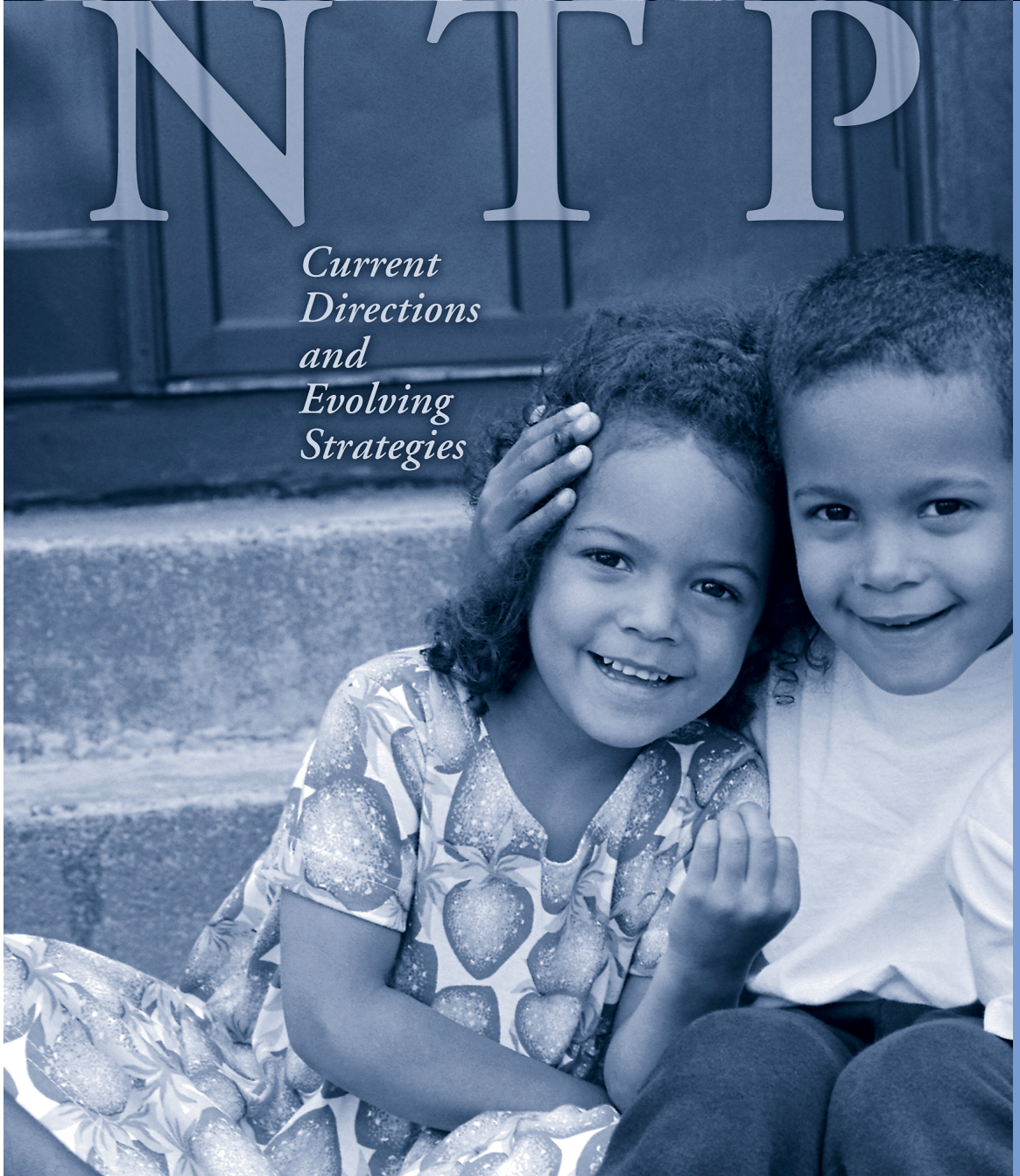
2001

NTP

Current Directions and Evolving Strategies



National Toxicology Program

NTP

Current Directions and Evolving Strategies

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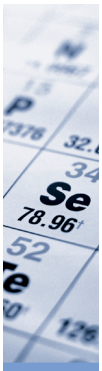
U.S. Department of
Health and Human Services
Public Health Service
National Toxicology Program

Overview

Mission and Goals

More than 80,000 chemicals are registered for use in commerce in the United States, and an estimated 2,000 new ones are introduced annually for use in everyday items such as foods, 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. Although relatively few chemicals are thought to pose a significant risk to human health, safeguarding public health depends on identifying the effects of these chemicals and the levels of exposure at which they may become hazardous to humans.

The National Toxicology Program (NTP) was established by the Department of Health and Human Services (DHHS) in 1978 to coordinate toxicological testing programs within the Department; strengthen the science base in toxicology; develop and validate improved testing methods; and provide information about potentially toxic chemicals to health regulatory and research agencies, the 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 development and the application of technology.

Focusing on the Future

The NTP provides information that improves the nation's ability to evaluate human health effects from chemical and physical exposures. The NTP maintains a number of complex interrelated research and testing programs that provide unique information and knowledge needed by health, regulatory, and research agencies to protect public health. All of these activities are executed in the public arena, including communication with all stakeholders. The NTP has always drawn strength and direction from the commitment of its scientists to open information exchange, adherence to

impartiality, and rigorous scientific peer review. This will remain a central priority of the program now and in the years to come.

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. In 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. Currently the NTP is focusing on 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 addressing potential safety issues associated with herbal medicines or DNA-based therapies. In general, these initiatives are broad-based and include various health-related end points.

The NTP continues to work to develop and validate alternative testing methods that will improve the identification of chemical hazards using fewer animals. This effort includes development of more efficient, mechanism-based testing strategies such as transgenic models for toxicology testing and implementation of microchip array-based technologies for genomics. Future initiatives in mechanism-based toxicology research will involve the integration of information from traditional and gene-expression-based studies to guide the development of new testing strategies for identification and study of environmental toxicants. 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.

