National Toxicology Program

NTP

CURRENT DIRECTIONS and EVOLVING STRATEGIES

National Toxicology Program

NTP

CURRENT DIRECTIONS and EVOLVING STRATEGIES

Good Science for Good Decisions

Table of Contents

4 Overview of the National Toxicology Program
10 Current Directions
16 Evolving Strategies
22 Centers
26 Report on Carcinogens
28 Outreach and Communication

ATSDR	Agency for Toxic Substances and Disease Registry
CDC	Centers for Disease Control and Prevention
CDM	Central Data Management
CERHR	Center for the Evaluation of Risks to Human Reproduction
CPSC	Consumer Product Safety Commission
DBPs	disinfectant by-products
DHHS	Department of Health and Human Services
EHP	*Environmental Health Perspectives*
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
ICCEC	Interagency Committee for Chemical Evaluation and Coordination
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
LLNA	Local Lymph Node Assay
MRI	Magnetic Resonance Imaging
NCEH	National Center for Environmental Health
NCI	National Cancer Institute
NCP	NTP Center for Phototoxicology
NCT	National Center for Toxicogenomics
NCTR	National Center for Toxicological Research
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute of Standards and Technology
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
PBPK	physiologically based pharmacokinetic
RoC	Report on Carcinogens
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
UV	Ultraviolet

Overview

Mission and Goals

More than 80,000 chemicals are registered for use in the United States. Each year, an estimated 2,000 new ones are introduced for use in such everyday items as foods, personal care products, prescription drugs, household cleaners, and lawn care products. We do not know the effects of many of these chemicals on our health, yet we may be exposed to them while manufacturing, distributing, using, and disposing of them or when they become pollutants in our air, water, or soil. Relatively few chemicals are thought to pose a significant risk to human health. However, safeguarding public health depends on identifying both what the effects of these chemicals are and at what levels of exposure they may become hazardous to humans—that is, understanding their toxicology.

The National Toxicology Program (NTP) was established by the U.S. Department of Health and Human Services (DHHS) in 1978 to (1) coordinate toxicology testing programs within the DHHS, (2) strengthen the science base in toxicology, (3) develop and validate improved testing methods, and (4) provide 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 tools of modern toxicology and molecular biology. The program maintains an objective, science-based approach in dealing with critical issues in toxicology and is committed to using the best science available to prioritize, design, conduct, and interpret its studies. To that end, the NTP is continually evolving to remain at the cutting edge of scientific research and to develop and apply new technologies.

Current Activities

The NTP maintains a number of complex, interrelated research and testing programs that provide unique and critical information needed by health, regulatory and research agencies to evaluate potential human health effects from chemical and physical exposures. All of the NTP's activities are open to public scrutiny, including communications with all interested parties. The NTP has always drawn strength and direction from the commitment of its scientists to exchange information openly, maintain impartiality, and apply rigorous scientific peer review. This is a central priority of the program now and will remain so in the future.

The NTP seeks to maintain a balanced research and testing program that provides data on a wide variety of issues important to public health. In particular, the NTP seeks nominations of studies that (1) fill significant gaps in the knowledge of the toxicity of chemicals or classes of chemicals, (2) address mechanisms of toxicity, or (3) enhance the predictive ability of future NTP studies. Currently, the NTP is focusing on

several areas that have received inadequate attention in the past. Some examples include photoactive chemicals, contaminants of finished drinking water, endocrine-disrupting agents, and certain complex occupational exposures. The NTP is addressing potential safety issues associated with herbal medicines, radiofrequency radiation emissions from cellular telephones, hexavalent chromium, and nanoscale materials. In general, these initiatives are broad-based and investigate various health-related effects.

The NTP continues to work to develop and validate alternative testing methods that will help identify chemical hazards using fewer test animals. This effort includes developing more efficient, mechanism-based testing strategies, such as genetically engineered models for toxicology testing, and implementing microchip-based gene expression technologies. These methods hold the promise of providing a true mechanistic basis for identifying and studying environmental toxicants.

The NTP also evaluates whether human exposures to environmental agents cause adverse effects on reproduction, development, and the immune, respiratory, and central nervous systems. The NTP is expanding its effort to include routine investigations of changes in the immune system and the nervous system from exposures occurring during fetal development and early life.

The NTP continues to expand activities designed to place research and testing results from animals in a perspective that is more relevant to human health. This includes human exposure assessment, toxicokinetics, mechanism-based pharmacokinetic modeling, and interpreting results in molecular epidemiology for use in identifying human hazards (e.g., the Report on Carcinogens and the NTP's Center for the Evaluation of Risks to Human Reproduction). The NTP is also coordinating an effort to obtain "real-world" information about worker practices, complex occupational exposures, and potentially related adverse health effects. We need such information to identify areas for research and to design better laboratory studies on the potential health effects of chemicals, complex mixtures, and exposures people encounter in the workplace.

Vision for the 21st Century

In its 25 years of existence, the NTP has been a leader in toxicology testing and research within the United States and has contributed significantly to the scientific knowledge upon which public health decisions are based. The NTP has focused upon using the best testing strategies for evaluating agents of public health concern. In the 1990s, the program undertook efforts to develop, evaluate, and validate tools for mechanism-based toxicology and incorporate them into its testing strategies. Mechanism-based

toxicology has led to some changes in the scientific basis for public-health decisions, but it has not dramatically reduced the need for the classical tests developed in the 1970s and 1980s. The last decade of the 20th century and the turn of the 21st century have produced dramatic advances in molecular biology and computer science. The NTP realizes that it is again time to evaluate its key activities. In a focused and concerted effort, the NTP needs to determine how best to (1) incorporate these new scientific technologies into its research and testing strategies and (2) broaden scientific knowledge on the linkage between mechanism and disease.

The NTP's vision for the 21st century is to move toxicology from a predominantly observational science at the level of disease-specific models to a predominantly predictive science focused on a broad inclusion of target-specific, mechanism-based, biological observations. Only through a concerted focus on the links between mechanism and disease will toxicology be able to replace disease-specific testing models with mechanism-based assays. Such assays are more informative, faster, and more closely linked to how diseases begin and advance.

The NTP intends to develop a plan for implementing this vision and will seek input to this plan from many groups, including its federal partners, its advisory committees, and the public. Implementing this vision should continue the NTP's leadership in toxicology and enable the NTP to provide better science for better public health decisions that will serve to protect human health and the environment.

Partnerships

Over the past two decades, the NTP has developed an increasingly interactive relationship with regulatory agencies. Through this relationship, the NTP plays an important, although indirect, role in shaping public health policy. Federal and state government agencies rely on the scientific knowledge base provided by the NTP to make credible decisions that protect public health without unnecessarily increasing the regulatory burden on industry. The NTP plays a critical role in providing needed scientific data, interpretations, and guidance on the appropriate uses of these data. The NTP also plays an important role in (1) fostering interagency collaborations in research and exposure assessment, (2) providing information to regulatory agencies about alternative methods for toxicity screening, and (3) exploring new technologies for evaluating how environmental agents cause disease.

The NTP is increasingly active in developing international partnerships to establish efficient means for avoiding duplication of effort(s) in toxicology testing. The NTP is collaborating with the European Ramazzini Foundation of Oncology and Environmental Sciences to create similar protocols, quality assurance, and reporting for laboratory studies on the health effects associated with long-term exposure to environmental agents. The two groups share common interests in identifying agents that cause cancer and in understanding the interaction and synergism between genetic susceptibility to cancer and exposure to cancer-causing agents. The NTP is working with the Korean government to help establish a national toxicology program similar to the NTP. The NTP is also participating in the World Health Organization's International Electric and Magnetic Fields Project to facilitate internationally coordinated research on the health effects of electric and magnetic fields, including those generated by cellular telephone technologies.

Organizational Structure and Oversight

Three agencies form the core of the NTP: (1) the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health (NIH), (2) the National Institute for Occupational Safety and Health (NIOSH) of the Centers for Disease Control and Prevention (CDC), and (3) the National Center for Toxicological Research (NCTR) of the Food and Drug Administration (FDA) (Figure 1) Each agency voluntarily provides resources to support NTP research, testing, centers, and outreach.

Program contacts for each agency are Dr. Christopher J. Portier (NIEHS/NIH), Dr. Albert E. Munson (NIOSH/CDC), and Dr. William T. Allaben (NCTR/FDA). The Director of the NIEHS/NIH, Dr. Kenneth Olden, serves as Director of the NTP and reports to the Secretary, HHS. The National Cancer Institute of the NIH was a charter agency for the NTP and continues to serve on the NTP Executive Committee.

