice: Tg.AC (FVB/N) Hmz	Water		39 weeks
				Mice: p53 +/- (C57BL/6) Mice: Tg.AC (FVB/N) Hmz	Gavage		39 weeks
Dichloroacetic Acid	79-43-6	Melnick	Chhabra/ Vallant	Mice: p53 +/- (C57BL/6) Mice: Tg.AC (FVB/N) Hmz	Water	Micronuclei	26 weeks
				Mice: Tg.AC (FVB/N) Hmz Mice: FVB/N	Topical	15 mice receive TPA as a positive control	14 days & 26 weeks; 39 weeks
				Mice: p53 +/- (C57BL/6) Mice: Tg.AC (FVB/N) Hmz	Water		39 weeks
Sodium Bromate	7789-38-0	Melnick	Chhabra/ Vallant	Mice: p53 +/- (C57BL/6) Mice: Tg.AC (FVB/N) Hmz	Water		26 weeks
				Mice: Tg.AC (FVB/N) Hmz Mice: FVB/N	Topical	14-day range finding study - 15 mice receive TPA as a positive control	14 days & 26 weeks; 39 weeks
				Mice: p53 +/- (C57BL/6) Mice: Tg.AC (FVB/N) Hmz	Water		39 weeks
Studies proposed to start in FY2001 as of 10/01/00; Others may be scheduled as protocols are finalized							
Prevention 5							
Melatonin	73-331-4	Rao	Ney	Mice: MMTV/NEU (Tg.NK)	Feed		26 weeks

[1] Project Leader: NIEHS/NIH staff scientist (unless otherwise indicated) who oversees each chemical's evaluation

[2] Contract Project Officer: NIEHS/NIH staff scientist (unless otherwise indicated) who coordinates research activities with the contract laboratory

[3] Hmz: hemizygous; Hom: homozygous

[4] Special Studies:

Micronuclei: genetic toxicity testing. Induction of micronuclei in mouse peripheral blood erythrocytes. Mice are treated with chemical and blood smears are made at the time of animal sacrifice.

[5] Study Length:

14 days or 17 days: Repeated dose study g used for determining the dose range for the subchronic study. The doses (usually five doses plus control) cover a wide dose range; 5 animals/group, 2 genders, 2 species; complete necropsies; histopathologic evaluations on organs/tissues showing grow evidence of treatment-related lesions with corresponding tissues in control animals.

26 or 39 weeks: Transgenic carcinogenesis study to identify chemicals for carcinogenic potential. One or more transgenic stains may be used and the route of administration will depend upon the strain(s); generally 15 animals/group, 2 genders; complete necropsies with histopathologic evaluation on all animals.

The Tg.AC transgenic mouse skin model (see page 73) appears useful for studying the molecular mechanisms that underlie multi-stage tumorigenesis. Studies are underway to identify the common factors induced by chemicals, wound repair, or radiation that would lead to activation of the *v-Ha-ras* transgene. Efforts are focusing on identifying and characterizing epidermal stem cell markers to visualize and monitor progression of skin cancer development from the very earliest stages of disease (Tennant, NIEHS/NIH). The Tg.AC mouse is an appropriate model to evaluate the effects of diet or the efficacy of anti-tumor agents or co-carcinogens that can modulate the tumor response. Inflammation (carcinogen or tumor-induced oxidative stress) is an intrinsic component to the progression of cancer. Studies are using Tg.AC models to examine modulation of oxidative stress by dietary antioxidants during various stages of pathogenesis (French, NIEHS/NIH).

The Tg.AC and p53 (see page 73) models are used to evaluate the mechanisms and molecular genetics of benzene, a ubiquitous environmental carcinogen associated with development of aplastic anemia that often leads to development of acute myelocytic leukemia. Studies are in progress for understanding the genetic events associated with benzene-induced cancer (French, NIEHS/NIH).

Breast cancer is a multi-faceted disease that is influenced by many factors including genetics and environment. Some components of diet, such as vitamin A and its analogues (retinoids), and therapeutic agents, such as tamoxifen, may delay or prevent mammary cancer. The transgenic mouse model, Tg.NK, (see page 73) is being used at the NIEHS/NIH for studying the ability of fiber, retinoids, melatonin, and linolenic acid to delay mammary tumor development; results for all but linolenic acid were positive. Future plans include studies with dietary supplements, therapeutic agents, and less toxic combinations of previously tested agents (Rao, NIEHS/NIH).

Epidemiology studies have established an association between elevated arsenic levels in drinking water and the incidence of urinary bladder transitional cell carcinomas. A study at NIOSH/CDC is investigating the possible molecular mechanisms of arsenic-induced urinary bladder cancer in transgenic mice exposed to 0.01% sodium arsenite in drinking water (Luster, NIOSH/CDC).

A pilot study to assess the effects of dietary restriction on serum insulin-like growth factor-1 (IGF-1) levels and progression of prostate cancer in the TRAMP (Transgenic Adenocarcinoma Mouse Prostate) transgenic model (see page 73) is in its final stages of evaluation at the NIEHS/NIH. This study will form the basis for a subsequent investigation of environmental factors that might influence the development of prostate cancer (Barrett, NIEHS/NIH).

Big Blue lacl assays measure gene mutations in somatic cells, and are sensitive to fundamentally different spectra of mutagenic events than are detected in the micronucleus assay. In the Big Blue rat model, the lacl gene is integrated into the genome of every cell of the animal and can be retrieved as a reporter gene for mutational analysis. The lacl assay has the major advantage of being able to measure the mutations (base pair substitutions, frameshifts and small deletions) in any tissue from which DNA can be isolated. Moreover, mutant induction in a neutral reporter gene, like lacl, can accumulate with time during chronic exposure and, thus, be a sensitive indicator of genotoxic damage (Manjanatha, NCTR/FDA).

Comparative Carcinogenesis

Leiomyomas

Uterine leiomyomas or "fibroids" are benign tumors clinically diagnosed in 20-30% of U.S. women during their third to fourth decade of life. Uterine leiomyomas pose a major public health cost in terms of outpatient care and hospital costs for surgical procedures (hysterectomies). Research is ongoing at the NIEHS/NIH to delineate some of the basic biological and molecular processes important in uterine fibroid development and growth that might be applied to the development of alternative treatment regimens. Archival mouse and human tissues are being used to determine the presence of growth factors in uterine leiomyomas and leiomyosarcomas. These studies have focused on evaluating the role of cell proliferation or prolonged cell survival in the growth of uterine leiomyomas, and other studies are underway to determine the role of modulators of apoptosis such as BCl-x and Mcl-1 (Dixon, NIEHS/NIH).

Ovarian Cancer and Dysfunction

Exposure to environmental chemicals has the potential to cause ovarian dysfunction and ovarian cancer in women. Disruption of ovarian function greatly impacts the reproductive, endocrine, and ultimately general health of women. NIEHS/NIH studies are focusing on identifying the ovarian target cell(s) and biochemical and molecular mechanisms by which synthetic or naturally-occurring environmental chemicals cause ovarian cancer or dysfunction, determining key genes and signaling molecules involved in disease etiology, and exploring how modifications in these pathways may ameliorate these disorders. Ovarian toxicants, such as phthalates and glycol ethers, and ovarian carcinogens, such as dioxins and furans, are being studied. Current efforts are also defining the role of genes and signaling molecules using transgenic animal models (Davis, NIEHS/NIH).

Areas for Future Initiatives and Resources

Future initiatives in mechanistic-based carcinogenesis research will involve an integration of information from traditional and microchip array-based studies to guide the development of new models for carcinogen identification and study. The approach towards gaining insight into critical molecular changes occurring during the carcinogenic process will be two pronged, *i.e*. examinations of gene activation and repression following acute chemical treatment, coupled with examination of the profiles of gene expression in fully developed tumors. As the NTP progresses in understanding the complex signaling pathways that are activated or repressed during carcinogenesis, the Program will be able to select transgenic animal models that best mimic processes occurring in human tissues, providing a firm foundation for cross species extrapolations of hazard. The NIEHS/NIH plans to participate in an initiative currently ongoing at the National Cancer Institute/NIH to evaluate approximately 50 existing transgenic or knockout models of cancer-related genes for their more immediate application to hazard identification studies.

RISK ASSESSMENT EVALUATIONS

Health, research, and regulatory agencies make decisions regarding the protection of public health based on scientific information from a variety of sources. In the evaluations of human risk, several areas of uncertainty often exist:

- adequacy of animal models to detect toxicologically-induced disease endpoints,
- adequacy of animal models to accurately reflect human risk,
- adequacy of mathematical models used to extrapolate high dose effects to environmental or occupational exposure levels,
- adequacy of information about human exposures to environmental toxicants, and
- adequacy of information about inter-individual variability and sensitive sub-populations.