The NTP also evaluates the potential for human exposures to result in 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 competence of the immune system and functioning of the nervous system from exposures occurring during fetal development and early life. End points evaluated in reproductive studies are being refined to address emerging knowledge concerning the subtle effects of low doses of endocrine-active chemicals.

The NTP continues to expand activities designed to place research and testing results from animals into a more relevant human health perspective. This encompasses such efforts as human exposure assessment, toxicokinetics, mechanism-based pharmacokinetic modeling, and the interpretation of results in molecular epidemiology for use in human hazard identification (e.g., Report on Carcinogens and NTP 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. 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.

Good Science for Good Decisions

Dr. Kenneth Olden, Director NTP

Role in Shaping Public Health Policy

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 science 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 concerning the appropriate uses of these data to regulatory agencies and other groups involved in health-related research. The NTP also plays an important role in fostering interagency collaborations in research and exposure assessment, providing regulatory agencies information about alternative methods for use in toxicity screening, and exploring new technologies that can be used to strengthen the knowledge base of how chemicals cause disease.

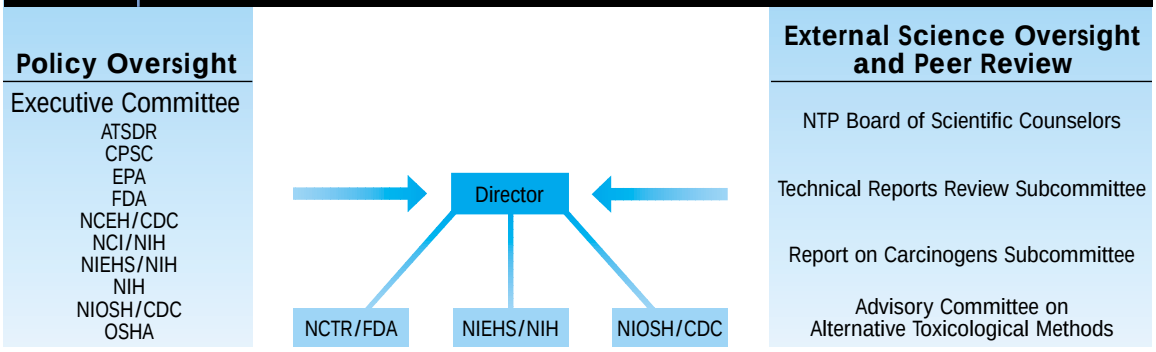
Organizational Structure and Oversight

Three agencies form the core of the NTP: 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) (Figure 1). Each voluntarily provides resources in support of NTP research, testing, centers, and outreach. The NTP is administratively located at the NIEHS/NIH. The Director of the NIEHS/NIH, Dr. Kenneth Olden, serves as Director of the NTP and reports to the Secretary, DHHS. The National Cancer Institute of the National Institutes of Health (NCI/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 of Federal research and regulatory agencies.

External advisory groups provide review and advice to the NTP (Figure 1). The NTP Board of Scientific Counselors ("the Board"), its subcommittees, and the Advisory Committee on Alternative Toxicological Methods (ACATM) assure regular scientific and public peer review and input to the NTP about its activities and priorities. Members of the Board and ACATM are scientists from the public and private sectors selected by the Secretary, DHHS. These bodies each meet once or twice annually, and all meetings are open to the public.

The NTP Board of Scientific Counselors provides primary scientific oversight to the Director regarding the NTP and the NTP Center for the Evaluation of Risks to Human Reproduction and evaluates the scientific merit

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.

of intramural and collaborative programs of the NTP. The Technical Reports Review Subcommittee of the Board provides peer review of NTP long-term toxicology and carcinogenesis studies and 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.

ACATM specifically advises the NTP on the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods and the Interagency Coordinating Committee on the Validation of Alternative Methods on their priorities, opportunities for alternative test methods, development of more efficient processes for determining the scientific validity and acceptability of new test methods, and the fostering of partnerships with stakeholders.

Toxicology and Carcinogenesis Evaluation Process

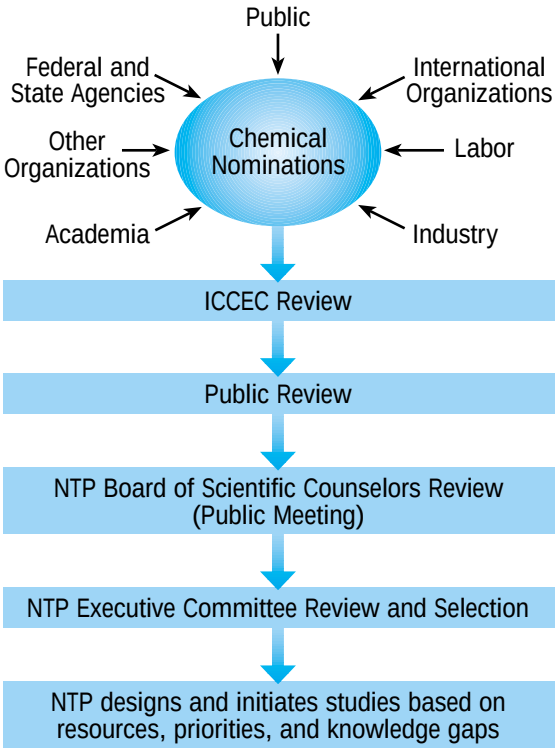
The NTP has a broad mandate to provide toxicological characterizations for chemicals and agents of public health concern. The NTP continually solicits and reviews nominations for toxicology studies; specific categories are given in Table 1. The nomination process is open to all interested individuals 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 Nominations and Selection, NIEHS/NTP, P.O. Box 12233, MD B3-10, Research Triangle Park, NC 27709; ntpnomin@niehs.nih.gov.

Nominations undergo several levels of review before agents are selected for study and toxicological studies are designed and implemented (Figure 2). Representatives from Federal agencies on the Interagency Committee for Chemical Evaluation and Coordination (ICCEC) and the NTP Board of Scientific Counselors, an external scientific advisory body, all participate. In addition, the NTP solicits and considers public comment on nominations throughout the process. At the final step

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 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

Figure 2 **NTP Chemical Nomination and Selection Process**



of this formal process, the NTP Executive Committee reviews and evaluates public comments and the testing recommendations and makes its own recommendations to the NTP: to test, to not test at this time, or to defer testing until additional information is received and considered. This multi-step process helps ensure that the NTP's testing program addresses toxicological concerns pertinent to all areas of public health and that there is balance among the types of substances being evaluated.