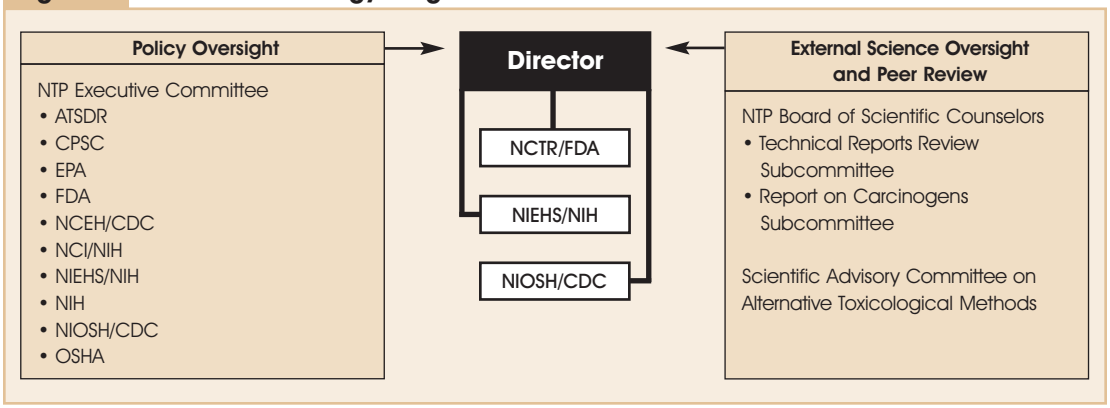
The NTP Executive Committee (Figure 1) provides oversight to the NTP for policy issues. This committee is composed of the heads (or their designees) of federal research and regulatory agencies. The NTP Board of Scientific Counselors ("the Board"), its subcommittees, and the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) assure regular scientific and public peer review and input about NTP activities and priorities.

The Board is a federally chartered advisory committee whose members are appointed by the Secretary, HHS. The Board provides primary scientific oversight to the Director for the NTP and evaluates the scientific merit of the NTP's intramural and collaborative programs. The Technical Reports Review Subcommittee of the Board provides peer review for (1) the NTP long-term toxicology and carcinogenesis studies, (2) studies conducted in genetically modified models, and (3) short-term toxicity study reports. The Report on Carcinogens Subcommittee of the Board provides external scientific evaluation of substances nominated for listing in or delisting from the Report on Carcinogens. These groups each meet once or twice each year, and all meetings are open to the public.

SACATM provides advice to the Director of the NIEHS, the Interagency Coordinating Committee on the Validation of Alternative Methods, and the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods. This advice addresses priorities and directives related to the development, validation, scientific review, and regulatory acceptance of new or revised toxicological test methods and ways to foster partnerships and communication with interested parties. The NIEHS Director appoints members to this federally chartered advisory committee. SACATM meets twice each year, and all meetings are open to the public.

The NTP also uses special emphasis panels, as needed, to provide independent scientific peer review and advice to the NTP. These panels help ensure transparent, unbiased, and scientifically rigorous input to the program for its use in making credible decisions about human health hazards, setting research and testing priorities, and evaluating test methods for toxicity screening.

Figure 1 **National Toxicology Program**



Toxicology and Carcinogenicity Evaluations

The NTP has a broad mandate to characterize chemicals and other agents of public health concern. The NTP continually solicits and reviews nominations for toxicology studies in the specific categories given in Table 1. The nomination process is open to all interested individuals and groups. Information about nominating a substance for testing by the NTP is available on the NTP web site (http://ntp-server.niehs.nih.gov) or by contacting Dr. Scott Masten, Office of Chemical Nomination and Selection (for contact information, see back flap).

Nominations undergo several levels of review before the NTP selects agents for study (Figure 2). Representatives from federal agencies on the Interagency Committee for Chemical Evaluation and Coordination (ICCEC) and the Board participate in the selection process. The NTP also solicits and considers public comment on nominations throughout this formal process. As the final step of selection, the NTP Executive Committee reviews and evaluates the testing recommendations and the public comments for each nomination. The committee makes its own recommendations on the nominations to test, not to test at this time, or to defer testing until more information is received and considered. These steps help ensure that the NTP's testing program addresses toxicological concerns pertinent to all areas of public health and maintains balance among the types of substances evaluated.

The Executive Committee's recommendation of a substance for study does not automatically commit the NTP to its evaluation. The NTP strives to balance its selection of substances for study (e.g., occupational exposures, environmental pollutants, food additives, consumer products, and pharmaceuticals) and initiates studies as time and resources permit. In reviewing and selecting nominated substances for study, the NTP also considers legislative mandates that require responsible private sector and commercial organizations to evaluate their products for human and environmental health effects. Also, a nomination selected for study may be deferred at any time if suitable data become available, if higher priority studies are identified, or if a study proves impractical.

The NTP evaluates substances for a variety of health-related effects, among them, general toxicity, reproductive and developmental toxicity, genotoxicity, immunotoxicity, neurotoxicity, metabolism, disposition, and carcinogenicity. The NTP generally uses rodent models for study and conducts short-term studies for up to thirteen weeks and long-term studies for up to two years. For each agent studied, a project leader designs a comprehensive testing strategy to address the identified research and testing needs. A project review committee evaluates the testing strategy and proposes an appropriate mechanism for sponsoring the study (e.g., grant, contract, etc.).

The NTP publishes results of short-term rodent toxicology studies in the NTP Toxicity Report series, and results of longer-term studies, generally two-year rodent toxicology and carcinogenicity studies, as NTP

Table 1 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 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

Technical Reports or in peer-reviewed scientific journals. During 2003, the NTP initiated a new technical report series for reporting the results from studies conducted in genetically modified models, such as genetically engineered mice. The Technical Reports Review Subcommittee of the Board (Figure 1) formally reviews these reports. Table 2 lists candidates for peer review in 2003 and 2004. Completed reports are available electronically from the NTP web site or can be purchased in hard copy from *Environmental Health Perspectives* (for contact information, see back flap).

Figure 2 NTP Toxicology Study Nomination and Selection Process

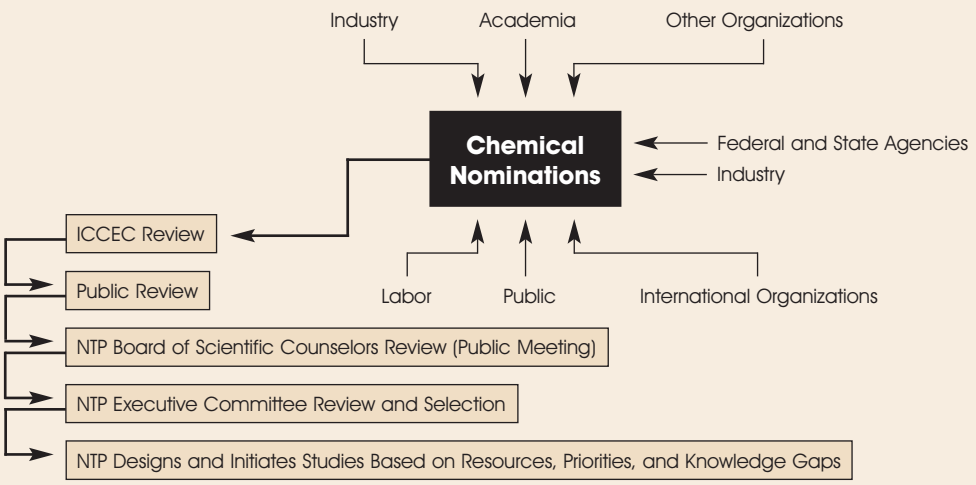


Table 2 Candidate Chemicals for Peer Review*

Winter 2004	
• Anthraquinone	• 2,3,4,7,8-Pentachlorodibenzofuran (PeCDF)
• 2,2-bis(Bromomethyl)-1,3-propanediol (studied in fish)	• TCDD, PCB 126 and PeCDF Mixture
• Malachite green and Leucomalachite green	• 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)
• Nitromethane (studied in fish)	• 1,2,3-Trichloropropane (studied in fish)
• 3,3′,4,4′,5-Pentachlorobiphenyl (PCB 126)	
Fall 2004	
• Azidothymidine (AZT)	• 2,2′,4,4′,5,5′-Hexachlorobiphenyl (PCB 153)
• Benzophenone	• PCB 126 and PCB 153 Mixture
• Bromodichloromethane	• 2,3,4,4′,5-Pentachlorobiphenyl (PCB 118) and PCB 126 Mixture
• Genistein (multigenerations)	• Sodium chlorate
Summer 2005	
• Benzene	• Glycidol
• Dibromoacetic acid	• Glycolic acid plus simulated solar light
• Dichloroacetic acid	• 4-Methylimidazole
• Dicyclohexylcarbodiimide	• Methyl isobutyl ketone
• Dilsopropylcarbodiimide	• Phenolphthalein
• Divinylbenzene	• Salicylic acid plus simulated solar light
• Genistein	• Sodium bromate

* Studies are conducted in rodent models unless otherwise indicated.