The NTP's effort in risk assessment is closely tied to its growing initiatives in mechanism-based toxicology and carcinogenesis. This linkage provides opportunities to improve priority setting, to use mechanistic response relationships in the "low dose" range, to select the most appropriate experimental systems for estimating risk, and to develop scientifically based models for specific subpopulations (*e.g.,* age, gender, genetic predisposition, ethnicity, etc.). Increased knowledge about the mechanisms responsible for environmentally induced disease coupled with the development of sensitive and specific biomarkers of exposure and tests for biological effect are important in detecting and monitoring the early insult(s) of environmental toxicants and in evaluating those effects under low dose exposures. All of these components can contribute scientific data about potential toxicity and carcinogenicity of environmental agents and strengthen the science base for risk assessment.

EPIDEMIOLOGY

Current Research Initiatives

The presence of toxicants in the environment is a potential threat to human health and the extent of that threat is unclear. Environmental toxicants may produce a variety of health effects depending upon the timing of exposure, dose, individual susceptibility, and other, yet unidentified, factors. Many of the studies currently underway or planned by the NTP investigate occupational or environmental exposure to toxicants as potential risk factors for specific health effects. Table 17 lists specific exposures and health effects presently under consideration.

In the past, environmental research often has studied the crudest, most easily measurable health effects (cancer or death). More subtle damage (*e.g.,* infertility, neurological function, or endocrine imbalance) is often harder to detect although such effects may be more common and impact a greater number of individuals. Increasingly, the NTP looks for ways to improve its ability to detect potential health effects from environmental exposures. Efforts are underway by the NTP to develop sensitive techniques for measuring the phenotypic effects of exposure and studying genetic changes associated with disease etiology, to identify new genes involved in response to environmental toxicants, and to identify genetic polymorphisms. Some of the important tools in this effort come from recent advances in biotechnology that include more sensitive methods for measuring low dose exposures, detecting early stages of disease, determining genetic susceptibility, and evaluating illnesses for which the causes are largely unknown but environmental etiology is plausible (*e.g.*, neurologic disease). In addition, the development of novel statistical tools is aiding epidemiologic investigations.

Table 17. Agents under Consideration as Risk Factors for Specific Human Health Effects

Exposure	Health Effect
Air pollution	Lung cancer, adult and childhood respiratory diseases
Arsenic	Lung cancer and reproductive disorders
Arylamines	Bladder cancer
Asbestos	Lung cancer
Beryllium	Chronic beryllium disease
1-Bromopropane	Reproductive effects in males
Fertilizers (nitrates)	Reproductive disorders
Halogenated aromatic compounds	Birth defects, childhood neurologic deficits, adolescent body habitus
Jet fuel	Female reproductive health and outcomes
Latex rubber	Allergy and asthma
Lead	Amyotrophic lateral sclerosis, succimer and childhood neurologic deficits, attention deficit hyperactivity disorder, reproductive disorders
Magnetic fields	Breast cancer
Mercury	Renal disease and systemic lupus erythematosis
Nickel	Reproductive effects in males
Organochlorines (PCBs, dioxin)	Birth defects, childhood neurologic deficits, adolescent body habitus, thyroid function, reproductive disorders, and pancreatic cancer
Pesticides (insecticides, herbicides, fungicides, and fumigants)	Cardiovascular, renal, and other chronic diseases, neurotoxicity, neurobehavioral function, adult neurologic deficits, attention deficit hyperactivity disorder, primary intracranial gliomas, male reproductive health effects, and respiratory disease
Perchloroethylene	Cervical cancer
Phenolphthalein	Colorectal polyps
Phthalates	Reproductive disorders and birth defects
Polyaromatic hydrocarbons (PAHs)	Bladder cancer, lung cancer and disorders, and reproductive disorders
Radon	Lung cancer and childhood leukemia
Radiation	Reproductive disorders
Uranium mining	Lung and other cancers
Water disinfection by-products	Birth defects, attention deficit hyperactivity disorder

Gene/Environment Interactions

Humans can vary widely in how they respond to environmental exposures. Genetic susceptibility may play an important role in many aspects of environmental carcinogenesis as well as non-cancer diseases. The NIEHS Environmental Genome Project is a multi-center effort to identify systematically in the U.S. population the alleles of 200 or more environmental disease susceptibility genes. For this NTP-related project, researchers at the NIEHS/NIH are looking for polymorphisms in several types of genes: receptors, carcinogen metabolism, DNA repair, and hormone metabolism in relation to risk for carcinomas of the lung, bladder, liver, or pancreas that are known to have strong associations with environmental exposures. In addition, efforts will also address how genetic susceptibility to carcinogens may differ by age, ethnicity, gender, or lifestyle factors (*e.g.,* smoking, alcohol consumption) in order to better integrate environmental and genetic factors in understanding human disease etiology (Taylor, NIEHS/NIH; Bell, NIEHS/NIH).

NCTR/FDA scientists are conducting research to provide new knowledge on the identification of subpopulations that are not only more susceptible to chemical carcinogens, but also those that are likely to experience adverse drug reactions or decreased therapeutic drug efficacy. This research should 1) facilitate a better understanding of the mechanisms of human carcinogenesis; 2) provide an estimation of human exposure to direct and indirect-acting carcinogens; 3) assess the importance of inter-individual differences in carcinogen and drug bio-activation, detoxification, or induced changes in gene expression; and 4) suggest

intervention strategies for human cancer prevention, Projects on the etiology of human cancers of the colon/rectum, pancreas, larynx, breast, ovary, prostate, prostate, lung, urinary bladder, bone marrow, and esophagus are ongoing (Kadlubar, NCTR/FDA).

Immune Function

There is increased interest at the NIEHS/NIH in identifying environmental factors that might impact the immune system and development of autoimmune disease. Currently animal studies are ongoing or proposed (see page 34). The Carolina Lupus Study is a population-based case control study of recently diagnosed patients living in eastern North Carolina or South Carolina. It focuses on measurement of endogenous hormone exposure, exogenous sources of estrogen, and occupational exposures. These are factors that have been linked to the development of system lupus erythematosus or other autoimmune disease (Cooper, NIEHS/NIH). Natural rubber latex (NRL) allergy is an important cause of occupationally related allergy and asthma in health care workers. Work at NIOSH/CDC has focused on development and application of immunologic assays for establishing exposure-response relationships related to development of NRL allergy. One project is currently evaluating NRL-specific serum IgG as a biomarker of exposure in serum of health care workers stratified by high versus low exposure. This project is a collaborative effort with investigators from the Virginia Health Care System (Weissman, NIOSH/CDC).

Lung Disease

The NIEHS/NIH is exploring the etiologic role of genetic susceptibility for lung cancer through investigations of several diverse populations: African-Americans, U.S. Caucasians, and Chinese. Another study is examining cancer risk in Czech uranium miners and investigating their cancer incidence using linkage with a population-based cancer registry (Sandler, NIEHS/NIH). The relationship between residential radon exposure and risk for lung cancer and/or childhood leukemia is also being studied (Sandler, NIEHS/NIH).

Both the NIEHS/NIH and NIOSH/CDC are interested in identifying factors that might increase risk for non-cancer diseases. A NIEHS/NIH study is examining the role of genetic factors, diet, and environmental exposures relative to risk for non-malignant respiratory disease using a cohort of over 50,000 older adults of Chinese ethnicity in Singapore (London, NIEHS/NIH). Another project is addressing childhood respiratory disease globally by examining several populations with varying prevalence of asthma: Southern California; Mexico City; and Wuhan, China (London, NIEHS/NIH). Currently a multi-facetted, international study being conducted by NIOSH/CDC is evaluating potential environmental and genetic determinants for a variety of occupational diseases including cancer, chronic beryllium disease, asthma, and silicosis (Kreiss, NIOSH/CDC).

Men's Health

An initiative at NIOSH/CDC is examining genetic polymorphisms in prostate cancer patients and controls to see if any might predispose the carrier to development of disease (Reynolds, NIOSH/CDC).

Neurologic Disease and Function

Several investigations are examining the role of environmental toxicants on neurologic disorders. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting the motor neurons of the brain stem and spinal cord for which there is evidence of possible environmental etiology. A current case-control study at the NIEHS/NIH is examining the

effects of cumulative lifetime exposure to lead and other neurotoxins including mercury, solvents, and pesticides on risk for ALS (Kamel, NIEHS/NIH).