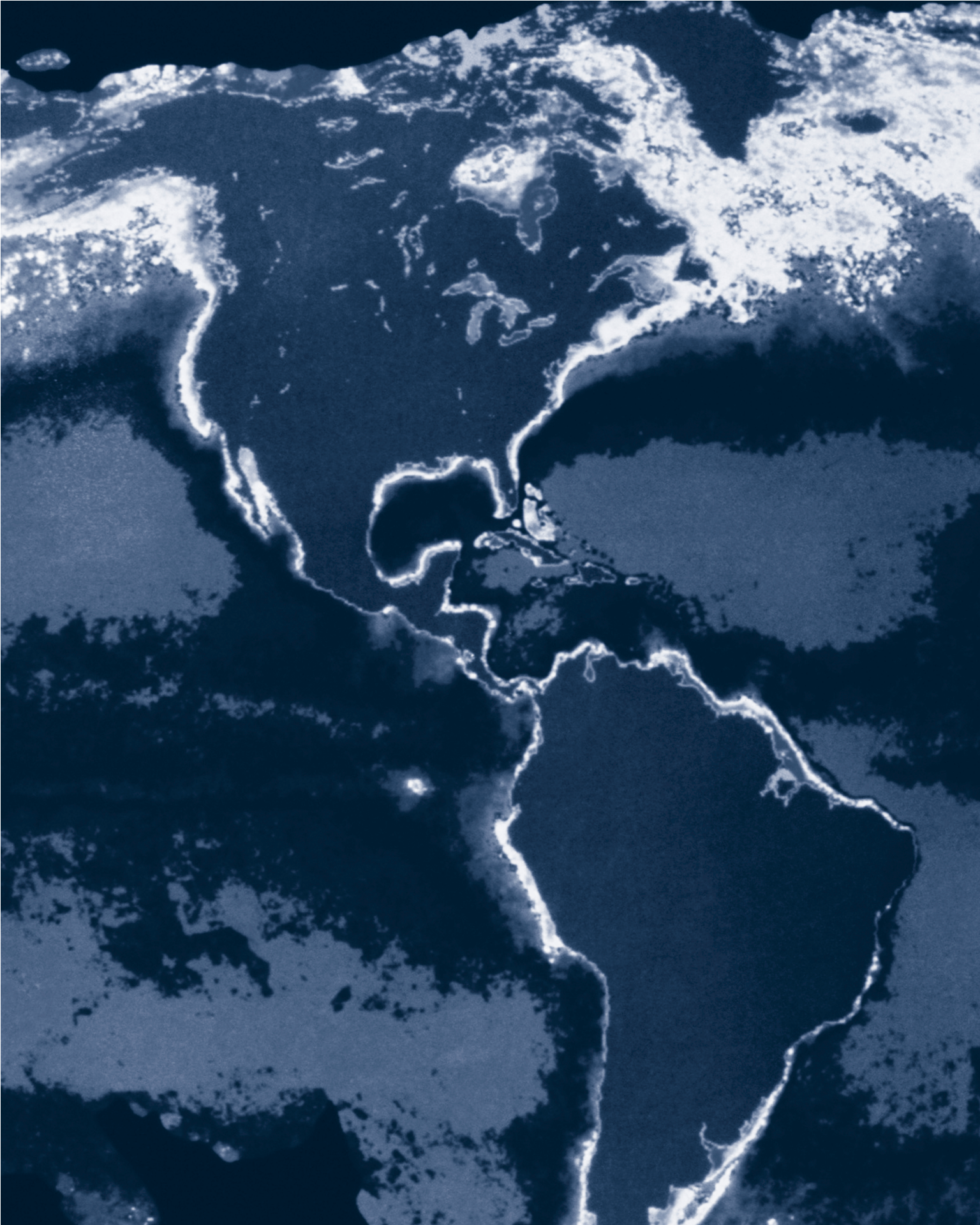
The Executive Committee's recommendation that a substance be selected for study does not automatically commit the NTP to its evaluation. The NTP strives to balance the selection of chemical sources (e.g., occupational, environmental, food additives, agricultural chemicals, and pharmaceuticals), and studies are initiated as time and resources permit. When selecting chemicals for study, the NTP operates under the principle that industry will evaluate chemicals or other agents for health and environmental impacts as mandated by Congress under legislative authorities. At any time during the selection process, a chemical or study may be withdrawn if suitable data become available, higher priority studies are identified, or a study proves impractical.

Substances may be studied for a variety of health-related effects, including but not limited to reproductive and developmental toxicity, genotoxicity, immunotoxicity, neurotoxicity, metabolism, disposition, and carcinogenicity. Rodent models are generally used for NTP studies. Pre-chronic, short-term studies generally are performed for two or thirteen weeks. Chronic, long-term bioassays last up to two years. Each agent studied is assigned an NIEHS/NIH study scientist who designs a comprehensive testing strategy (design, methods, hypothesis, etc.). A project review committee evaluates the testing strategy and proposes a vehicle for execution (grant, contract, etc.).

The results of short-term rodent toxicology studies are published as a Toxicology Study Report. Longer-term studies, generally two-year rodent studies, are published as NTP Technical Reports and may also be published in peer-reviewed scientific journals. Both types of studies undergo peer review prior to publication. The NTP Technical Reports Review Subcommittee (Figure 1) evaluates the Technical Reports from carcinogenicity and toxicity studies in open public meetings. Candidates for peer review in 2001 are listed in Table 2.

Table 2 Candidate Chemicals for Peer Review in 2001

May 2001	October 2001
Acrylonitrile	2,4-Hexadienal
Citral	Riddelliine
Methacrylonitrile	Urethane + Ethanol
o-Nitrotoluene	Vanadium pentoxide
p-Nitrotoluene	



II Current Directions

The NTP conducts research on a broad range of high priority agents and issues of public health concern. The following are brief overviews of some current NTP initiatives.