Current Directions

The NTP conducts research on a broad range of high-priority agents and issues of public health concern. Information about NTP studies, including standard protocols, fact sheets, and data from completed studies, is available on the NTP web site (http://ntp-server.niehs.nih.gov). Below are brief overviews of some current initiatives.

Radiofrequency Radiation Emissions from Cellular Phones

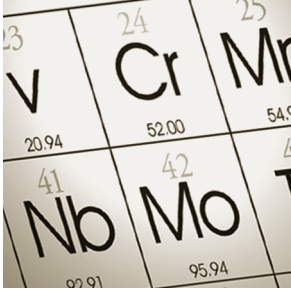
More than 100 million Americans currently use wireless communication devices, with thousands of new users added daily. Personal (cellular) telecommunications is a rapidly evolving technology that uses microwave radiation to communicate between a fixed base station and a mobile user. Most systems employ a hand-held cellular telephone, with the radiation antenna held close to the user's head.

The Federal Communication Commission requires cellular phones and other wireless communication devises to meet its guidelines for exposure to radiofrequency radiation. These guidelines are based on protecting the user from immediate injury from the heat produced by radiofrequency radiation. We currently do not have enough data to determine whether these guidelines will also protect against potential adverse effects of long-term exposure.

Studies in laboratory animals are crucial for understanding whether exposure to radiofrequency radiation may pose a danger to human health. Other research groups are performing several long-term animal studies addressing this issue. Also, the NTP plans to conduct laboratory research to help clarify any potential health hazard for the U.S. population. The NTP will study the toxic and carcinogenic effects of chronic exposure to cell phone radiofrequency radiation emissions in laboratory animals. The NTP has worked with technical experts from the National Institute of Standards and Technology (NIST) to test the suitability of various radiofrequency radiation exposure systems for these studies. NTP-sponsored studies performed at NIST have demonstrated the suitability of using reverberation chambers to expose animals to uniform fields of cell phone radiofrequency radiation.

Children's Health

The NTP continues to be a leader in issues related to children's health through research and the NTP's Center for the Evaluation of Risks to Human Reproduction (CERHR, see page 22). The NTP has ongoing efforts to evaluate effects of various agents on developing immune and nervous systems through laboratory studies of pesticides, water disinfectant by-products, and endocrine-disrupting agents. The program has expanded these efforts by establishing study protocols where perinatal animals will be given these agents



and then examined for developmental immunotoxicology, neurotoxicology, and reproductive effects. Toxicokinetic data from animal studies of mothers, fetuses, and newborns will be used to develop pharmacokinetic models based on physiology. These models will help evaluate risks to humans from exposure to environmental toxicants during early development.

Hexavalent Chromium

Chromium is a naturally occurring element present in various valence states. Trivalent chromium is an essential nutrient, and in nature chromium occurs most commonly in this state. Hexavalent chromium compounds are the next most stable form; however, they rarely occur naturally and are typically associated with industrial sources.

Because of concerns by a number of California legislators, the California Environmental Protection Agency, and the California Health and Human Services Agency, the NTP is studying the potential of hexavalent chromium given in drinking water to cause cancer. Hexavalent chromium is an established human carcinogen in certain occupational settings, presumably as a result of inhalation exposure. However, we do not know the long-term consequences of exposure to hexavalent chromium compounds in the water supply. Data currently available on the chronic toxicity and carcinogenicity of hexavalent chromium given orally are not sufficient to establish or characterize any hazard. The NTP studies include both short- and long-term administration of hexavalent chromium to laboratory animals as sodium dichromate dihydrate in drinking water, as well as studies on hexavalent chromium's tissue absorption.

Phototoxicology

The U.S. public is increasingly exposed to ultraviolet (UV) radiation from sunlight due to more leisure time spent in outdoor activities and also from other sources (e.g., tanning booths). The NTP is coordinating an effort between the NIEHS/NIH and NCTR/FDA to study the phototoxicology and photocarcinogenicity of substances nominated to the NTP, including those of high priority to the FDA. In general, these studies investigate the effects on gene expression, toxicity, and carcinogenicity of sunlight combined with either topically or systemically applied substances in the SKH-1 hairless mouse. Much of this research is being carried out at the NTP Center for Phototoxicology (NCP; see page 22).

Phototoxicology studies are in progress at the NCP for several topically applied compounds. Many cosmetics include alpha-hydroxy and beta-hydroxy acids as chemical exfoliating agents to correct or improve the appearance of "sun-aged" skin. The relation of skin cancer to their continuous use combined with exposure

to sunlight is not known. The NCP is also studying the possible acute toxicity and carcinogenicity of topically applied plant fractions of the aloe vera plant or topically applied retinyl palmitate in combination with simulated sunlight. Many products, including cosmetics and dietary supplements, contain portions of the aloe vera plant. Retinyl palmitate is included in some cosmetics as an "anti-wrinkle" compound, and its safety in the presence of sunlight needs further study. Also, the NCP is designing studies of other cosmetic ingredients, including topically applied Padimate O used in cosmetic and sunscreen preparations, furocoumarin compounds found in lemon and lime oils, and nanoscale particles used in sunscreens (zinc oxide and titanium dioxide).

The NCP is involved in examining the utility of site-specific mutations in the p53 gene as predictors (biomarkers) of the development of squamous cell carcinoma after exposure to simulated solar light. Several animal models for studying how cutaneous melanoma starts and progresses have been developed over the past several years. The NCP is currently examining how well genetically engineered animals containing the TP-ras (+) p16/INK4a (+/–) gene predict the development of UV-induced melanoma.

Herbal Medicines

Medicinal herbs are among our oldest medicines, and their increasing use in recent years is evidence of public interest in alternatives to conventional medicine. About one third of the U.S. population is believed to use some form of alternative medicine, including herbal remedies. The use of herbal medicines and other dietary supplements has increased substantially since passage of the 1994 Dietary Supplement Health and Education Act. Although about 1,500 botanicals are sold as dietary supplements or ethnic traditional medicines, herbal formulations are not subjected to FDA premarket approval to ensure their safety or efficacy.

The NTP is planning or conducting research on several medicinal herbs and compounds found in herbs (listed in Table 3) to examine carcinogenicity, reproductive toxicity, neurotoxicity, immunotoxicity, or toxic effects associated with exposures to high acute doses and chronic low doses.

Occupational Exposures

The NTP is coordinating an effort between the NIEHS/NIH and NIOSH/CDC to better understand worker exposures, educate workers, and identify occupational health research gaps. Current efforts are addressing worker exposure to welding fumes and 1-bromopropane.

Studies of diseases in workers suggest that occupational exposure to welding fumes may cause adverse health effects. More information is needed to evaluate the relationship between timing and amount of exposure and the adverse effects and to understand the specific causes of these effects. The NIOSH/CDC has constructed a computer-controlled, automated robotic welding fume inhalation system that will expose laboratory animals to tightly controlled, well-characterized welding fumes caused by different welding processes and materials. The physical and chemical composition of the generated fumes and gases will be characterized. Also, studies will be performed to evaluate which exposure conditions, generator parameters, and welding processes and materials cause acute responses in laboratory animals by assessing lung injury, inflammation, and changes in the immune system.

An industry consortium has petitioned the EPA to list 1-bromopropane as an alternative for ozone-depleting solvents. This could vastly increase the exposure of workers and the public to this compound. To obtain information on exposures to this chemical, the NIOSH/CDC is conducting an industry-wide study targeting industries that use adhesives, the metal degreasing and electronics industry, and chemical, aerosol, and adhesive manufacturers. Study sites are selected based on quantity and type of 1-bromopropane use, number of workers exposed, type of manufacturing process, and how well the site represents the industry.