Several projects are targeting agricultural workers and the potential health effects of pesticide exposures. A NIEHS/NIH collaboration with NCI/NIH and EPA investigating licensed pesticide applicators provides a unique opportunity to explore non-cancer health effects associated with potent neurotoxins contained in pesticides (Sandler, NIEHS/NIH). Another study is examining the effects of occupational pesticide exposure in farm workers of central Florida to elucidate the effects of pesticide exposure on neurologic function and other health problems (Kamel, NIEHS/NIH). A NIOSH/CDC initiative is trying to identify what environmental factors might increase susceptibility of rural residents for primary intracranial gliomas (brain cancer) (Ruder, NIOSH/CDC).

NIOSH/CDC has undertaken a project to improve the test methods for evaluating cognitive, motor, and sensory functions for use in studies of workers exposed to agricultural chemicals. Currently, a three-part neurobehavioral performance battery is being evaluated in farm workers and their families (Steenland, NIOSH/CDC). Another project is working to improve methods used to assess neurobehavioral effects of organophosphate pesticides. This project is collaborative between NIOSH and Ohio State University and the Oregon Health Sciences University. Preliminary findings suggest that objective assessment methods may be critical for uncovering subtle neurobehavioral changes following pesticide exposures (Russo, NIOSH/CDC).

The NIEHS/NIH is also focusing efforts on children's neurologic health and environmental exposures. Data collection continues for one of the first population-based studies of attention-deficit hyperactivity disorder. This is part of an effort to describe environmental risk factors for this common childhood ailment (Rowland, NIEHS/NIH).

Reproduction and Development

An area of NTP interest is the potential impact of environmental toxicants on reproduction and development. Epidemiologic efforts at the NIEHS/NIH are targeted toward describing the basic biology of human reproduction, developing improved epidemiologic tools for detecting environmental damage, and identifying environmental factors that might interfere with human reproduction. An initiative is ongoing to develop methods that will allow identification of reproductive hazards affecting fertility, ovarian function, and pregnancy (Baird, NIEHS/NIH). In preparation for a large scale epidemiology field study on the adverse effect of lipid peroxidation on reproduction, the NIEHS/NIH is working to identify sensitive, specific, and clinically relevant oxidative stress markers (Little, NIEHS/NIH).

Using a population-based registry data from Norway, the NIEHS/NIH is studying the impact of birth defects on survival and reproduction as well as the risk of birth defects in the offspring of affected parents (Wilcox, NIEHS/NIH). A current investigation of pregnant women in the Ukraine is part of the European Longitudinal Study of Pregnancy and Childhood. This study targets understanding the relationships between environmental pollution and pregnancy, breast milk contamination, and reproductive outcome. Such information should help to elucidate the impact of environmental exposures on the health of pregnant women and their offspring (Little, NIEHS/NIH; Gladen, NIEHS/NIH).

In concert with the NTP's Safe Drinking Water Program (see page 16), efforts are also underway at the NIEHS/NIH to study whether water disinfection by-products found in drinking water might affect the occurrence of birth defects and attention deficit hyperactivity disorder (Wilcox, NIEHS/NIH). Parallel to examining effects of endocrine disrupting agents in animals, the NIEHS/NIH is conducting several epidemiology studies on this topic (see page 21).

Women's Health

NIOSH/CDC is investigating breast cancer incidence among female workers exposed to polychlorinated biphenyls (PCBs). These compounds are suspected breast carcinogens because of their estrogenic and lipophilic properties (Whelan, NIOSH/CDC). A pilot study is also underway at NIOSH/CDC to investigate whether an increased risk among dry-cleaning workers for cervical cancer mortality, which was found in both NIOSH/CDC and NCI/NIH studies, might be associated with perchloroethylene exposures (Ruder, NIOSH/CDC). Researchers at the NCTR/FDA and the FDA's Office of Women's Health are collaborating on a study of breast cancer in African-American women that is examining metabolic modification of dietary and hormonal factors. The study is a post-market surveillance of chemical toxicants found in foods, drugs, cosmetics, and medical devices and their relationship to human breast cancer risk (Kadlubar, NCTR/FDA).

Women's reproductive health is the focus of initiatives at both NIEHS/NIH and NIOSH/CDC. As part of the Agricultural Health Study, the NIEHS/NIH is examining whether pesticide and other farm exposures affect menstrual cycle abnormalities, fetal loss, and preterm births among women living on farms (Rowland, NIEHS/NIH). A new project is being initiated at the NIEHS/NIH to identify determinants of phthalate body burden in women of reproductive age and to evaluate the feasibility of assessing phthalate exposure with laboratory measurements in population-based studies (Hoppin, NIEHS/NIH). Another study is relating environmental exposure to clinical outcomes following *in vitro* fertilization (Weinberg, NIEHS/NIH). Several efforts are underway at the NIOSH/CDC to examine potential effects of occupational exposures on women's health. One is to develop biological markers of female reproductive health. Some of these biomarkers are being applied in an investigation of the effects of jet fuel exposure on female reproductive health in a case-control study of female air force personnel. This study is also evaluating potential differences in toxicity between Caucasian and African-American women (Kesner, NIOSH/CDC). Another study is examining the effects of radiation and circadian rhythm disruption on reproductive outcome in female flight attendants (Whelan, NIOSH/CDC).

Areas for Future Initiatives and Resources

Molecular Epidemology

Molecular epidemiology studies will be progressively more important to the NTP. For example, such studies will be used increasingly in the *Report on Carcinogens* (see page 77) to support listing agents as known human carcinogens. It is therefore of paramount importance to develop principles for evaluating and interpreting these studies, and the NTP proposes two types of approaches. First, the NTP will compile and synthesize existing information on this topic and then prepare articles, reports, and other educational materials to disseminate the results of this effort. For example, the Epidemiology Section of the American Industrial Hygiene Council has prepared guidelines for the conduct of molecular epidemiology studies that will be reviewed. Second, the NTP proposes to convene a future workshop to discuss issues involved in conducting molecular epidemiology studies and to develop principles for evaluating and interpreting these studies. The workshop would be a public forum and the results of the workshop would be available to the public in multiple formats.

Studies of Specific Agents

Analytic epidemiology studies on the relationship of toxicant exposure to specific health effects often rely on the fortuitous identification of exposed populations. Agents of interest to the NTP can occasionally be studied in this way. An example is methyl tertiary butyl ether

(MTBE), which is presently used as a gasoline additive. Public concern has been generated by the potential for inhalation exposure and its presence in ground water. Animal studies show that MTBE causes hematopoietic, kidney, liver, and testicular tumors in rodents; however, little is known about its carcinogenic potential in humans. MTBE has been used clinically to treat gallstones. Researchers at the NIEHS/NIH are presently investigating whether a cohort of MTBE-treated patients can be identified, and if so, it will be followed for cancer incidence or mortality by linking with the Surveillance Epidemiology and End Results (SEER) Cancer Registries or the National Death Index.

EXPOSURE ASSESSMENT

The NTP recognizes that accurate and complete exposure assessment is critical both to the success of epidemiology studies of toxicant exposure and to the utility of such studies for risk assessment and public health policy. However, the availability of human data is often the weakest component of risk assessment and limits the effective utilization of experimental data for making decisions about chemical exposures. Increased knowledge about the mechanisms that are responsible for environmentally induced diseases coupled with both sensitive and specific biomarkers and tests of biological effect from exposure are important in detecting and monitoring the early insult(s) of environmental toxicants and in evaluating those effects under low-dose exposure.

Current Research Initiatives

Within NIOSH/CDC is a coordinated effort to collect and analyze data on toxic compounds to which workers are potentially exposed and to help set priority rankings for additional toxicology research, risk characterization and assessment, and development of safety and health criteria or recommendations for future testing. This project serves as an internal NIOSH/CDC resource (Ahlers, NIOSH/CDC).

Exposure Assessment Surveys

Advances in analytical methodologies now enable the detection of environmental and occupational chemicals in small biological samples (*e.g.*, blood, urine, and hair). Taking advantage of such advances, the NTP is leading a new interagency human exposure assessment initiative in collaboration with NCEH/CDC, NIOSH/CDC, and EPA to quantify the body burdens of chemicals released into the environment and workplace. Discussions are underway about the scope and feasibility of such an effort.

The NIEHS/NIH and NCEH/CDC are collaborating on a pilot project for quantifying approximately 70 chemicals found in either human blood or urine that are considered to be endocrine disruptors. Information about this project is given on page 21.