Safe Drinking Water Program

It is estimated that more than 200 million Americans use municipally treated drinking water, so the availability of safe drinking water is of enormous public health significance. Although chlorination is considered one of the major public health advances of the twentieth century, chemical disinfection by-products (DBPs) of the chlorination or other disinfection process may cause health problems such as cancer. In addition, there are agents found naturally in water or that are present by contamination of public water systems that pose a threat to public health.

To provide scientific data for setting sound water quality standards, 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, toxicological evaluation of DBPs focusing on reproductive toxicity, immunotoxicity, neurotoxicity, and carcinogenicity. Selection of DBPs for study is based on their presence in drinking water, occurrence with different disinfection processes, chemical structures, and representation of the different DPB families: trihalomethanes, haloacetic acids, and haloacetonitriles. DBPs under study by the NTP are listed in Table 3.

Besides DBPs, a complex array of agents occur naturally (e.g., arsenic, aluminum), as a result of contamination (e.g., methyl tertiary butyl ether, pesticides, organotins), or with environmental changes (e.g., algal blooms resulting in mycotoxins in surface waters). Several of these have been nominated to the NTP for evaluation, including aluminum complexes, organotins, and the two most common blue-green algal toxins, microcystin-LR and cylindrospermopsin.

Phototoxicology

A new phototoxicology research and testing laboratory, a joint endeavor between the NIEHS/NIH and the FDA, has been established: the NTP Center for Phototoxicology. Currently a study is underway to test whether creams containing alpha-hydroxy acids (glycolic acid) or beta-hydroxy acid (salicylic acid) affect the carcinogenicity of ultraviolet (UV)-containing light on SKH-1 mouse skin.These chemicals are found in over-the-counter cosmetics as dermatological

chemoexfoliants, and the impact of their continuous use for risk of solar light (e.g., UV)-induced skin cancer is not known. Protocols are in development to study possible phototoxic effects of products derived from the aloe vera plant.

Endocrine-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, shut off, or modify signals that hormones carry and thus affect the normal functions of tissues and organs. The NTP is involved in several efforts that help to strengthen the science base within this field.

The NIEHS/NIH and the National Center for Environmental Health of the Centers for Disease Control and Prevention are collaborating on a pilot project to quantify approximately 70 chemicals found in human blood or urine that are considered endocrine-disrupting agents, including phthalates and phytoestrogens. The biological samples are collected under the National Health and Nutrition Examination Survey, which includes men and women from a range of age, socioeconomic, and ethnic groups. These data will provide estimates of human exposures to endocrine-disrupting agents within the U.S. population and will help identify agents of public health concern. This study complements the recently completed scientific peer review by the NTP Center for the Evaluation of Risks to Human Reproduction of the potential reproductive and developmental toxicity of phthalate esters.

Table 3 **Water Disinfection By-Products under Study**

Bromochloroacetic acid	Dibromoacetonitrile
Bromodichloroacetic acid	Dibromochloroacetic acid
Bromodichloromethane	Dichloroacetic acid
Chloramine	MX (a furanone)
Chloroform	Sodium bromate
Dibromoacetic acid	Sodium chlorate
	Sodium chlorite

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 phytoestrogen genistein, the pesticides methoxychlor and vinclozolin, and the plastic additive nonylphenol. These studies assess effects on reproduction, development of hormone-sensitive organs, and cancer end points in rodents over multiple generations. Behavioral and immunological end points are also being evaluated.

As required by the 1996 Food Quality Protection Act, the EPA is in the process of choosing appropriate assays to screen endocrine-active agents and develop standardized, validated protocols for those assays. One concern is the adequacy of current guidelines for assessing the reproductive and developmental toxicities of chemicals at environmental levels. On behalf of the EPA, the NTP organized a scientific peer review to evaluate reported effects and dose-response relationships for endocrine-disrupting chemicals studied using dosing paradigms lower than those normally recommended under the EPA's standard toxicity testing guidelines. The panel examined data from major selected studies (excluding studies on dioxin and dioxin-like compounds), discerning the presence or absence of low-dose effects in laboratory animals that could be relevant for human health assessments. The panel also evaluated the dose-response curve for endocrine-active substances in the low-dose region. The report from this peer-review panel is anticipated in 2001.

The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods is involved in preparing background documents for the EPA on current *in vitro* methods for assessing androgenic and estrogenic activities of chemicals. A peer review of these methods is tentatively planned for 2002.

Herbal Medicines

Medicinal herbs are among our oldest medicines, and their increasing use in recent years is evidence of a public interest in alternatives to conventional medicine. Approximately 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. However, although approximately 1,500 botanicals are sold as dietary supplements or ethnic traditional medicines, herbal formulations are not subjected to FDA pre-market toxicity testing to ensure their safety or efficacy.

The NTP is planning or conducting research (Table 4) on several medicinal herbs and compounds found in herbs that focus on carcinogenicity, reproductive toxicity, neurotoxicity, immunotoxicity, and effects associated with acute exposures to high doses and chronic exposures to low doses.

DNA-Based Products

Although DNA-based therapies are being developed for the treatment of a wide range of human diseases, by their very nature they pose a risk of interacting with the host genome or disrupting normal cellular processes in unexpected and unpredictable ways with potentially adverse consequences. Examples of DNA-based products 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. Presently the NTP is collaborating with the FDA to study the safety of DNA-based products and address life-long risks presented by their use, potential for reproductive toxicities, transmission of altered genetic material to subsequent generations, and the potential for DNA-based products to cause autoimmune disease or immune dysfunction.

Occupational Exposures

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

Asphalt fumes generated during road paving have been linked to acute irritation of mucous membranes and skin, but to date no cancer risk has been established. Using a system designed to produce asphalt fumes similar to those found in the field, NIOSH/CDC has developed methods for characterizing these fumes and for monitoring asphalt fume exposure in inhalation toxicity studies. Laboratory inhalation studies are underway to evaluate the effects of exposure to asphalt fumes in cells and animals.