Table 3 Herbals and Herbal Components under Study by the NTP*

Aloe vera gel	Widely used as a dietary supplement and component of cosmetics. Ninth highest in sales in 2002. The gel has been used for centuries as a treatment for minor burns and is increasingly being used in products for internal consumption.
Black cohosh	Used to treat symptoms of pre-menstrual syndrome, dysmenorrhea and menopause. Ranked 11th in sales in 2002.
Bladderwrack	A source of iodide used in treatment of thyroid diseases and also used as a component of weight-loss preparations.
Comfrey	Herb consumed in teas and as fresh leaves for salads; however, it contains pyrrolizidine alkaloids (e.g., symphatine), which are known to be toxic. Used externally as an anti-inflammatory agent in the treatment of bruises, sprains, and other external wounds. Based in part on NTP studies on the alkaloid components of comfrey, the FDA has recommended that the manufacturers of dietary supplements containing this herb remove them from the market.
***Echinacea purpurea* extract**	The most commonly used medicinal herb in the United States in 2002. Used as an immunostimulant to treat colds, sore throat, and flu.
Ephedra	Also known as Ma Huang; 21st in sales in 2002. Traditionally used as a treatment for symptoms of asthma and upper respiratory infections. Often found in weight loss and "energy" preparations, which usually also contain caffeine. Use has been associated with side effects such as heart palpitations, psychiatric and upper gastrointestinal effects, and symptoms of autonomic hyperactivity such as tremor and insomnia, especially when taken with other stimulants.
***Ginkgo biloba* extract**	Ranked 4th in sales in 2002. Ginkgo fruit and seeds have been used medicinally for thousands of years. The extract of green-picked leaves has shown 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	Ranked 13th in sales of medicinal herbs in 2002. Ginsenosides are thought to be the active ingredients in ginseng. 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	Ranked 17th in sales in 2002; Traditionally used to treat wounds, digestive problems, and infections. Current uses include as a laxative, tonic, and diuretic. Mistakenly thought to disguise the presence of other drugs in drug tests.
Green tea extract	Used for its antioxidative properties, 15th in sales in 2002.
Kava kava	The 25th most widely used medicinal herb in 2002, has psychoactive properties, and is sold as a calmative and antidepressant. A recent report of severe liver toxicity has led to restrictions of its sale in Europe and apparently affected sales in the United States. Some components may alter efficacy/toxicity of therapeutic agents.
Milk thistle extract	Ranked 8th in sales in 2002. Used to treat depression and several liver conditions, including cirrhosis and hepatitis, and to increase breast milk production.
Pulegone	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.
Senna	Laxative with increased use due to the removal of one of the widely used chemical-stimulant type laxatives from the market.
Thujone	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.

* Ranking of sales is from The American Herb Association Vol. 18(3), p. 7, 2002.
(Those entries without ranking are not in the top 36 in sales for 2002.)

Exposure is studied using breath and other biological measures. The CERHR (see page 22) has evaluated the potential reproductive and developmental toxicities of 1-bromopropane and 2-bromopropane, and NTP-CERHR monographs on these chemicals are available.

The NIOSH/CDC is planning a National Exposures at Work Survey that will be conducted in a nationally representative sample of workplaces across all industries, starting with the health services industry. This survey will collect data on chemical, physical, and biological agents to which workers could be exposed, as well as data on exposure controls and health and safety practices. Information from this initiative will be used to educate workers, identify occupational health knowledge gaps, and help target areas where research is likely to reduce workplace illness.

Safe Drinking Water Program

More than 200 million Americans are estimated to use municipally treated drinking water, so the availability of safe drinking water is of enormous importance to public health. Although chlorination is one of the major public health advances of the 20th century, by-products of chlorination or other disinfection processes (disinfection by-products, DBPs) may cause health problems such as cancer. Also, some agents found naturally in water or that contaminate public water systems may pose a threat to public health.

The EPA is responsible for setting water standards for DBPs. To provide scientific data for setting sound standards for water quality, the NTP is collaborating with the EPA on a research program to assess potential risks from human exposure to DBPs. This program includes a systematic, mechanism-based evaluation of DBPs focusing on reproductive toxicity, immunotoxicity, neurotoxicity, and carcinogenicity. The program selects DBPs for study based on their presence in drinking water, occurrence with different disinfection processes, chemical structures, and class: trihalomethanes, haloacetic acids, and haloacetonitriles. Table 4 lists DBPs currently under study by the NTP.

Besides DBPs, many agents occur (1) naturally (e.g., arsenic, aluminum) in water, (2) because of contamination (e.g., methyl tert-butyl ether and other gasoline additives, pesticides, organic tin compounds), or (3) with environmental changes (e.g., cyanobacterial toxins from algal blooms in surface waters). The NTP is designing long-term toxicology and toxicokinetic studies on several of these agents, including aluminum complexes, organic tin compounds, and the two most common cyanobacterial toxins (microcystin-LR and cylindrospermopsin).

Nanoscale Materials

Nanotechnology in recent years has become an increasing focus of U.S. and global research and development efforts. As with many technological advances, new materials are created, and as a result, the potential exists for new and unanticipated human exposures for which the impact on human health is unknown. The NTP is developing a broad-based research program to address potential human health hazards associated with

Table 4 Water Disinfection By-Products under Study

- Bromochloroacetic acid
- Bromodichloroacetic acid
- Bromodichloromethane
- Dibromoacetic acid
- Dibromoacetonitrile
- Dichloroacetic acid
- Sodium bromate
- Sodium chlorate

the manufacture and use of nanoscale materials. This research program will include studies of nanoscale materials that apply existing toxicology testing methods and that also explore the development of novel toxicological methods to assess potential health effects.

Nanoscale materials are a broadly defined set of substances where at least one critical dimension is less than 100 nanometers. Ultrafine particulate matter is a well-known example of ambient nanoscale particles; however, the NTP's research program will initially focus on manufactured nanoscale materials of current or projected commercial importance. Nanoscale materials can in theory be engineered from nearly any chemical substance; semiconductor nanocrystals, organic dendrimers, and carbon fullerenes and carbon nanotubes are a few of the many examples. They are already appearing in commerce as industrial and consumer products and as novel drug delivery formulations.

The intent of the NTP's research program is to evaluate the toxicological properties of major nanoscale materials classes which represent a cross-section of composition, size, surface coatings, and physico-chemical properties, and use these as model systems to investigate fundamental questions concerning if and how nanoscale materials can interact with biological systems. Some of these fundamental questions include: What are the appropriate methods for detection and quantification of nanoscale particles in tissues? How are nanoscale materials absorbed, distributed in the body and taken up by cells? Are there novel toxicological interactions? As part of this research program, studies to evaluate the biological disposition of nanoscale crystalline fluorescent semiconductors ("quantum dots"), long-term toxicology studies of carbon-based nanoscale materials (e.g., single- or multi-walled nanotubes, fullerenes), and phototoxicology studies of representative nanoscale metal oxide particles used in industrial settings and consumer products (e.g. titanium dioxide) are currently being considered (see Phototoxicology, page 11).

DNA-Based Products

DNA-based therapies are being developed to treat a wide range of human diseases. However, by their very nature, they pose a risk of interacting with the host's genes or disrupting normal cellular processes in unexpected, unpredictable, and potentially harmful ways. Examples of DNA-based products include (1) vaccines against viruses and bacteria that have been made from plasmid DNA, (2) synthetic oligonucleotides to modulate gene expression, and (3) viral carriers for gene therapy. Presently, the NTP is collaborating with the FDA and sister NIH institutes to study the safety of DNA-based products. These studies address life-long risks presented by their use and the potentials for reproductive toxicities, for transmission of altered genetic material to subsequent generations, and for DNA-based products to cause autoimmune disease or immune dysfunction.

Endrocrine-Disrupting Agents

Endocrine disruptors are naturally occurring or man-made substances that may mimic or interfere with natural hormones in the body. Endocrine disruptors may turn on, turn off, or change signals that hormones carry and so affect the normal functions of tissues and organs. The NTP is involved in several efforts to strengthen the scientific knowledge within this field.

Endocrine-disrupting chemicals are of interest to the FDA, and through an interagency agreement, the NIEHS/NIH supports toxicology studies being conducted at the NCTR/FDA. Chemicals under study include the plant-based estrogen (phytoestrogen) genistein, the pesticide vinclozolin, the drug ethinyl estradiol, and the industrial chemical nonylphenol. These studies assess effects on reproduction, development of hormone-sensitive organs, and cancer in rodents over several generations, as well as behavioral and immunological effects. The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods is evaluating several methods for use in identifying potential endocrine-disrupting agents (see page 23).

Evolving Strategies

Considering the large number of chemicals in commercial use, the NTP must continually set priorities and develop research strategies to characterize toxicants and identify hazards that make the best use of available resources. Using new testing strategies that provide more or better information can strengthen the scientific knowledge on which regulatory decisions are based. The NTP core agencies are developing and validating new testing methods, and university-based researchers are also participating in these efforts through NIEHS/NIH extramural grants.

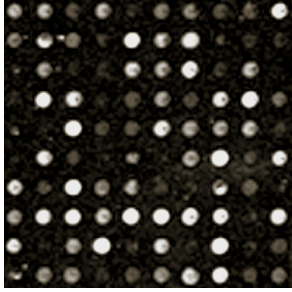
Many testing strategies focus on more rapid screening tests, alternative or complementary *in vivo* tests for rodent bioassays, and less use of two-year rodent studies to determine toxicities. Strategies include molecular screening methods, non-mammalian test species, genetically engineered animal models, genetically engineered *in vitro* cell systems, microchip-based genomic technologies, and computer-based predictive toxicology models. Such techniques can provide insight into the molecular and biological events associated with a chemical's toxic effect, as well as mechanistic information for assessing human risk. They can also help clarify dose-response relationships, make species comparisons, and identify sources of variations among individuals. Below are brief overviews of some current and emerging NTP initiatives to make better use of these new research tools.