NIOSH/CDC is currently planning to conduct a national, cross-sectional, on-site survey of establishments and workers (Boiano, NIOSH/CDC). Details about this survey are available on page18. Another current project is focusing specifically on identifying workers exposed to reproductive toxicants (Moorman, NIOSH/CDC, see page 18).

The NIEHS/NIH through the National Allergen Survey is assessing the allergen types and levels in the nation's housing to provide estimates of allergen exposure in the U.S. population. Information from this survey should facilitate future studies examining exposure/disease relationships and evaluation of regional, ethnic, socioeconomic, and housing characteristic differences in the allergen burden (Zeldin, NIEHS/NIH).

Exposure Assessment Monitoring

The NIOSH/CDC has several ongoing projects aimed at monitoring the health of workers and determining whether methods for reducing exposure are effective. Based upon animal toxicology data, pesticides have potential effects on neurologic function, skin reaction, and may be carcinogens. A current project is assessing pesticide exposures of greenhouse workers, specifically while applying pesticides and harvesting roses, in order to determine if early contact with pesticide-treated roses poses a health risk, to identify the areas of the body with the greatest exposure and to test the effectiveness of personal protective equipment worn to reduce exposures (Sanderson, NIOSH/CDC).

Through an interagency agreement with the NIEHS/NIH, a major effort is underway at the NIOSH/CDC to better characterize worker exposure by obtaining "real world" information about worker practices, exposures, and possibly related health effects. Initiatives currently underway target asphalt fumes and 1-bromopropane. Additional information is available on page 18.

The NIEHS/NIH is taking part in an effort with the United States Agency for International Development that includes working with technical experts from Egypt, Jordan, Israel, and the Palestine Autonomous Territories in a collaborative program to promote the safe and minimized use of agricultural pesticides. Activities under this agreement include formation of technical teams in each country and joint meetings, implementation and use of communication technologies to improve communications, selection of agricultural areas and crops for study, and development of a health questionnaire. Training courses in safe pesticide use are ongoing and monitoring of air, dust, and crops for pesticide residues has begun; data are being shared by all participating countries (Abdo, NIEHS/NIH).

Exposure Assessment Biomarker and Methods Development

The NIOSH/CDC has several efforts underway targeting a variety of toxicants (*e.g.*, perchloroethylene, herbicides, chromium, asphalt fumes, hexane, riddelliine) to identify biomarkers for internal dose, biologically effective dose, susceptibility, and detection of early effects so that exposure and its consequences can be accurately determined in occupationally exposed groups. Many of these biomarkers will address biological monitoring of genotoxicity, protein adduct formation, and genetic polymorphisms as well as internal exposure monitoring of biological specimens (*e.g.,* blood, urine). Such biomarkers should have application for human field studies (DeBord, NIOSH/CDC; Butler, NIOSH/CDC; Teass, NIOSH/CDC; Fu, NCTR/FDA). Two biomarkers of DNA damage from asphalt fumes have been developed and are being applied to evaluating exposure, early DNA damage, and host susceptibility confounding factors in roofing asphalt workers (DeBord, NIOSH/CDC). Biological monitoring methods for urinary detection of mercapturic acid conjugates are under development for the herbicides alachlor, metolachlor, acetochlor, atrazine, and cyanazine (Teass, NIOSH/CDC).

Several efforts are aimed toward development of technologies to improve exposure assessment. A new generation of high performance quadrupole time-of-flight mass spectrometry (Q-TOF MS) coupled to nanoflow liquid chromatography is being developed to characterize DNA/protein adducts induced by *in vivo* exposure to asphalt fumes. This should allow macromolecular identification and characterization of adduct formation both qualitatively and quantitatively because it is more sensitive and accurate than previously used methods (Wang, NIOSH/CDC).

Some initiatives target improvements in urinary biological monitoring technology. One project is testing a novel technique, Fluorescence Microbead Immunosorbent Assay (FMIA) as a viable tool for multi-analyte biological monitoring that allows simultaneous analysis of multiple pesticide analytes and cytokines in urine. Assays for atrazine, hetochlor and

metolachlor mercapturate are ongoing (Biagini, NIOSH/CDC). Validation studies for enzyme-linked immunosorbent assays (ELISA), which measure metabolites of pesticides in urine, are also ongoing. Methods are currently under development to measure exposure to the pesticide chlorpyrifos and azinphos-methyl piperalin and acetochlor for use in field studies (Striley, NIOSH/CDC). Another project is addressing the use of human hepatocyte model systems for studying interperson variability in how chemicals are metabolized. A hepatocyte library characterized for its xenobiotic metabolism has been compiled. In the future such a system might be used to identify which metabolites for specific chemicals would be most appropriate for urinary biomonitoring. Future arrays could also be constructed to simulate specific populations at risk (Striley, NIOSH/CDC).

The NIOSH/CDC is addressing concerns about occupational allergies. Occupational exposure to organic substances can result in the development of immunologically mediated hypersensitivity reactions that may affect the upper airways (allergic rhinitis) or lower airways (asthma and hypersensitivity pneumonia). Researchers at NIOSH/CDC are working to develop and validate assays for assessing the immune response of workers exposed to complex organic substances and for better identifying the antigenic or allergenic substance(s). Efforts are targeting new technologies for measurement of airborne endotoxin, development of immunochemical assays, and inter-laboratory comparisons studies for development of standardized analytical procedures (Lewis, NIOSH/CDC).

Efforts are continuing at the NIOSH/CDC to develop state-of-the-art methods for assessing male reproductive health for use in field investigations. Male reproductive hazards appear to target at least one of four major sites (endocrine system, the testes, the accessory glands, and sexual function). A goal is to implement new technologies and clinical methods for assessing these sites to measure toxic effects in clinical studies. These methods are being field-tested in bicycling policemen and in FY2001 field studies are planned for assessing the reproductive health of men exposed to 1-bromopropane. Another study of the reproductive effects of nickel is being initiated with McMaster University, Canada (Schrader, NIOSH/CDC).

Areas for Future initiatives and Resources

Exposure Assessment Methods

A great need exists for simple and low-cost exposure assessment techniques that could be used in population-based studies of disease etiology. Both laboratory-based and questionnaire methods are essential. The NTP is considering several approaches to develop such techniques. First, the Program will look for opportunities to interface with existing or planned exposure initiatives, such as National Health Exposure Assessment Survey (NHEXAS) or the Interagency Human Exposure Assessment Initiative currently being developed by the Center for Environmental and Nutritional Research (CENR). As available, the NTP will use data being collected by these projects to develop and validate exposure assessment techniques. Second, the NTP will interface with existing or planned health surveys such as the National Health and Nutrition Examination Survey (NHANES) to encourage the incorporation of occupational histories and other exposure measures into such surveys. Analysis of the resulting data could also be useful for developing and validating exposure assessment techniques. Finally, the NTP will continue to review ongoing laboratory research within its Program and determine whether this might be applicable to the development of biomarkers useful for population-based studies.

Surveys of Occupational Cohorts

There are many agents where carcinogenicity has been demonstrated in animal studies but no published studies exist of cancer in humans. The NTP is interested in whether it would be possible to fill this data gap by surveying occupationally exposed populations and is moving forward with a strategy to address this issue. The first step will be to use the NIOSH National Occupational Exposure Survey (NOES) to identify workers who are exposed to specific agents and to determine how many workers are exposed and in what industries. This information will then be used together with the *Report on Carcinogens* to identify and prioritize agents for further study. Mortality studies for selected agents could then be conducted by linking workers previously employed in specific industries to the Surveillance Epidemiology and End Results (SEER) Cancer Registries or the National Death Index. Initial studies could use ever employment in an industry as a marker of exposure; however, more detailed subsequent studies could use job titles and duration of employment to make more specific inferences about the relationship of exposure to cancer mortality. Although the preliminary surveys will be relatively straightforward, the mortality studies will be complex and require both considerable resources and access to industry records. Collaboration with NIOSH/CDC is a potential mechanism for gaining access to industry records.

TOXICOKINETIC AND BIOCHEMICAL MODELING

Current Research Initiatives

The NIEHS/NIH is involved in the development of toxicokinetic and biochemical models to strengthen the scientific basis for estimating human health risks associated with exposure to adverse environmental agents. Models vary greatly in their complexity and utility depending on available data and on the mechanistic understanding about how an exogenous agent may perturb processes that maintain good health and normal development. Biologically based dose response models are used to: 1) characterize and analyze relationships between exposure to environmental agents and tissue dosimetry, 2) characterize and analyze tissue response in relation to tissue dose, and 3) characterize and analyze dose-response relationships for exposure-related effects. The development of biologically realistic models is an iterative process. These models are used for understanding the biological basis of agent-induced adverse effects and for identifying factors contributing to inter-individual variability in responses to exposure.