An industry consortium has petitioned the EPA to list 1-bromopropane as an alternative for ozone-depleting

Table 4 Herbs and Herbal Ingredients under Study by the NTP

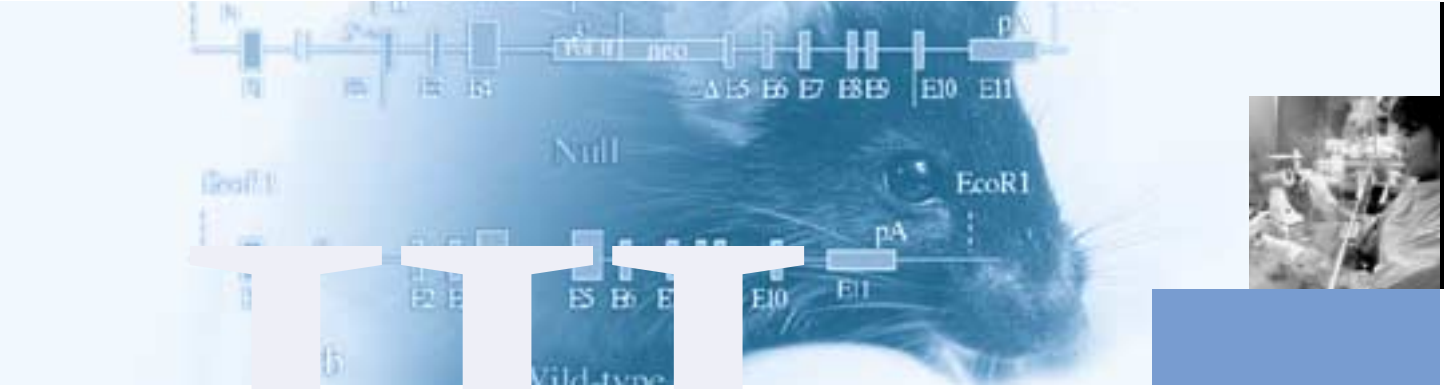
Aloe vera gel	Seventh most widely used herb; used as both a dietary supplement and component of cosmetics.
Black walnut extract	Found in hair dye formulations and walnut oil stain. Juglone is a major constituent.
Echinacea purpurea extract	Most commonly used medicinal herb in the United States.
Ginkgo biloba extract	Among the five or six most frequently used medicinal herbs.
Ginseng and ginsenosides	Fourth most widely used medicinal herb. Gensenosides are thought to be the active ingredients. Ginseng has been associated with various adverse health effects.
Goldenseal	Second or third most popular medicinal herb used in this country.
Kava kava	Reported to be the fifth most widely used medicinal herb. Has psychoactive properties. Sold as a calmative and antidepressant.
Milk thistle extract	Used to treat depression and several liver conditions and to increase breast milk production.
Pulegone	A toxic compound found in pennyroyal.
Thujone	A toxic compound of wormwood.

solvents. If this occurs, there is the potential for a vast increase in the exposure of workers and the public to this compound. To obtain information on exposures to this chemical, NIOSH/CDC is conducting an industry-wide exposure assessment. The target population is a variety of industrial sectors: chemical, aerosol, and adhesive manufacturers; adhesive users; and the metal degreasing and electronics industries. Study sites will be selected on the basis of quantity and manner of 1-bromopropane use, number of workers exposed, type of manufacturing process, and representativeness of the industry. Exposure will be characterized using inhalation, exhaled breath, and biological measures. Following completion of this survey and identification of the major sites for occupational exposure to 1-bromopropane, a multifaceted health and exposure evaluation of workers at those sites will take place.

NIOSH/CDC is planning to conduct a national, cross-sectional, on-site survey of establishments and workers. The survey will include all industry sectors covered by OSHA and the Mine Safety and Health Administration and will gather nationally representative 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.

Children's Health

The NTP continues to be a leader in issues related to children's health through research and the NTP Center for the Evaluation of Risks to Human Reproduction. The NTP has ongoing efforts to evaluate effects of various agents on developing immune and nervous systems through laboratory studies of pesticides, water DBPs, and endocrine-disrupting agents. The Program is expanding these efforts establishing study protocols where animals will be dosed routinely in perinatal exposure scenarios to examine developmental immunotoxicology, neurotoxicology, and reproductive end points. Toxicokinetic data collected from dams, fetuses, and neonates will be used to develop physiologically based pharmacokinetic models of risks to humans during perinatal developmental periods from environmental toxicants.



III Evolving Strategies

Considering the large number of chemicals in commercial use, the NTP continually must set priorities and develop strategies that best use its available resources. Implementing new strategies that provide additional or more accurate information can strengthen the science base on which regulatory matters are decided. Development and validation of a wide range of testing methods are ongoing at the NTP's core agencies, and in addition, university-based researchers are involved through NIEHS/NIH extramural grants.

Many testing strategies focus on providing more rapid screening, developing alternative or complementary *in vivo* tests to use with rodent bioassays, and decreasing dependence on two-year rodent bioassays for determining toxicities. Targeted systems include molecular screening methods, non-mammalian test species, transgenic animal models, genetically engineered *in vitro* cell systems, microchip array technology, and computer-based predictive toxicology models. Such strategies can provide insight into the molecular and biological events associated with a chemical's toxic effect and provide mechanistic information that can be used to assess human risk. These models can be used to clarify dose-response relationships, make species comparisons, and identify sources of inter-individual variability. The following are brief overviews of some current emerging NTP strategies.

Toxicogenomics

With the advent of novel molecular technologies, the NTP is moving into the arena of toxicogenomics, a new scientific field that examines how the entire genome is involved in biological responses of organisms exposed 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 in response to these toxicants. It combines information from studies of genomic-scale mRNA profiling (by microarray analysis), cell-wide or tissue-wide protein profiling (proteomics), genetic susceptibility, and computational models to illustrate the roles of gene-environment interactions in disease. This field could have a revolutionary impact on environmental health, drug safety, and risk assessment.