Caenorhabditis elegans

The NTP is interested in developing rapid, sensitive, and specific tests for screening environmental agents. A current focus is the nematode *C. elegans*. The NTP is investigating the response of *C. elegans* when exposed to known or suspected toxicants and monitoring the effects of these toxicants on its growth, size, reproduction, and movement. The goal is to determine the possibility of using *C. elegans* as a practical and efficient model in toxicology studies.

NTP Research Databases

A primary goal of the NTP is to evaluate agents of public concern for their potential toxicity or carcinogenicity. Some studies address general toxic effects in laboratory animal species, whereas others focus on specific immune, neurological, reproductive, and developmental effects. Data from general toxicology and carcinogenicity studies are publicly available on the NTP web site (http://ntp-server.niehs.nih.gov). The NTP is expanding public accessibility to its data to include all study types (general toxicology, carcinogenesis, genetic toxicity, and organ systems). The NTP provides access to the data in a common format using web-based applications that allow users to query data, conduct simple statistical manipulations, and export the information. Basic tools are available on the web site to allow users to search for studies



on specific substances and retrieve data for individual animals. The NTP is also developing tools for conducting systematic searches both within and across studies based on which endpoints showed significant changes. This type of search will permit the comparison of effects among individual chemicals or classes of chemicals.

Toxicogenomics

New molecular technologies have brought the NTP into the arena of toxicogenomics, a new scientific field that examines how the entire genetic structure, or genome, is involved in an organism's response to environmental toxicants. Toxicogenomics applies genetic knowledge to environmental medicine by studying the effect of toxicants on gene activity and specific proteins produced by genes. It combines information from studies of genomic-scale messenger RNA profiling (by microarray analysis), cell-wide or tissue-wide protein profiling (proteomics), genetic susceptibility, and computational models. This information helps illustrate the roles of interactions between genes and the environment in disease. This field could revolutionize environmental health, drug safety, and risk assessment.

To centralize activities in toxicogenomics, the NIEHS/NIH established the National Center for Toxicogenomics (NCT) in 2000. Complementary DNA microchip-based technology enables the NCT to assess the genetic impact of toxicants. Microarrays containing genes from common test animals and organisms, including mice, rats, and yeast, are currently in use.

The NCT is evaluating gene arrays against known toxicants and building a database of gene expression information to determine the typical genetic changes or "signature" profiles produced by these toxicants. Identification of such changes in gene expression on a genome-wide basis could provide a global perspective on how an organism responds to a specific stress, drug, or toxicant. As this technology continues to improve, it will help NTP scientists evaluate and compare compounds under study. Such information could define cellular networks of responsive genes, identify target molecules of toxicity, provide future biomarkers and alternative testing procedures, and identify individuals who are sensitive to drugs or environmental agents.

Initial efforts by the NTP include profiling a classic liver toxicant acetaminophen and studying variables that affect gene expression. To facilitate interpretation of these data, the NTP is also collecting gene expression data from animals not exposed to acetaminophen to establish which genes are more variable and which genes are more stable.

Magnetic Resonance Imaging

Traditionally, in NTP toxicology and carcinogenicity studies, lesions are evaluated with conventional optical microscopy of collected tissue samples. Representative samples are collected because examining all the tissue involved is impractical. Because of recent advances in the technology for imaging, magnetic resonance imaging (MRI) of the entire body at microscopic resolution is now possible. The NTP is investigating MRI for imaging laboratory animals. MRI microscopy is three-dimensional, can examine the same specimens at different angles, and measures the volume of tissue and organs. MRI of live animals permits acquisition of imaging data at different times over an animal's lifetime. Because the images are digital, web-based viewing is easier.

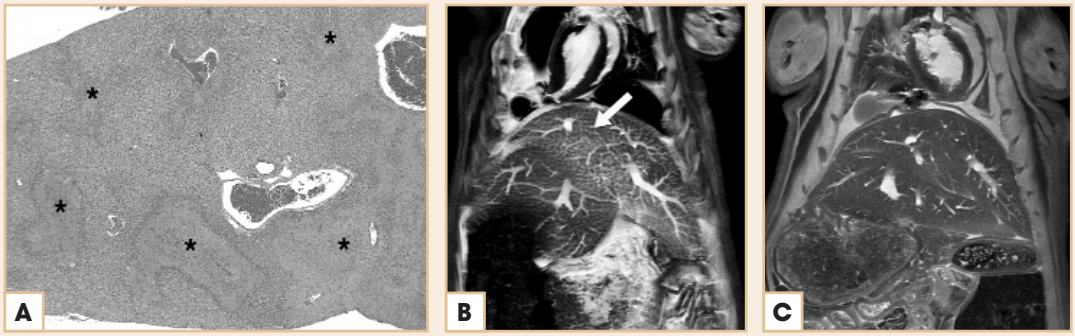
MRI is a noninvasive technique that will permit more complete and thorough examination of tissues and organs from test animals without destroying the samples and may also allow more information to be gathered from NTP studies than before. Anticipated uses include monitoring lesions and examining the morphology and functionality of genetically engineered mice. The NTP also hopes in the future to apply this technology to studies of birth defects. This technology is being applied to NTP microarray studies of acetaminophen-induced hepatotoxicity (Figure 3, see Toxicogenomics, page 17).

Risk Assessment Methodology

Risk assessment involves using facts to determine whether exposures to agents in the environment or workplace are hazardous to the health of individuals or populations, and if so, to what extent. Models based on mathematics and biology are useful for estimating human risk. These models represent physiological and biochemical processes known to occur in laboratory animals and humans. They can provide a scientifically sound basis for evaluating data from studies in animals and then applying that information across species to determine if and how exposure to an agent might cause health effects in humans. The NTP initiatives in assessing human exposures and advances in toxicogenomics should increase the amount of human and animal mechanistic data for developing and improving biologically based models.

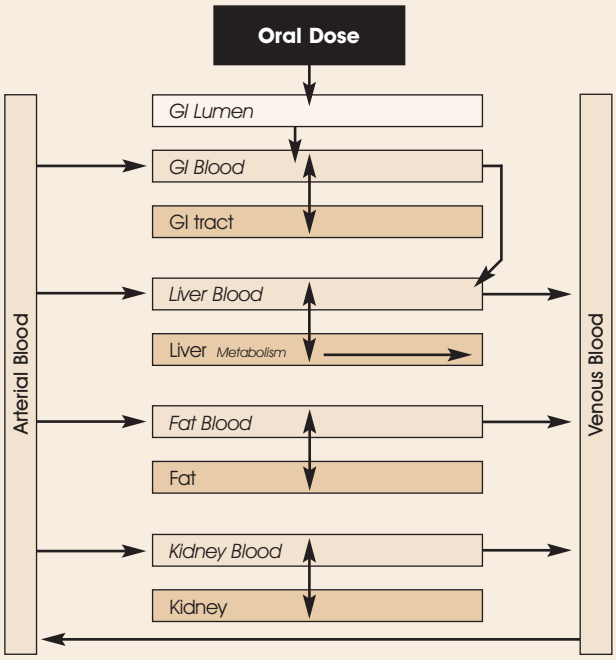
Physiologically based pharmacokinetic (PBPK) models measure the biological processes of absorption, distribution, metabolism, and elimination of an agent in animals or humans (Figure 4). The NIEHS/NIH continues to create and develop PBPK models to evaluate exposure-response relationships for carcinogenicity

Figure 3 Optical and MRI Microscopy of Liver from Acetaminophen-treated Rats



A. Photomicrograph showing small and large patches of necrosis (asterisks) 24 hours after dosing with acetaminophen.
B. MRI of acetaminophen-treated rat showing liver vasculature (white branching structures) and mottled appearance (arrow) of the affected liver.
C. MRI on a vehicle control rat in which the liver parenchyma has a homogeneous signal. White areas represent hepatic blood vessels.

Figure 4 **Physiologically Based Pharmacokinetic Model**



This model represents the uptake, distribution, and metabolism of an environmental agent administered orally.

Table 5 **Physiologically Based Pharmacokinetic Modeling**

Chemical	Route of Exposure
Anthraquinone	Oral in feed
Butadiene	Inhalation
Chromium	Oral in drinking water
Decalin	Inhalation
p-p′-Dichlorodiphenylsulfone	Oral in feed
Isoprene	Inhalation
Melatonin	Endogenous
Mercury	Inhalation
Methyleugenol	Oral by gavage
2-Methylimidazole	Oral by gavage, intravenous
4-Methylimidazole	Oral by gavage, intravenous
Naphthalene	Inhalation
2,3,4,7,8-Pentachlorodibenzofuran	Oral by gavage
Polybrominated diphenyl ethers	Oral by gavage
Polychlorinated biphenyls (209 congeners)	Multiple routes
Primidone	Oral in feed
Propylene glycol mono-*t*-butyl ether	Inhalation
Sodium nitrite	Oral in drinking water
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	Oral by gavage, dermal
Urethane	Oral by gavage, intravenous
Vanadium pentoxide	Inhalation

and developmental and reproductive toxicities (listed in Table 5). PBPK models are now often included in NTP Technical Reports. This information helps regulatory agencies assess how the health effects in experimental animals caused by exposure to environmental agents relate to effects observed in humans. Combining PBPK models with models that measure changes in cells in target tissues under different concentrations of test agents helps define dose-response relationships and determine the likelihood of adverse effects from "low-dose" exposure. These models also help to assess variation among individuals in specific groups (e.g., same or similar age, gender, genetic predisposition, or ethnicity).