Toxicokinetic Modeling

Complete dosimetry of a chemical or physical agent describes its absorption, distribution, metabolism, and elimination (ADME) at differing levels of exposure, over all ages, via multiple routes of exposure, and under varying genetic backgrounds in humans and test animals. In recent years, NTP has expanded its efforts in toxicokinetics to provide a better understanding of the behavior of chemicals under study in test animal species. ADME assessments (see page 26) are being done on a number of NTP test agents.

An ongoing initiative at the NIEHS/NIH is to integrate data from a number of levels to address knowledge gaps that create uncertainty in risk assessment for receptor-mediated toxicants. These studies focus on dioxin and its structural analogs as a prototypical receptor-mediated toxicant. Dioxin-like compounds are ubiquitous environmental contaminants and their persistence in the environment, their lipophilicity, and subsequent bioaccumulation through the food chain result in chronic human exposure. Dioxin has been classified as a known human carcinogen; however, considerable controversy exists over the potential human

health risk posed by daily exposure. Data from animal models, cell systems, and human studies are being used to develop risk assessment models for dioxin. These studies will attempt to identify sensitive subpopulations and develop strategies for replacing default methods for estimating the range of expected risks in the population (Portier, NIEHS/NIH; Walker, NIEHS/NIH).

Biochemical Modeling

For many chemicals or classes of chemicals, there is emerging data related to gene expression, protein levels, receptor binding and interaction, and cellular protein changes that should allow development of biochemical models more complex than simple ADME models. Such models can provide mechanistic insights into the origin of biological changes at the cellular and molecular levels resulting from a particular exposure and improve risk assessment. Research is underway to develop computational methods to provide detailed atomic level descriptions of biomolecules such as protein-DNA complexes (Darden, NIEHS/NIH). At the NIEHS/NIH mechanistic models are being constructed to characterize Ah receptor-dependent transcriptional activation of dioxin-responsive genes and enzyme induction in 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD) treated rats (Portier, NIEHS/NIH; Walker, NIEHS/NIH). Future efforts will address the development of a prostate growth and development model to evaluate the potential role of environmental agents in prostate carcinogenesis through their interference in endocrine signaling pathways.

Physiologically Based Pharmacokinetic/Toxicokinetic Models

Physiologically based pharmacokinetic/toxicokinetic (PBPK) models have an improved and realistic description of key physiological processes and biochemical activities that affect both ADME of the parent compound and its metabolites. PBPK models are being used increasingly to extrapolate animal doses to human doses. Substitution of human physiological and biochemical parameter estimates into models characterized for laboratory animals provides a sound scientific basis for extrapolations of tissue dosimetry across species, extrapolation from high exposures to low exposures, and extrapolation across different routes of exposure. Because PBPK models use parameters that are measurable in human populations, these models can also be used to evaluate the impact of inter-individual variability. Several PBPK models have been developed at the NIEHS/NIH for agents of interest to the NTP (Table 18). Data used to create these models were generated through NTP contracts and from literature sources (Kohn, NIEHS/NIH; Portier, NIEHS/NIH).

Table 18. Physiologically Based Toxicokinetic Modeling Ongoing by the NTP

Chemical	Exposure Route	NTP Technical Report Series No.
2,3,7,8-Tetrachloro-dibenzo-*p*-dioxin[1]	oral and Topical	
Anthraquinone	oral – feed	494
Butadiene[1]	Inhalation	
Isoprene	inhalation	486
Melatonin[1]	Endogenous	
Mercury (pregnant rat)[1]	inhalation	
Methyleugenol	oral – gavage	491
Naphthalene	inhalation	500
p,p'-Dichlorodiphenylsulfone	oral – feed	501
Polychlorinated Biphenyls (209 congeners)[1]	multiple	
Primidone	oral – feed	476
Sodium Nitrite	oral – drinking water	495

[1] Ongoing PBPK modeling research

Areas for Future Initiatives and Resources

Toxicokinetic Modeling

Researchers at the NIEHS/NIH are interested in developing a library of prototypical submodels of physiological and biochemical processes (*e.g.,* GI tract absorption, dermal absorption, placental transfer, urinary elimination, glutathione depletion and re-synthesis). Improvements in toxicokinetic modeling are also needed. The toxicokinetic models developed for several NTP chemicals have been based largely on blood time-course data of parent compounds and the distribution and elimination of radioactivity in animals treated with radiolabeled compound. The reliability of these models could be vastly improved with greater accounting for mass balance, direct information on the enzymatic kinetics of the metabolic elimination of the parent compound and its primary metabolites, and tissue time-course data of parent compound and its metabolites. The NTP is also interested in the development of mechanistically based dose-response models for non-cancer endpoints in order to improve the ability to estimate human risk from environmental toxicants. One proposed future effort is to characterize the disposition of environmental agents in dams and embryos during organogenesis and perinatal development.

Biochemical Modeling

Researchers at the NIEHS/NIH are interested in extending their current efforts in modeling tissue responses to active toxicants (*e.g.,* TCDD) by investigating alterations in gene expression. As molecular information becomes available, efforts will be made to characterize dose response relationships for DNA damage induced by epoxide and epoxide-forming chemicals (*e.g.,* 1,3-butadiene, isoprene, chloroprene, ethylene/ethylene oxide, propylene/propylene oxide, and styrene/styrene oxide).

Mechanistic information helps to provide links in extrapolating animal data to humans, and the NTP is interested in testing mechanistic hypotheses by biomathematical modeling of intracellular responses to toxicant exposures (*e.g.,* relationships among peroxisome proliferator activated receptor-mediated gene expression, peroxisome proliferation, and liver tumor induction). Future NIEHS/NIH research will target this effort.

ALTERNATIVE TEST SYSTEM DEVELOPMENT AND VALIDATION

A large number of chemicals (>80,000) are currently in use. The NTP continually faces the task of determining how to acquire the scientific information about a substance(s) being evaluated that will best address identification of any related hazard from exposure and strengthen the science base. Implementing new strategies, which provide additional or more accurate information, can strengthen the science base on which regulatory decisions are based. Through the NTP, efforts are focused on the development and validation of new alternative test systems (sensitive, specific, rapid) for toxicological research that will reduce, replace, or refine animal use.

Model systems under development include non-mammalian species, transgenic species, genetically engineered *in vitro* cell systems, microchip array technology, and computer-based predictive toxicology models. In addition, through the NTP Center for the Validation of Alternative Toxicological Methods, a concerted and coordinated Federal effort is being made to identify, validate, and promote regulatory acceptance of alternative test systems. University-based researchers are also involved in this alternative methods development and validation through the NIEHS/NIH extramural grants program.

TRANSGENIC MODELS

Transgenic Mouse Models

The conventional rodent bioassay has been used for over three decades and is accorded credibility in identifying carcinogens thought to pose risks for human health. An ongoing goal of the NTP is to seek other model systems for toxicology and carcinogenesis studies, especially those that can provide mechanistic information relative to understanding an agent's mode of action. The use of transgenic models holds promise for improving both the accuracy and efficacy of experimental assessment of the carcinogenic potential of chemicals. Genetically altered or "transgenic" mouse models carry activated oncogenes or inactivated tumor suppressor genes known to be involved in neoplastic processes both in humans and rodents. This trait may allow them to respond to carcinogens more quickly than conventional rodent strains. In addition, the neoplastic effects of agents can be observed in transgenic models within a time frame in which few, if any, spontaneous tumors would arise. The high incidences of spontaneous or background tumors, which occur most often late in the two-year rodent cancer studies, are among the most confounding factors for interpreting the findings of chemical carcinogenesis and their implications for human health. The use of target or reporter genes also allows for direct molecular and cellular analysis of a chemical's effects in these models and can provide additional mechanistic information about mode of action.

Over the past few years, the NIEHS/NIH and NTP have been actively evaluating transgenic strains in toxicological testing strategies. Based on current evaluations, the models with greatest potential usefulness at this time are the $p53^{def}$ (*p53*+/-heterozygous) and Tg.AC (*v-Ha-ras* transgene). The Tg.AC mice carry a *v-Ha-ras* oncogene that represents a class of oncogenes that plays a key role in signal transduction pathways, which regulate cell proliferation, and are detectable in the early stages of tumor induction. The heterozygous $p53^{def}$ mouse lacks a member of a class of suppressor genes that has an important role in cell cycle control; loss of function is associated with progression of tumors to malignancy. These strains show specificity for being able to identify genotoxic agents ($p53^{def}$) and both genotoxic and nongenotoxic agents (Tg.AC). Evaluation of the specificity and usefulness of these two

transgenic models is continuing (French NIEHS/NIH; Spalding NEIHS/NIH; Tennant, NIEHS/NIH).