In an effort toward centralizing activities in toxicogenomics, the NIEHS/NIH has established the National Center for Toxicogenomics (NCT). The NCT will coordinate toxicogenomic research, create a public database of gene responses to specific toxicants and the proteins synthesized by those genes, and develop computer

software to manage the growing toxicogenomics database. The NCT will accomplish its goals by supporting scientists at academic and other research institutions and in-house research and collaborations with public agencies, private healthcare, and biotechnology companies.

cDNA microarray technology is a research tool that will enable the NCT to assess the genetic impact of toxicants. The NIEHS/NIH has developed a human cDNA chip, ToxChip, that contains clones of about 2,000 human genes and a human Discovery Chip containing 12,000 clones. Microarrays are currently in use at the NIEHS/NIH from common test animals and organisms including mice, rats, and yeast. At NIOSH/CDC a hepatic microarray has been compiled.

The NIEHS/NIH and NIOSH/CDC are evaluating their arrays against known toxicants and building a database of expression information in order to determine the typical genetic changes or "signature" profiles they produce. 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 be used and improved, it will assist NTP scientists in evaluating and comparing compounds under study. Such information could define cellular networks of response genes, identify target molecules of toxicity, provide future biomarkers and alternative testing procedures, and identify individuals who are sensitive to drugs or environmental agents.

Transgenic Animals

For more than three decades, the conventional rodent bioassay has been used to identify carcinogens thought to pose risks to human health. With the advent of genomic technologies, transgenic models are being evaluated as alternative or complementary to the rodent bioassay. Genetically altered or transgenic mouse models carry activated oncogenes or inactivated tumor suppressor genes known to be involved in neoplastic processes in both humans and rodents. This trait may allow them to respond to carcinogens more quickly than conventional rodent strains. In addition, the neoplastic effects of carcinogenic agents can be observed in the transgenic models within a time frame in which few, if any, spontaneous tumors would arise. The use of target or reporter genes also allows for direct molecular and cellular analysis of a chemical's effects and can provide additional mechanistic information about its mode of action.

The NTP is evaluating the usefulness of a number of transgenic rodent models for studies of carcinogenesis [p16INK4a, p53def (p53 +/–), Tg.AC (v-Ha-ras), and Tg.NK (MMTV/c-Neu)] and mutagenesis [Tg.LAC1/C57Bl6 (Big Blue)]. The p53def and Tg.AC models appear to have the capacity to identify a selected group of known human carcinogens. The p53def has demonstrated preferential identification of genotoxic/mutagenic carcinogens, and the Tg.AC has responded to both genotoxic and nongenotoxic carcinogens. As the NTP's understanding of the complex signaling pathways activated or repressed during carcinogenesis progresses, the Program will be able to select transgenic animal models that best mimic human tissue processes, providing a firmer foundation for cross-species extrapolations of hazards. Efforts are also underway to develop transgenic cell strains and to evaluate the usefulness of transgenic fish as alternate models to mice or cultured cells.

Risk Assessment Methodology

Risk assessment involves synthesis of large amounts of diverse data to decide the plausibility and magnitude of hazards posed by environmental agents. Physiologically based pharmacokinetic (PBPK) models are mathematical models that quantify processes of absorption, distribution, metabolism, and elimination of an agent resulting from exposure in animals or humans. The development of these PBPK models, in combination with physiologically based pharmacodynamic models, is an iterative process

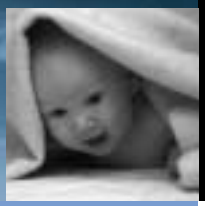
that builds on data related to the biological behavior of the agent in tissue samples, animal models, or humans and on the rapidly expanding knowledge of the physiological, cellular, and molecular events contributing to various disease processes. They may be used to define dose-response relationships, clarify these relationships in the "low-dose" region, make species comparisons, assess inter-individual variability, and create science-based models for specific subpopulations (e.g., age, gender, genetic predisposition, ethnicity). NTP initiatives in human exposure assessment and new toxicogenomic technologies should provide human and animal mechanistic data to develop and improve these models.

PBPK models have been developed or are under development at the NIEHS/NIH to assess carcinogenicity relationships between exposure, target tissue dosimetry, and tissue response. As such, they can provide a mechanistic basis for dose-response relationships for carcinogenicities and developmental and reproductive toxicities (Table 5). Inclusion of PBPK models in the NTP Technical Reports is becoming routine, and this information should aid regulatory agencies to extrapolate data across species.

As toxicogenomic data related to gene expression, protein levels, receptor binding and interaction, and cellular protein changes become available, biochemical models (more complex than absorption, distribution, metabolism, and elimination models) are being developed. Such mechanistic models have characterized Ah receptor-dependent transcriptional activation of dioxin-responsive genes, enzyme induction in 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin-treated rats, and the physiological and biochemical processes involved in renal accumulation of α2μ-globulin and in the deposition of α2μ-globulin-binding ligands in male rats.

Table 5 Physiologically Based Toxicokinetic Modeling

Chemical	Route of Exposure
Anthraquinone	Oral in feed
Butadiene	Inhalation
p-p´-Dichlorodiphenylsulfone	Oral in feed
Isoprene	Inhalation
Melatonin	Endogenous
Mercury (pregnant rat)	Inhalation
Methyleugenol	Oral by gavage
Naphthalene	Inhalation
Polychlorinated biphenyls (209 congeners)	Multiple routes
Primidone	Oral in feed
Sodium nitrite	Oral in drinking water
2,3,7,8-Tetrachloro-dibenzo-*p*-dioxin	Oral and dermal



IV Centers

NTP Center for the Evaluation of Risks to Human Reproduction

The NTP 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 for scientifically based, uniform assessments of the potential for adverse effects on reproduction and development caused by agents to which humans are exposed. The Center was established in 1998. Its assessments of individual chemicals are carried out through rigorous evaluations by independent, scientific panels and are intended to

- interpret for and provide information to the public about the strength of the scientific evidence that a given exposure or exposure 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.