Developing biologically based models relies on first developing a simple model and then testing predictions of the model experimentally. As more data become available from studies in cell cultures, animals, and humans, the model is continually adjusted or expanded. For example, the NIEHS/NIH has developed mechanistic models for changes in gene expression of the aryl hydrocarbon receptor resulting from exposure of animals to the carcinogen 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The EPA used this model in its TCDD cancer risk assessment, which serves as the agency's basis for regulating human exposure to this environmental hazard. TCDD is the most potent member of the dioxin class of chemicals. Dioxins occur widely as by-products of chemical processes that involve reactions of chlorine and hydrocarbons (e.g., produced by paper and pulp bleaching or incineration of hospital and municipal wastes) and are contaminants in some pesticides, herbicides, and wood preservatives.

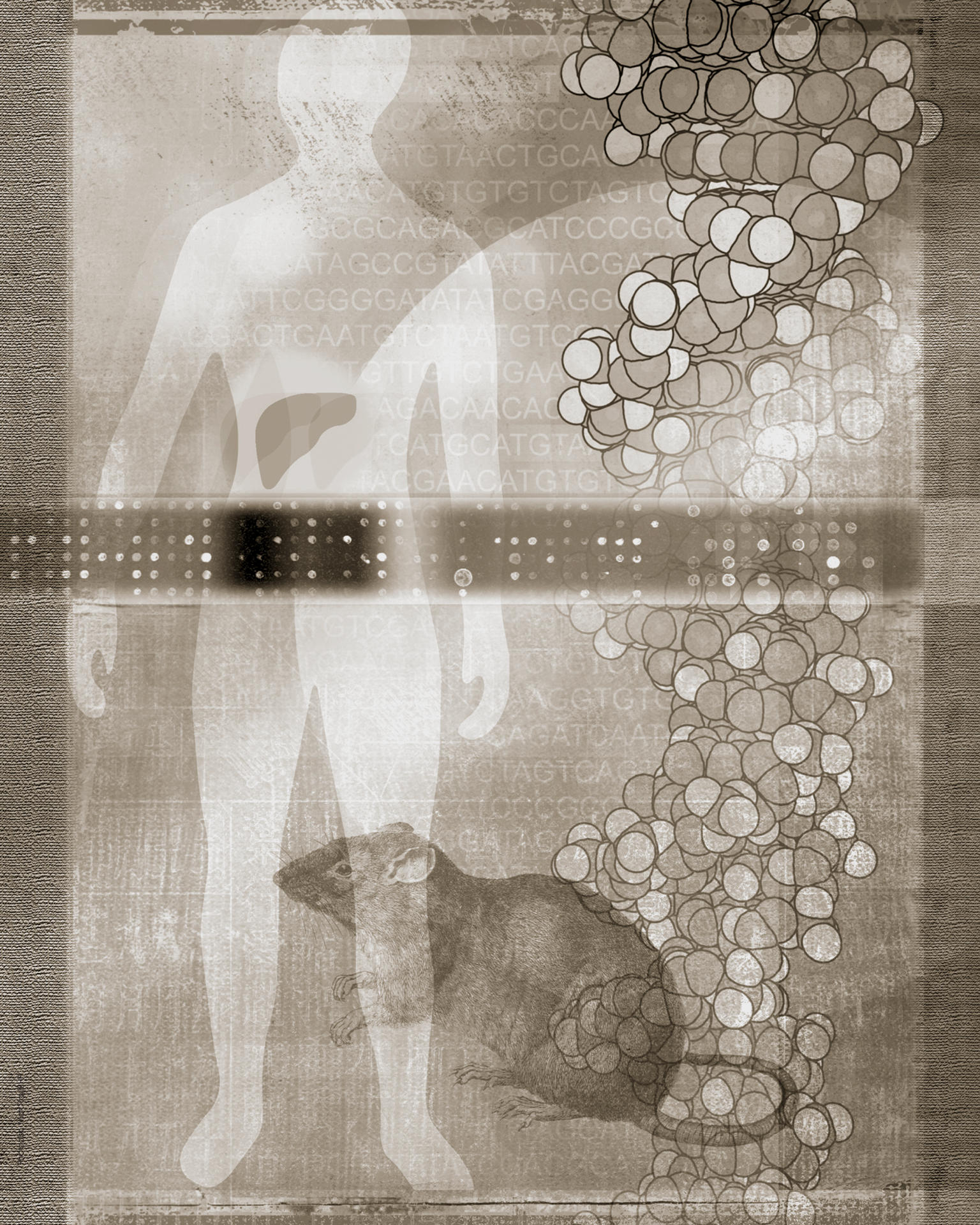
Transgenic Animals

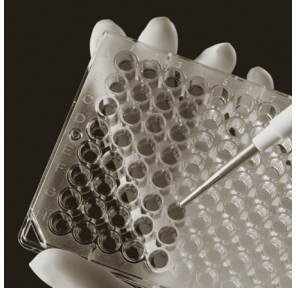
For more than three decades, the NTP has conducted studies in laboratory rodents (bioassays) to identify carcinogens thought to pose risks to human health. Genetically engineered animal models based on new gene technologies are being evaluated as alternatives or complements to rodent bioassays. Genetically altered or transgenic mouse models carry activated oncogenes or inactivated tumor suppressor genes known to be involved in neoplastic (tumor-causing) processes in both humans and rodents. This trait may allow these mice to respond or show the effects of carcinogens more quickly and more reliably than conventional rodent strains. Target or reporter genes also allow direct molecular and cellular analysis of a chemical's effects and can provide additional mechanistic information about its mode of action.

The NTP has sought input from its advisory groups and the public about the usefulness of a number of transgenic rodent models for short-term studies of carcinogenicity [p53(+/–), Tg.AC (v-Ha-ras), and RasH2]. In general, there is support for the p53(+/-) and RasH2 models for evaluating the carcinogenic potential of chemical or physical agents. The NTP will continue to consider transgenic models when designing the testing strategy for substances under study. As understanding increases of the complex signaling pathways turned on or off during carcinogenesis, the NTP will be able to select transgenic animal models that best mimic human tissue processes. This should provide a firmer foundation for applying hazard data from animals to humans. Efforts are also underway to develop transgenic cell lines and to evaluate the usefulness of transgenic fish as alternatives to mouse or culted cell models.

ScanScope–2D Imaging Technology for Pathological Evaluations

The NTP has now acquired a new technology for evaluation of lesions from its studies. ScanScope scans whole histopathology slides at high resolution and compresses the large file using JPEG2000. The resulting image can be viewed over the Internet and one is able to zoom in on any portion of the slide with magnifications similar to what is seen with a microscope using a 10, 20, or 40X objective. The result is that the computer becomes equivalent to a microscope and you can examine any portion of the whole image at microscopic resolution. This technology provides the NTP pathologists a tool to share treatment-induced lesions with colleagues anywhere in the world via the Internet and, as needed, obtain their diagnostic opinions.





Centers

NTP Center for Phototoxicology

The NTP Center for Phototoxicology (NCP), established in 2000, conducts research on how light affects the toxicology and carcinogenicity of substances nominated to the NTP and on the mechanisms that underlie these effects. Current research initiatives are described on page 11 under "Phototoxicology." Research in this area is very important because of the public's increasing exposure to ultraviolet (UV) radiation or sunlight through more frequent use of tanning booths and more leisure time spent in outdoor activities.

The NCP's state-of-the-art laboratory can study the potential toxic or carcinogenic effects of a test substance in combination with UV or visible radiation from several light sources. The NCP also conducts mechanistic studies to learn how these effects might occur. The laboratory can simulate sunlight using 6.5-kilowatt xenon-arc lamps that can mimic terrestrial solar light for most latitudes. Emulating terrestrial light enables researchers to experimentally duplicate human exposure conditions, which is critical for such research. The facility can also perform studies using light from different types of fluorescent tubes, such as those used in fluorescent lamps and suntan-bed lamps.

The NTP Board of Scientific Counselors advises the NCP on its programs and priorities. Substances selected for testing are nominated directly from the FDA and from outside submissions to the NTP. The FDA's Phototoxicology Chemical Selection Working Group prioritizes nominations and forwards them to the NTP for formal consideration in its nomination and selection process. More information about the NCP is available by contacting Dr. Paul C. Howard, Director, NCP (for contact information, see back flap).