The NIEHS/NIH is also evaluating the usefulness of other transgenic models including:

- TRAMP (Transgenic Adenocarcinoma Mouse Prostate) mice (Maronpot, NIEHS/NIH),
- p53 null mutants on an FVB/N background (Stasiewicz, NIEHS/NIH),
- p16 transgenic mice (carries targeted deletion in *Cdnk2a* locus that eliminates expression of $p16^{INK4a}$ and $p19^{ARF}$) (French, NIEHS/NIH),
- Tg.NK mouse transgenics (contains c-*neu*, the human breast cancer oncogene homologue of *erb*B2 and develops mammary tumors early in life) (Rao, NIEHS/NIH),
- COX 1 and COX2 knockout mice (study physiological functions of cyclooxygenase isoforms) (Langenbach, NIEHS/NIH),
- Tg.APC [contains a mutation in the mouse adenomatous polyposis coli (*Apc*) gene, the mutation site in the majority of human colon cancers] (Stasiewicz, NIEHS/NIH),
- Inherited defects in the *BRCA1* and *BRCA2* genes account for most hereditary-linked human breast and ovarian cancers. Efforts at the NIEHS/NIH are focusing on development of experimental mouse models for BRCA alterations. These mice should be useful to define functions of these genes and to provide important insights for understanding these cancers (Wiseman, NIEHS/NIH), and
- Mouse lines recessive for functional ER-alpha (estrogen receptor) and ER-beta receptor signaling systems (Korach, NIEHS/NIH).

Transgenic Fish Models

Efforts are underway to determine the usefulness of transgenic fish as an alternate model for mice and cultured cells. Transgenic technology has been applied to the study of induced somatic mutation directly at the DNA level using PhiX174 bacteriophage as an identical marker in rodents, fish (*Fundulus heterosclitus*), and cultured cells to compare dose, adduction, and DNA repair and mutation. Initial studies comparing mice and fish exposed to the potent carcinogen, 7,12-bis-hydroxymethylbenz[a]anthracene, are encouraging. Future studies include expanding the target sequence in the transgenic vector, investigating mutagenicity in mice exposed to certain environmental mixtures, and combining this approach with developmental and endocrine disruptor endpoints (Burkhart, NIEHS/NIH).

NTP Interagency Center for the Evaluation of Alternative Toxicological Methods

The development, validation, acceptance, and harmonization of new and revised toxicological test methods are coordinated in the Federal government through the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). ICCVAM was established in 1997 in response to the 1993 NIH Revitalization Act to reduce, refine, or replace the use of animals in research and testing. NICEATM was established in 1998 to collaborate with ICCVAM in the development, scientific review, validation, and achievement of regulatory acceptance of new and improved test methods applicable to the needs of Federal agencies. Dr. William Stokes is the Center Director.

On December 19, 2000, the ICCVAM Authorization Act of 2000 (PL 106-545) established ICCVAM as a permanent committee of the NIEHS/NIH under the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). ICCVAM will consist

of the heads of 15 Federal agencies (ATSDR, CPSC, Departments of Agriculture, Defense, Energy, Interior, and Transportation, EPA, FDA, NIOSH/CDC, NIH, NCI/NIH, NIEHS/NIH National Library of Medicine, and OSHA).

The purposes of ICCVAM are to 1) increase efficiency and effectiveness of Federal agency test method review; 2) eliminate unnecessary duplicative efforts and share experiences between Federal regulatory agencies; 3) optimize utilization of scientific expertise outside of the Federal government; 4) ensure that new and revised test methods are validated to meet the needs of Federal agencies; and 5) reduce, refine, or replace the use of animals in testing where feasible.

The Advisory Committee for Alternative Toxicological Methods (ACATM, see page 2) provides oversight to NICEATM. The ICCVAM Authorization Act establishes a permanent Scientific Advisory Committee to NICEATM and ICCVAM. The NTP will review ACATM's charter and revise as appropriate to meet the Act's requirement.

ICCVAM and NICEATM work to promote the validation and regulatory acceptance of toxicological test methods that are more predictive of human and ecological effects than those currently available and to communicate with stakeholders and the public. The desired outcomes from these new methods are an improvement in agencies' abilities to assess risk and make regulatory decisions and the refinement, reduction, and replacement of animals in toxicological testing. Workshops are held, as needed, for evaluation of the adequacy of existing methods, identification of areas needing alternative methods, and evaluation of proposed validation studies. A formal, scientific review process is in place for evaluation of the validation status of proposed alternative testing methods.

Information about ICCVAM and NICEATM is found at http://iccvam.niehs.nih.gov. Specific questions and inquiries about nominations of alternative testing methods can be addressed to: NICEATM, NIEHS/NIH, P.O. Box 12233, MD EC-17, Research Triangle Park, NC 27709, T: (919) 541-2384, F: (919) 541-0947, e-mail: iccvam@niehs.nih.gov.

Alternative Method Reviews

Local Lymph Node Assay

The murine Local Lymph Node Assay (LLNA), a method for assessing the allergic contact dermatitis causing potential of chemicals, was the first alternative test method to be evaluated using ICCVAM's formal process. The Expert Panel's conclusion - LLNA is a valid alternative to currently accepted guinea pig test methods and offers advantages of using fewer animals, eliminating pain and distress, taking less time, being mechanistically based, and providing dose-response information - was forwarded to the various ICCVAM agencies. Agency response has been overwhelmingly favorable and the CPSC, EPA, OSHA, and FDA concurred with the Expert Panel and announced acceptance of LLNA in October 1999.

In partnership with ILSI Health and Environmental Sciences Institute, ICCVAM organized a training workshop on the LLNA January 25-26, 2001 at NIH. The workshop's objective was to assist participants in gaining an understanding of the theory and application of the method.

International Workshop on *In Vitro* Methods for Assessing Acute Toxicity

ICCVAM/NICEATM held this workshop October 17-20, 2000 in Arlington, VA to assess the current status of *in vitro* test methods for evaluating the acute systemic toxicity potential of chemicals and to make recommendations for validation efforts necessary for characterizing the usefulness and limitations of existing methods.

Endocrine Disruptor Screening and Testing Program

At the request of EPA, ICCVAM and NICEATM are planning an Expert Panel Meeting in 2002 to assess the validation status of several *in vitro* assays for use in EPA's Endocrine Screening Program. NICEATM is preparing background documents on *in vitro* estrogen receptor and androgen receptor binding and transcriptional activation assays. This information will be used for evaluating the validation status of these assays.

Areas for Future Initiatives and Resources

Development and Validation of Models

The NTP is recognized internationally for its toxicology testing using rodent bioassays; however, the Program recognizes the need to expand its efforts toward understanding the mechanistic basis for toxicity. Resources will continue to be used toward the development, validation, and application of alternative models for NTP research. This includes both transgenic mouse models for use in carcinogenicity research, as well as alternative models such as cell systems and fish. Efforts to develop and evaluate transgenic animals for non-cancer endpoints has started [*e.g.*, the use of the Center as a resource for coordinating activities related to the development, validation, and application of transgenic models with other agencies, industry, and academia].

Center Activities

Working together, ICCVAM and NICEATM activity has been highly successful in providing an organized and productive means for coordinating activities among Federal agencies relative to the validation and regulatory acceptance of alternative toxicological test methods. The NTP is fully committed toward efforts in this area; however, with the numerous ongoing and planned activities, greater resources may be needed in the future. ICCVAM anticipates a future review of selected endocrine disruptor screening and testing methods proposed for evaluation of chemicals in the EPA High Production Volume Testing Program. The Center is also involved in developing a 5-10 year strategic plan to guide it in setting priorities and using resources for review and evaluation of alternative test methods. NICEATM and ICCVAM will continue to move forward with implementation of the ICCVAM Authorization Act.