The NTP Board of Scientific Counselors advises the Center on its processes, priorities, and direction. The Center follows a formal, open process for nomination, selection, and review of chemicals; public input is encouraged. Chemicals for review are selected based on the evaluation of several factors, including production volume, extent of human exposures, public concern about the chemical hazard, published evidence of reproductive or developmental toxicities, and other information.

The Center's first expert panel was formed in the summer of 1999 to evaluate evidence that seven selected phthalate esters (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) may pose a reproductive and/or developmental risk for exposed humans. The Expert Panel completed its review in 2000, and its reports are available on the Center's homepage, http://cerhr.niehs.nih.gov. The NTP is preparing its Center Report and anticipates its transmittal to Federal and State agencies, interested stakeholders, and the public in 2001.

The Center will conduct its next Expert Panel evaluation for potential reproductive and developmental toxicity on methanol. Methanol is a commercially

important, high-production volume chemical with potential for occupational, consumer, and environmental exposure. It is used in chemical syntheses and as an industrial solvent. It is also found in a variety of consumer products such as paints, antifreeze, cleaning solutions, and adhesives and is a by-product of sewage treatment, fermentation, and paper production. Methanol is currently used in race car fuels and there is potential for expanded use in the future as a vehicle fuel or fuel additive. Ethylene glycol, 1-bromopropane, and 2-bromopropane are being considered for future evaluations.

The Center's web site has information on various environmental exposures and their potential to affect pregnancies and child development, and it has links to other resources. The Center welcomes the nomination of chemicals for review or scientists for its expert registry. Information about the Center and the nomination process can be obtained from its homepage or by contacting Dr. Michael Shelby, Director, CERHR, NIEHS/NTP, P.O. Box 12233 MD EC-32, Research Triangle Park, NC 27709; Telephone: (919) 541-3455; Fax: (919) 316-4511; shelby@niehs.nih.gov.

NTP Center for Phototoxicology

The NTP Center of Phototoxicology (NCP) was created in 2000 to conduct mechanistic-based research and photocarcinogenesis studies on compounds of regulatory importance to the FDA and to address NTP nominations that require phototoxicology testing. Research conducted through this center should have a significant impact on public health by generating scientific data that address potential phototoxicities or photocarcinogenicities of the combination of therapeutics, cosmetics, devices, or food additives with sunlight.

The Center's state-of-the-art laboratory is designed for testing the effects of UV radiation or simulated solar light-induced toxicity and cancer on drugs, chemicals used in cosmetic preparations, and other agents (e.g., sun block additives, tanning enhancers, skin colorants, and tattoo inks). The light simulated closely matches the spectrum of terrestrial solar light and can emulate the conditions to which humans are exposed. The facility is also capable of performing studies using different types of fluorescent tube-generated light, such as those used in fluorescent lamps and suntan-bed lamps. With the public's increasing exposure to UV radiation or sunlight through more frequent use of tanning booths and leisure time spent in outdoor activities, research in this area is becoming increasingly important.

Chemicals selected for testing are nominated directly from the FDA and from outside submissions to the NTP. An FDA committee, the Phototoxicology Chemical Selection Working Group, prioritizes nominations and forwards them to the Interagency Committee for Chemical Evaluation and Coordination for entry into the NTP nomination and selection process. The standing Toxicology Study Selection and Review Committee reviews the experimental design and the progress of studies. Current research initiatives are described on page 8 under "Phototoxicology."

Information and questions can be directed to Dr. Paul C. Howard, Director, NCP, NCTR/FDA, HFT-110, 3900 NCTR Road, Jefferson, AR 72079; phoward@nctr.fda.gov.

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. 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 first established in 1997. The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) was established in 1998 to collaborate with the ICCVAM to facilitate the development, scientific review, and validation of novel toxicological methods that predict human health risks while reducing, refining, and/or replacing animal tests and to promote communication with stakeholders.

In December of 2000, Congress enacted the ICCVAM Authorization Act of 2000 (Public Law 106-545) and established ICCVAM as a permanent NIEHS/NIH committee under the NICEATM. ICCVAM consists of the heads of 15 Federal agencies or their designees (Table 6).

The purposes of ICCVAM are to:

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

Public Law 106-545 establishes a Scientific Advisory Committee and requires agencies to determine that alternative test methods are valid. Currently the Advisory Committee on Alternative Toxicological Methods provides ICCVAM and NICEATM advice on activities and priorities.

NICEATM and ICCVAM convene workshops to evaluate the adequacy of existing methods, identify areas needing alternative methods, and evaluate proposed validation studies. ICCVAM and NICEATM recently organized an international workshop to review and evaluate *in vitro* methods for assessing acute systemic toxicity and a peer review meeting on the Up-and-Down Procedure (UDP) for assessing acute oral toxicity. The UDP is a proposed replacement for the conventional LD50 test. ICCVAM and the Health and Environmental Sciences Institute of the International Life Sciences Institute held a training workshop on the local lymph node assay (LLNA) in January 2001. LLNA, a method for assessing allergic contact dermatitis of chemicals, was the first method to be evaluated under the ICCVAM peer-review process. The independent peer-review panel concluded that the LLNA is a valid substitute for currently accepted guinea pig test methods. EPA, OSHA, and FDA announced their acceptance of the LLNA as an alternative method in October 1999. A future peer review meeting is being planned to assess the validation status of several *in vitro* assays proposed for use in the EPA's Endocrine Disruptor Screening Program.

Additional information about ICCVAM, NICEATM, meeting schedules, meeting reports/minutes, and information on the nomination process of alternative toxicological methods can be obtained from the ICCVAM/NICEATM homepage (http://iccvam.niehs.nih.gov) or by contacting Dr. William S. Stokes, Director, NICEATM, NIEHS/NTP, P.O. Box 12233, MD EC-17, Research Triangle Park, NC 27709; Telephone: (919) 541-2384; Fax: (919) 541-0947; iccvam@niehs.nih.gov.