Center for the Evaluation of Risks to Human Reproduction

Established in 1998, the NTP's Center for the Evaluation of Risks to Human Reproduction (CERHR) serves as an environmental health resource to the public and to regulatory and health agencies. The NTP Board of Scientific Counselors advises the CERHR on its processes, priorities, and direction. The CERHR web site (http://cerhr.niehs.nih.gov) has information on various environmental exposures and their potential to affect pregnancy and child development, as well as links to other resources.

The CERHR provides scientifically based, uniform assessments of the potential for adverse effects on reproduction and development caused by chemicals to which humans are exposed. It follows a formal, open process for nomination, selection, and review of chemicals; public input is encouraged. The CERHR selects chemicals for review based on several factors, including production volume, extent of human exposures, public concern about the chemical hazard, and published evidence of reproductive or

developmental toxicities. The CERHR carries out these assessments through rigorous evaluations by independent scientific panels in public forums. These evaluations are intended to

- interpret scientific evidence to the public and provide information about the strength of the evidence that a given exposure or circumstance poses a hazard to reproduction or the health and welfare of children;
- provide regulatory agencies with objective and scientifically sound assessments 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 these assessments; and
- identify knowledge gaps to help establish research and testing priorities.

Chemicals reviewed to date by the CERHR expert panels are listed in Table 6.

The NTP has begun a monograph series for the chemicals evaluated by the CERHR. Each monograph includes the NTP Brief, the Expert Panel Report, and public comments on the Expert Panel Report. The brief gives the NTP's interpretation of the potential for the chemical to cause adverse reproductive and/or developmental effects in exposed humans and is based on the Expert Panel Report, public comments on those reports, and any new information that becomes available after the expert panel meeting. NTP-CERHR monographs are transmitted to appropriate federal and state agencies and made available to the public. Expert panel reports and NTP-CERHR monographs, as completed, are posted on CERHR's web site and are available in hard copy and CD-ROM from the CERHR.

The CERHR conducts workshops, as needed, to bring together experts and discuss important topics relative to effects of environmental agents on reproduction and development. The CERHR recently sponsored a workshop to discuss test methods for assessing chemical-induced effects on the thyroid system and how results obtained in laboratory animal studies could best be used to predict effects that might occur in humans. The meeting report is available on the CERHR web site.

The CERHR welcomes nominations of chemicals for review as well as scientists for its registry of experts. Information about the CERHR and the nomination process is available from its web site or by contacting Dr. Michael Shelby, Director, CERHR (for contact information, see back flap).

NTP Interagency Center for the Evaluation of Alternative Toxicological Methods

Toxicity testing is absolutely necessary to assess the hazards and safety of substances in our food, air, and water, in the workplace, and at home. Developing, validating, accepting, and harmonizing new, alternative, and revised toxicological test methods are coordinated throughout the federal government by the Interagency

Coordinating Committee on the Validation of Alternative Methods (ICCVAM). ICCVAM was established in 2000 (ICCVAM Authorization Act of 2000: Public Law 106-545) as a permanent interagency coordinating committee of the NIEHS/NIH under the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). ICCVAM consists of the designated representatives from 15 federal agencies (Table 7). NICEATM provides scientific and operational support for ICCVAM and related activities. The Scientific Advisory Committee on Alternative Toxicological Methods, established in January 2002, provides advice on the activities of ICCVAM and NICEATM.

NICEATM and ICCVAM work together to evaluate the usefulness and limitations of new, revised, and alternative toxicological test methods that may (1) predict human health risks better than currently used methods, (2) save time and money, and (3) refine (cause less pain and distress), reduce, or replace animal use. NICEATM also promotes information sharing and communication among government agencies, industry, the public, and the international community.

ICCVAM has a formal process for nominating and submitting new, revised, and alternative toxicological test methods for evaluation. Once test methods are accepted for evaluation, NICEATM and ICCVAM convene independent scientific peer review panels to assess their usefulness and limitations. Workshops and expert panel meetings are also convened to (1) evaluate how well current safety assessment methods are working, (2) identify areas needing improved or new methods, (3) assess the current validation status of new methods, and (4) recommend appropriate research, development, and validation. These meetings are open to the public and provide an opportunity for public comment. Meeting reports, public comments, and ICCVAM recommendations on the scientific validity and potential acceptability of alternative test methods are forwarded to federal agencies for their consideration. Each agency determines the regulatory acceptability of a method according to its own statutory mandates. ICCVAM also works to achieve international acceptance of test methods that it finds to be scientifically valid for specific uses.

Table 6 Chemicals Reviewed to Date by CERHR Expert Panels

Acrylamide	A neurotoxicant, shown in animal studies to be a carcinogen, germ cell mutagen, and reproductive toxicant; present in starchy foods treated at high temperatures (e.g., French fries and potato chips).
1-Bromopropane	Various industrial uses and is being considered as a replacement for ozone-depleting chemicals, such as hydrochlorofluorocarbons and chlorinated solvents.
2-Bromopropane	No industrial uses in the United States, but is a contaminant in 1-bromopropane.
Ethylene glycol	A high-production-volume chemical used chiefly as an intermediate in the production of polyester compounds and as antifreeze for heating and cooling systems.
Fluoxetine	Widely prescribed antidepressant (Prozac, Sarafem) and recently prescribed to treat premenstrual dysphoric disorder in women of childbearing age; more recently approved by the FDA for use in children 7-17.
Methanol	Commercially important, high-production-volume chemical with potential for occupational, consumer, and environmental exposure.
Propylene glycol	Used as an intermediate in the production of polyester resins, approved for use in food, cosmetics, and pharmaceutical products, and as antifreeze and a deicing agent.
Seven selected phthalates*	Widely used as plasticizers in consumer products such as shower curtains, medical devices, upholstery, raincoats, and soft-squeeze toys.

* 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 Local Lymph Node Assay (LLNA) was the first method evaluated by the ICCVAM process. ICCVAM recommended that the LLNA could be used as a valid substitute for currently accepted guinea pig test methods. Accepted by U.S. agencies in 1999, LLNA was adopted by the international Organisation for Economic Co-operation and Development in 2002. Other test methods evaluated by ICCVAM are listed in Table 8.

ICCVAM has developed a process for establishing performance standards for validated and accepted test methods. These standards can be used to determine the acceptability of other proposed test methods that are based on similar scientific principles and measure or predict the same biological or toxic effect. They (1) identify components that are essential to a proposed similar test method, (2) list the minimum reference chemicals required for assessing the accuracy and reliability of a proposed similar test method, and (3) establish the accuracy and reliability that a similar test method should achieve with the reference chemicals.

More information about NICEATM and ICCVAM, meeting schedules, meeting reports and minutes, and information on nominating alternative toxicological methods are available through the ICCVAM/NICEATM web site (http://iccvam.niehs.nih.gov) or by contacting Dr. William S. Stokes, Director, NICEATM (for contact information, see back flap).

Table 7 ICCVAM

- Agency for Toxic Substances and Disease Registry
- Consumer Product Safety Commission
- Department of Agriculture
- Department of Defense
- Department of Energy
- Department of the Interior
- Department of Transportation
- Environmental Protection Agency
- Food and Drug Administration
- National Cancer Institute/NIH
- National Institute of Environmental Health Sciences/NIH
- National Institutes of Health (NIH)
- National Library of Medicine/NIH
- National Institute for Occupational Safety and Health/Centers for Disease Control and Prevention
- Occupational Safety and Health Administration

Table 8 Test Methods Evaluated by ICCVAM

Test Method	Regulatory Application
Local Lymph Node Assay	Substitute for currently accepted guinea pig test methods for allergic contact dermatitis.
Up-and-Down Procedure	Alternative method for assessing acute oral toxicity; as a replacement for the conventional LD50 test for hazard classification testing.
In vitro (non-animal) cytotoxicity methods	Used to estimate doses for assessing acute systemic toxicity in animals.
In vitro estrogen receptor and transcriptional activation assays	EPA's Endocrine Disruptor Screening Program for identifying potential endocrine-disrupting chemicals.
In vitro androgen receptor and transcriptional activation assays	EPA's Endocrine Disruptor Screening Program for identifying potential endocrine-disrupting chemicals.
Corrositex®, EpiDerm™, EPISKIN™, and Transcutaneous Electrical Resistance Assay	*In vitro* methods to determine dermal corrosivity.
FETAX (Frog Embryo Teratogenesis Assay: *Xenopus*)	*In vitro* method to determine the developmental toxicity of chemicals and mixtures.

RoC

The Report on Carcinogens (RoC) is prepared every two years in response to Section 301 of the Public Health Service Act, as amended. The RoC lists all substances that either are known to be human carcinogens or may reasonably be anticipated to be human carcinogens, and to which a significant number of people living in the United States are exposed. The Secretary, HHS delegated responsibility for preparing the RoC to the NTP, which prepares the report with help from other federal health and regulatory agencies and nongovernmental institutions.