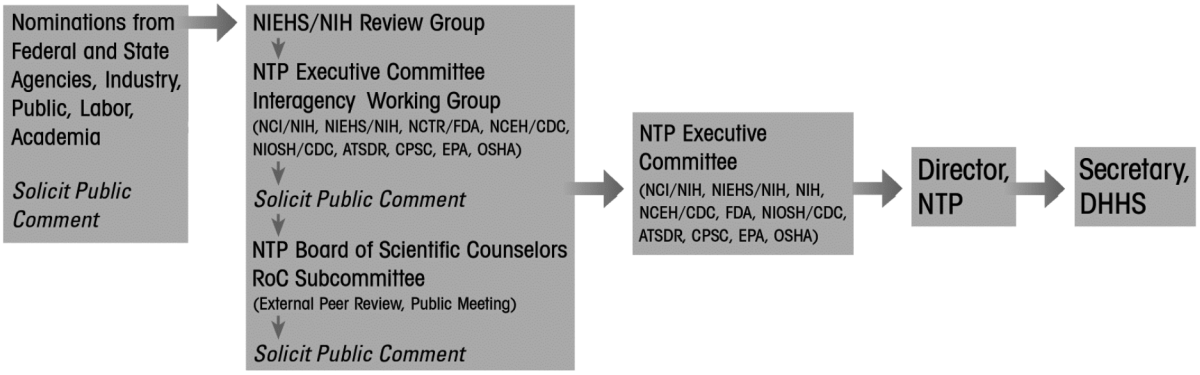
REPORT ON CARCINOGENS

The *Report on Carcinogens* (RoC) is a Congressionally mandated listing of known human carcinogens and reasonably anticipated human carcinogens, and the Secretary, DHHS delegated responsibility for its preparation to the NTP. The nomination of chemicals, substances, mixtures, or exposure circumstances for listing/delisting in the RoC is open to all interested individuals and groups. Dr. C.W. Jameson, NIEHS/NIH oversees preparation of the RoC. As shown in Figure 6, the review of nominations to the Report is a multi-step and open process. The scientific review of nominations involves three separate reviews (NIEHS/NTP Review Group, NTP Executive Committee Interagency Working Group, NTP Board of Scientific Counselors RoC Subcommittee) followed by review and comment by the NTP Executive Committee. Public comments are solicited multiple times during the process and are provided to each review group as available. The NTP Director receives the input from all reviews plus the public comments and makes his recommendations on the nominations to the Secretary, DHHS for review and approval. Additional information about the RoC is available on the NTP web site: http://ntp-server.niehs.nih.gov.

The review of nominations for the 10th Edition of the RoC began in 1999 and continued during 2000. Table 19 lists the nominations being considered for the 10th Report. Completion of the review process and submission of the NTP's recommendation on nominations to the 10th RoC is anticipated in 2001.

Requests for additional information about the RoC and the submission of nominations for consideration can be directed to Dr. C.W. Jameson, Report on Carcinogens, NIEHS, P.O. Box 12233 EC-14, 79 T.W. Alexander Dr., Research Triangle Park, NC 27709; T: (919) 541-4096; F: (919) 541-0144; jameson@niehs.nih.gov.

Figure 6. Report on Carcinogens Review Process



ATSDR, Agency for Toxic Substances and Disease Registry; CPSC, U.S. Consumer Product Safety Commission; EPA, U.S. Environmental Protection Agency; FDA, Food and Drug Administration; NCEH/CDC, National Center for Environmental Health of the Centers for Disease Control and Prevention; NCI/NIH, National Cancer Institute of the National Institutes of Health; NCTR/FDA, National Center for Toxicological Research of the FDA; NIEHS/NIH, National Institute of Environmental Health Sciences of the National Institutes of Health; NIH, National Institutes of Health; NIOSH/CDC, National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention; OSHA, Occupational Safety and Health Administration.

Table 19. Summary for Nominations Being Reviewed for Consideration of Listing In or Delisting from the Tenth Report on Carcinogens

Nomination (CAS No.)	Primary Uses or Exposures	To Be Reviewed For
Nominations considered in the first round of reviews		
Beryllium and Beryllium Compounds (7440-41-7)	Used in fiber optics and cellular network communications systems, aerospace, defense and other industry applications.	Possible updating of current listing of beryllium and certain beryllium compounds to *known to be a human carcinogen*
2,2-Bis-(bromomethyl) –1,3-propanediol (Technical Grade) 3296-90-9	Used as a fire retardant in unsaturated polyester resins, in molded products, and in rigid polyurethane foam	Listing as *known to be a human carcinogen*
2,3-Dibromo-1-propanol (96-13-9)	Used as a flame retardant, as an intermediate in the preparation of the flame retardant tris(2,3-dibromopropyl) phosphate, and as an intermediate in the manufacture of pesticides and pharmaceutical preparations.	Listing as *reasonably anticipated to be a human carcinogen*
Dyes metabolized to 3,3'-Dimethylbenzidine	Dyes mainly used in textile industries with other applications in paper, plastics, and rubber industries.	Listing as *reasonably anticipated to be a human carcinogen*
Dyes metabolized to 3,3'-Dimethoxybenzidine	Dyes mainly used in textile industries with other applications in paper, plastics, and rubber industries.	Listing as *reasonably anticipated to be a human carcinogen*
IQ (2-Amino-3-methylimidazo[4,5-f]quinoline) (76180-96-6)	Found in cooked meat and fish and in cigarette smoke	Listing as *reasonably anticipated to be a human carcinogen*
Styrene-7,8-oxide (96-09-3)	Used mainly in the preparation of fragrances and in some epoxy resin formulations	Listing as *reasonably anticipated to be a human carcinogen*
Vinyl Bromide (593-60-2)	Used primarily in the manufacture of flame retardant synthetic fibers	Listing as *reasonably anticipated to be a human carcinogen* by two review groups[1] and listing as *known to be a human carcinogen* by a third review group[2]
Vinyl Fluoride (75-02-5)	Used in the production of polyvinylfluoride which is used for plastics	Listing as *reasonably anticipated to be a human carcinogen* by two review groups[1] and listing as *known to be a human carcinogen* by a third review group[2]
Nominations considered in the second round of reviews		
Broad Spectrum UV Radiation (UVR), and UVA and UVB, and UVC	Solar and artificial sources of ultraviolet radiation	Listing of UVR as *known to be a human carcinogen* Listing of UVA as *reasonably anticipated to be a human carcinogen* Listing of UVB as *reasonably anticipated to be a human carcinogen* Listing of UVC as *reasonably anticipated to be a human carcinogen*
Chloramphenicol (56-75-7)	Used widely as an antibiotic since the 1950s. Veterinary use of chloramphenicol has resulted in the occurrence of residues in animal-derived food.	Listing as *reasonably anticipated to be a human carcinogen*
Estrogens, Steroidal	Estrogens are widely used in oral contraceptives and	Listing as *known to be a*

Nomination (CAS No.)	Primary Uses or Exposures	To Be Reviewed For
	in post-menopausal therapy for women.	*human carcinogen*
Methyleugenol (93-15-2)	Flavoring agent used in jellies, baked goods, nonalcoholic beverages, chewing gum, candy, and ice cream. Also used as a fragrance for many perfumes, lotions, detergents and soaps	Listing as *reasonably anticipated to be a human carcinogen*
Nickel (Metallic) and Certain Nickel Alloys	Widely used in commercial applications for over 100 years	Listing of Metallic Nickel as *reasonably anticipated to be a human carcinogen* Listing of Certain Nickel Alloys as *reasonably anticipated to be a human carcinogen* by one review group[3] and not listing in the RoC by two review groups[4]
Talc (14807-96-6) Non-Asbestiform and Asbestiform	Both forms of talc occur in various geological settings around the world. Occupational exposure occurs during mining, milling and processing. Exposure to talc non-asbestiform in the general population occurs through use of products such as cosmetics.	Listing Talc Asbestiform as *known to be a human carcinogen* by one review group[3] and listing as *reasonably anticipated to be a human carcinogen* by one review group[5] and a tie vote for this recommendation by one review group[2]. Listing Talc Non-Asbestiform as *reasonably anticipated to be a human carcinogen* by two review groups[1] and not to list as *reasonably anticipated to be a human carcinogen* by one review group[2]
Trichloroethylene (79-01-6)	Trichloroethylene is widely used as a solvent, with 80-90% used worldwide for degreasing metals.	Upgrade to *known to be a human carcinogen* by one review group[3] and not to change from *reasonably anticipated* by two review groups[4]
Wood Dust	It is estimated that at least two million people are routinely exposed occupationally to wood dust worldwide. Non-occupational exposure also occurs. The highest exposures have generally been reported in wood furniture and cabinet manufacture, especially during machine sanding and similar operations	Listing as *known to be a human carcinogen*

[1] The NIEHS Review Committee for the Report on Carcinogens and the NTP Executive Committee Interagency Working Group for the Report on Carcinogens

[2] The NTP Board of Scientific Counselor Report on Carcinogens Subcommittee

[3] The NIEHS Review Committee for the Report on Carcinogens

[4] The NTP Executive Committee Interagency Working Group for the Report on Carcinogens and the NTP Board of Scientific Counselor Report on Carcinogens Subcommittee