Table 6 ICCVAM

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

RoC

Report on Carcinogens

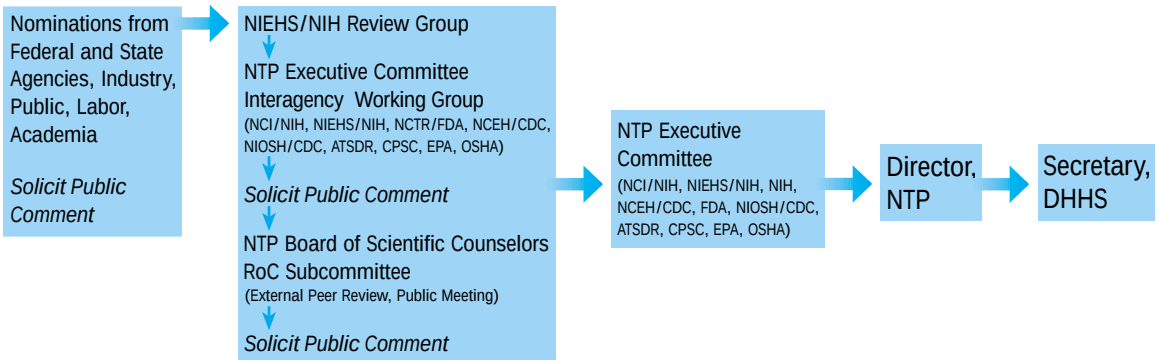
The Report on Carcinogens (RoC) is prepared biennially in response to Section 301 of the Public Health Service Act as amended. The RoC contains a list of all substances (i) that either are known to be human carcinogens or may reasonably be anticipated to be human carcinogens; and (ii) to which a significant number of persons residing in the United States are exposed. The Secretary, DHHS has delegated responsibility for preparation of the RoC to the NTP Director. The listing of a substance in the RoC is descriptive in nature and represents an initial step in hazard identification, which is generally considered the first step in the analytical process known as risk assessment.

The NTP solicits and encourages broad participation from interested parties in nominating agents, substances, mixtures, or exposure circumstances for listing in or delisting from the RoC. Nominations should be submitted to Dr. C.W. Jameson, Head, Report on Carcinogens, NIEHS/NIH, P.O. Box 12233, MD EC-14, Research Triangle Park, NC 27709; Telephone: (919) 541-4096; Fax: (919) 541-0144; jameson@niehs.nih.gov. Information about the RoC is also available through the NTP homepage (http://ntp-server.niehs.nih.gov).

Specific criteria are used to assess whether a nomination should be listed in the RoC. The review of nominations for listing in or delisting from the RoC involves a multi-phased, peer-review process with participation by representatives from Federal agencies (NIEHS/NIH Review Group, NTP Executive Committee Interagency Working Group, and the NTP Executive Committee) and the NTP Board of Scientific Counselors Report on Carcinogens Subcommittee (Figure 3). The NTP Director evaluates all review group recommendations, public comments, and other information in developing his recommendation to the Secretary, DHHS.

The 9th RoC was released May 15, 2000 and is available from the Environmental Health Information Service (see page 22 under “Communication and Public Outreach”). Table 7 lists the nominations under consideration for the 10th RoC. The preparation and review process for the RoC extends over approximately a two-year period; publication of the 10th Edition is anticipated for completion in 2002.

Figure 3 **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 7 **Summary of the Agents, Substances, Mixtures, or Exposure Circumstances Nominated for Possible Listing in or Delisting from the 10th Report on Carcinogens**

Reviewed in January 2000	Reviewed in December 2000
2-Amino-3-methylimidazo[4,5-f]quinoline	Broad-spectrum UV radiation and UVA and UVB and UVC
Beryllium and beryllium compounds	Chloramphenicol
2,2-bis-(Bromomethyl)-1,3-propanediol	Estrogens, Steriodal
2,3-Dibromo-1-propanol	Metallic nickel and certain nickel alloys
Dyes metabolized to 3,3´-dimethoxybenzidine (dimethoxybenzidine dyes as a class)	Methyleugenol
Dyes metabolized to 3,3´-dimethylbenzidine (dimethylbenzidine dyes as a class)	Talc Asbestiform and Non-Asbestiform
Styrene-7,8-oxide	Trichloroethylene
Vinyl bromide	Wood dust
Vinyl fluoride	

VI Outreach

Communication and Public Outreach

Open communication with Federal and State agencies, industry, stakeholders, academia, and the public is crucial for the success of NTP projects. Partnerships with sister Federal agencies are increasing, 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 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 serves as the focal point for receiving input to the Program and oversees the distribution of information about programs, workshops, initiatives, etc.

NTP testing and research results, program plans, and other publications are distributed through mailings, *Federal Register* announcements, and the NTP homepage, http://ntp-server.niehs.nih.gov. In addition, individuals can subscribe free of charge to the NTP listserver by registering online through the homepage or by sending e-mail to ntpmail-request@list.niehs.nih.gov with "subscribe" as the message. The NTP homepage also offers access to information about the NTP, and links are available that detail and highlight ongoing and future initiatives and NTP centers.

The Central Data Management (CDM) Office oversees distribution (on request) of specific chemical study information and NTP documents, including 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 for study, and minutes from meetings of the NTP Board of Scientific Counselors and its subcommittees. To request any of these documents, contact CDM, NIEHS/NIH, P.O. Box 12233 MD E1-02, 111 T.W. Alexander Drive, Research Triangle Park, NC 27709; Telephone: (919) 541-3419; Fax: (919) 541-3687; cdm@niehs.nih.gov.

Online, searchable access and printed copies of NTP publications, including the Report on Carcinogens, NTP Technical Reports, and NTP Toxicology Reports, are available through the Environmental Health Information Service (EHIS). Subscription packages to EHIS include access to NTP publications as well as *Environmental Health Perspectives* (primary issues and supplements), the Rodent Historical Controls Database, and the Chemical Health and Safety Database. For additional information, contact EHIS, NIEHS/NIH, P.O. Box 12233 MD EC-15, 79 T.W. Alexander Drive, Research Triangle Park, NC 27709; Telephone: (800) 315-3010; Fax: (919) 541-0763; ehis@niehs.nih.gov.

NTP

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