The RoC is an informational, scientific, and public health document that identifies and discusses agents, substances, mixtures, and exposure circumstances that may pose a carcinogenic hazard to human health. It compiles meaningful and useful data on the listed agents, including carcinogenicity, genotoxicity, and biological mechanisms in humans and/or animals, the potential for exposure to them, and federal regulations to limit exposures.

The NTP solicits and encourages broad participation from groups or individuals interested in nominating agents, substances, mixtures, or exposure circumstances for listing in or delisting from the RoC. Anyone may submit a nomination for consideration by the NTP.

The preparation and review process for each RoC takes about three years. Review of the nominations for listing in or delisting from the RoC follows a formal process that includes many phases of scientific peer review and many opportunities for public comment (Figure 5). The review groups evaluate each nomination according to specific RoC criteria. The NTP Director evaluates all review group recommendations, public comments, and other information in developing a recommendation to the Secretary, HHS.

The 10th RoC was released December 11, 2002, and is available on the *Environmental Health Perspectives* web site (http://ehp.niehs.nih.gov; for other contact information, see back flap). The scientific review of nominations to the 11th RoC is completed, and publication is anticipated in 2004 (see list in Table 9).

The NTP periodically holds public meetings to gain input regarding the procedures used for the review of nominations for listing in or delisting from the RoC and on the criteria used for evaluation of the nominations. These meetings provide an opportunity for the public to present their views to the NTP and to comment on the views expressed by others. The NTP held the first such public meeting in October 1999 that resulted in some modifications to the nomination review process. Another public meeting was held in January 2004. Information concerning this recent meeting, including the public comments received, the meeting transcript, and the NTP response to issues identified at this meeting, will be posted on the NTP web site (http://ntp-server.niehs.nih.gov), as available.

More information about the RoC, how to obtain copies of the report, and how to submit a nomination for listing in or delisting from the RoC, is available through the NTP web site or by contacting Dr. C.W. Jameson, Head, RoC (for contact information, see back flap).

Figure 5 **Report on Carcinogens Review Process for the 11th Report**

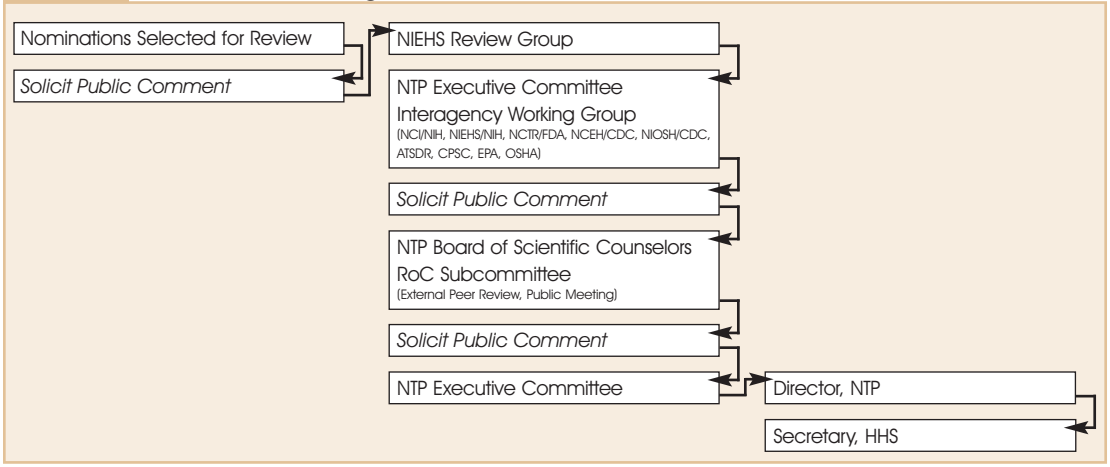


Table 9 **Nominations for Possible Listings in the 11th Report on Carcinogens**

Reviewed in 2002	Reviewed in 2003
• 1-Amino-2,4-dibromoanthraquinone • 2-Amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ) • 2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) • 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) • Cobalt Sulfate • Diethanolamine • Naphthalene • Nitrobenzene • Nitromethane • 4,4'-Thiodianiline	• Diazoaminobenzene • Hepatitis B Virus • Hepatitis C Virus • Human Papillomaviruses, genital mucosal types • Lead and Lead Compounds • Neutrons • X-Radiation and Gamma (γ)-Radiation

Outreach

Open communication with federal and state agencies, industry, academia, and the public is crucial for the success of NTP activities. Partnerships with sister federal agencies are ongoing, and the NTP continues to collaborate with the private sector. NTP conferences and workshops give researchers, regulators, policy makers, and the public the chance to examine issues together, exchange information, and reach agreement on future directions for toxicology and risk assessment.

The NTP is interested in input from the public and all interested parties on its programs and priorities. Nominations, inquiries, and comments are welcome at any time. The NTP Liaison and Scientific Review Office collects input, represents the program through exhibits at national and international meetings, publishes the quarterly newsletter *NTP Update,* and oversees the distribution of information about programs, workshops, initiatives, and other projects. In addition, this office manages scientific peer review for the NTP and organizes workshops on scientific and public health topics. General inquiries and requests for information can be directed to this office (for contact information, see back flap).

The NTP web site (http://ntp-server.niehs.nih.gov) offers access to information about the NTP, with links that detail and highlight ongoing and future initiatives and the NTP centers. The NTP distributes testing and research results, program plans, and other publications through mailings, *Federal Register* announcements, and the NTP web site. Also, individuals can subscribe free of charge to the NTP listserv by registering online through the web site or by sending e-mail to ntpmail-request@list.niehs.nih.gov with "subscribe" as the message. The NTP list-serv notifies subscribers by e-mail about the release of new NTP publications and about upcoming events, such as advisory committee meetings, peer reviews, expert panel meetings, and workshops.

The Central Data Management (CDM) oversees distribution (on request) of specific chemical study information and NTP documents, including the NTP Annual Plan, NTP study status reports, copies of draft NTP Technical Reports, background documents for chemicals nominated to the NTP for study, and minutes from meetings of the NTP Board of Scientific Counselors and its subcommittees. To request any of these documents, contact the CDM (for contact information, see back flap).

The *Environmental Health Perspectives* (EHP) web site (http://ehp.niehs.nih.gov) provides searchable access and printed copies of NTP publications, including the RoC, NTP Technical Reports, and NTP Toxicity Reports. EHP subscription packages include access to NTP publications, EHP journals, and the Rodent Historical Controls Database. For more information, contact EHP (for contact information, see back flap).

Contact Information

Primary Contact:

NTP Liaison and Scientific Review Office
P.O. Box 12233, MD A3-01
Research Triangle Park, NC 27709
Telephone: (919) 541-0530
E-mail: liaison@starbase.niehs.nih.gov

Program Contacts:

NTP Center for the Evaluation of Risks to Human Reproduction
P.O. Box 12233, MD EC-32
Research Triangle Park, NC 27709
Telephone: (919) 541-3455
E-mail: cerhr@niehs.nih.gov
Web site: http://cerhr.niehs.nih.gov

NTP Center for Phototoxicology
NCTR/FDA, HFT-110
3900 NCTR Road
Jefferson, AR 72079
Telephone: (870) 543-7672
E-mail: phoward@nctr.fda.gov
Web site: http://www.fda.gov/nctr/science/phototox.htm

NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
P.O. Box 12233, MD EC-17
Research Triangle Park, NC 27709
Telephone: (919) 541-2384
E-mail: niceatm@niehs.nih.gov
Web site: http://iccvam.niehs.nih.gov

Office of Chemical Nomination and Selection
P.O. Box 12233, MD A3-01
Research Triangle Park, NC 27709
Telephone: (919) 541-5710
E-mail: ntpnomin@niehs.nih.gov

Report on Carcinogens
P.O. Box 12233, MD EC-14
Research Triangle Park, NC 27709
Telephone: (919) 541-4096
E-mail: jameson@niehs.nih.gov

Central Data Management
P.O. Box 12233, MD EC-03
Research Triangle Park, NC 27709
Telephone: (919) 541-3419
E-mail: cdm@niehs.nih.gov

Environmental Health Perspectives
1001 Winstead Drive
Cary, NC 27513
Telephone: (866) 541-3841
E-mail: ehponline@niehs.nih.gov
Web site: http://ehp.niehs.nih.gov

National Toxicology Program

NTP

U.S. Department of Health and Human Services

headquartered at the
National Institutes of Health
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC 27709
Web site: http://ntp-server.niehs.nih.gov

U.S. Department of
Health and Human Services
Public Health Service
National Toxicology Program

Printed in 2004 on recycled paper