[5] The NTP Executive Committee Interagency Working Group for the Report on Carcinogens

APPENDIX 1

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APPENDIX 2

BOARD OF SCIENTIFIC COUNSELORS

Name and Title	Affiliation	Term Ends	Board Service[1]	Expertise
George Bailey, Jr., Ph.D. Professor of Food Toxicology and Director, Marine/ Freshwater Biomedical Sciences Center	Oregon State University Corvallis, OR	6/30/02	Board	Alternative Methods, Xenobiotic Metabolism
George Bonney, Ph.D. Professor, Microbiology Director Statistical Genetics and Bioinformatics Unit, National Human Genome Center	Howard University Washington, DC	6/30/04	Board RoC	Genetic Epidemiology, Carcinogenesis, Biostatistics
Hillary M. Carpenter, III, Ph.D. Toxicologist	Minnesota Department of Health St. Paul, MN	06/30/04	Board RoC	Toxicology, Risk Assessment, Public Health
Linda A. Chatman, D.V.M. Principal Pathologist	Pfizer, Inc. Groton, CT	6/30/01	TRRS	Pathology, Carcinogenesis
Harold Davis, Ph.D., D.V.M. Director of Toxicology	Amgen, Inc. Thousand Oaks, CA	6/30/01	TRRS	Pathology, Lab Animal Medicine
Yvonne P. Dragan Assistant Professor	Ohio State University Columbus, OH	6/30/03	RoC TRRS	Toxicology, Experimental Carcinogenesis
Norman R. Drinkwater, Ph.D. Professor of Oncology McArdle Laboratory for Cancer Research	University of Wisconsin-Madison Madison, WI	6/30/02	Board TRRS	Experimental Carcinogenesis
Clay Frederick, Ph.D. Senior Research Fellow, Mechanistic Toxicology Group	Rohm and Haas Company Spring House, PA	6/30/01	Board RoC	Toxicology, Animal Models
John R. Froines, Ph.D. Professor and Director UCLA Center for Occupational and Environmental Health	UCLA School of Public Health Los Angeles, CA	6/30/03	RoC	Occupational Health
Lynn Goldman, M.D. Visiting Scholar Department of Health Policy and Management	Johns Hopkins University, School of Hygiene and Public Health Baltimore, MD	6/30/02	Board	Pediatrics, Health Regulation
Stephen S. Hecht, Ph.D. Wallin Professor of Cancer Prevention	University of Minnesota Cancer Centers Minneapolis, MN	6/30/01	RoC TRRS	Human Disposition of Environmental Carcinogens, Carcinogenic Mechanisms
Karl T. Kelsey, M.D. Occupational Health Program	Harvard School of Public Health and Medical School Boston, MA	6/30/01	RoC	Occupational & Molecular Epidemiology
James E. Klaunig, Ph.D. Director, Division of Toxicology Professor, Department of Pharmacology and Toxicology	Indiana University School of Medicine Indianapolis, IN	6/30/03	TRRS	Toxicology, Experimental Carcinogenesis, Public Health
Robert A. LeBoeuf, Ph.D. Associate Director, Human and Environmental Safety	The Procter and Gamble Company Cincinnati, OH	6/30/03	Board	Molecular Carcinogenesis, Cancer Research Models
Grace K. Lemasters, Ph.D. Professor and Director Division of Epidemiology and	University of Cincinnati, College of Medicine Cincinnati, OH	6/30/02	Board	Occupational & Reproductive Epidemiology

Name and Title	Affiliation	Term Ends	Board Service[1]	Expertise
Biostatistics, Department of Environmental Health				
David E. Malarkey, DVM, Ph.D. Assistant Professor of Pathology, Department of Microbiology, Pathology and Parasitology	North Carolina State University Raleigh, NC	6/30/04	TRRS	Pathology, Lab Animal Medicine
Donald R. Mattison, M.D. Medical Director	March of Dimes, Birth Defects Foundation White Plains, NY	6/30/03	Board	Reproductive/ Developmental/ Risk Assessment
Michele Medinsky, Ph.D. Toxicology Consultant	Durham, NC	6/30/01	RoC TRRS	Metabolism, Toxicokinetics
Rafael Moure-Eraso, Ph.D, CIH Associate Professor Department of Work Environment	University of Massachusetts Lowell, College of Engineering Lowell, MA	6/30/02	Board RoC	Industrial Hygiene Worker Health
Jill C. Pelling, Ph.D. Professor, Department of Pathology and Laboratory Medicine	University of Kansas Medical Center Kansas City, KS	6/30/01	RoC	Mechanisms of Carcinogenesis
Walter W. Piegorsch, Ph.D. Professor of Statistics Director of Undergraduate Studies Department of Statistics	University of South Carolina Columbia, SC	6/30/04	RoC TRRS	Biostatistics, Risk Assessment
Jose Russo, M.D. Director, Breast Cancer Research Labs	Fox Chase Cancer Center Philadelphia, PA	6/30/00	RoC TRRS	Human Pathology
Allan H. Smith, M.D., Ph.D. Professor of Epidemiology	University of California, Berkeley Berkeley, CA	6/30/03	RoC	Epidemiology
Mary Anna Thrall, DVM Professor, Department of Pathology	Colorado State University Fort Collins, CO	6/30/04	TRRS	Clinical Pathology, Lab Animal Medicine
Shelia H. Zahm, Sc.D. Deputy Director, Division of Cancer Epidemiology and Genetics	National Cancer Institute Rockville, MD	6/30/01	RoC	Cancer Epidemiology
Expert Consultants				
David H. Phillips Ph.D., DSc, FRCPath Research Scientist, Institute of Cancer Research	Haddow Laboratories Sutton, England	6/30/03	RoC TRRS	Molecular Epidemiology, Carcinogenesis
Hiroshi Yamasaki, Ph.D. Chief, Unit of Multistage Carcinogenesis	International Agency for Research on Cancer Lyon, France	12/31/00	Board RoC	Experimental Carcinogenesis

[1]Board Service
Board = Serves as member on the parent Board
RoC = Serves on the NTP Board of Scientific Counselors Report on Carcinogens Subcommittee
TRRS = Serves on the NTP Board of Scientific Counselors Technical Reports Review Subcommittee

APPENDIX 3

ADVISORY COMMITTEE ON ALTERNATIVE TOXICOLOGICAL METHODS

Name and Title	Affiliation	Term Ends	Expertise
Paul T. Bailey, Ph.D. Toxicology Consultant Toxicology Division	Exxon Mobil Biomedical Sciences Inc. Annandale, NJ	09/01	Regulatory Toxicology, Immunotoxicology
Rodger D. Curren, Ph.D. Institute for in Vitro Sciences, Inc.	Gaithersburg, MD	09/01	*in vitro* Toxicology
Michael S. Denison, Ph.D. Professor, Department of Environmental Toxicology	University of California – Davis Davis, CA	09/01	Molecular Biology
Elaine Faustman, Ph.D. Professor and Associate Chair, Department of Environmental Health	University of Washington Seattle, WA	09/01	Developmental Toxicology
Alan M. Goldberg, Ph.D. Associate Dean, Corporate Affairs; Professor of Toxicology; Director, Center for Alternatives to Animal Testing	Johns Hopkins University Baltimore, MD	09/01	*in vitro* Toxicology, Neurotoxicology
Sidney Green, Ph.D. Department of Pharmacology	Howard University Washington, DC	09/01	Pharmacology, Regulatory Toxicology
A. Wallace Hayes, Ph.D. Vice President, Corporate Product Integrity	The Gillette Company Boston, MA	09/01	General Toxicology
Roger McClellan, D.V.M. Consulting Toxicologist	Albuquerque, NM	09/01	General Toxicology
Kenneth Ramos, Ph.D. Department of Physiology and Pharmacology, College of Veterinary Medicine	Texas A & M University College Station, TX	09/03	Molecular Toxicology, Cell Biology
Andrew N. Rowan, Ph.D. Senior Vice President	Humane Society of the United States Gaithersburg, MD	09/01	Animal Welfare, Bioethics
Katherine A. Stitzel, D.V.M. Associate Director, Human Safety	The Procter & Gamble Company Cincinnati, OH	09/01	*in vitro* Toxicology, Regulatory Toxicology
Peter Theran, V.M.D. Director, Center for Laboratory Animal Welfare	Massachusetts Society for the Prevention of Cruelty to Animals Boston, MA	09/03	Animal Welfare, Comparative Medicine