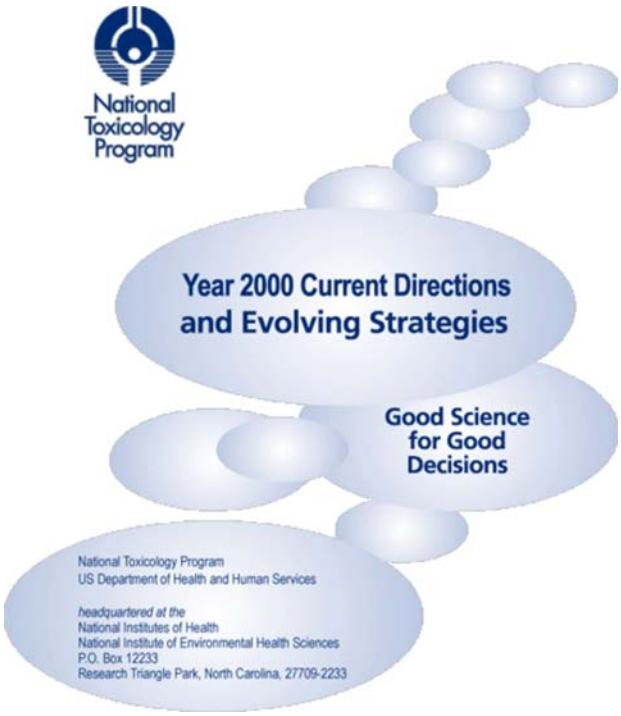

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OVERVIEW OF THE NATIONAL TOXICOLOGY PROGRAM

Mission and Goals

Today more than 80,000 chemicals are registered for use in commerce in the United States, and an estimated 2,000 new ones are introduced annually to be used in products we encounter in our daily lives 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. While relatively few such chemicals are thought to pose a

significant risk to human health, the safeguarding of public health depends upon identifying the effects of these chemicals and the levels of exposure at which they may become potentially hazardous to humans.

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

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

The Program uses these goals to set priorities as it moves forward to improve the nations ability to evaluate human health effects from chemical exposures. Its vision, leadership, and commitment to the concept of good science for good decisions create an atmosphere that allows the NTP to be flexible and innovative in its approach toward addressing public health concerns related to chemical exposures at home and work and in our environment. Nationally, the NTP rodent bioassay is recognized as the standard for identification of carcinogenic agents. However, the NTP has expanded its scope beyond cancer to include examining the impact of chemicals on non-cancer toxicities such as those affecting reproduction and development, and the immune, respiratory, and nervous systems. As part of this effort, The NTP Center for Evaluation of Risks to Human Reproduction was created. The NTP has also worked to reduce the use of experimental animals and to develop and validate alternative testing methods leading to creation of the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods.

The strengthening of existing partnerships and the forging of new ones are important to the NTPs achievement of its goals. Partnerships with sister Federal agencies are increasing and the NTP continues to collaborate with the private sector. Examples include co-sponsorship of numerous workshops, interagency initiatives in exposure assessment, and establishment of an interagency committee to oversee validation of alternative testing methods. The NTP recognizes that initiatives, which address critical knowledge gaps in toxicological evaluation, offer the best opportunities for preventing environmentally mediated diseases. Therefore, the Programs testing of chemicals is evolving to include more mechanism-based toxicology studies that focus on understanding the mechanistic actions of chemical agents. The NTP also recognizes that the use of human data in toxicological evaluations is imperative in order to be at the forefront in studying and developing risk assessment methodologies for quantifying the sequence of events that starts with chemical exposure and ends with toxicity. An initiative in human exposure assessment and an interagency agreement on evaluating occupational exposures contribute to this effort. Moving forward into 2000, the NTP is evolving to remain at the cutting edge of science as a means to meet its commitment to good science for good decisions.

Organizational Structure and Oversight

Three agencies, the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH), the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA) form the core for this program (Figure 1). The NTP is located administratively at the NIEHS/NIH and the Director of the NIEHS/NIH, Dr. Kenneth Olden, serves as Director of the NTP and as such reports to the Secretary, DHHS. The National Cancer Institute of the National Institutes of Health (NCI/NIH) was a charter agency and continues to serve on the NTP Executive Committee.

The NTP Executive Committee provides oversight to the NTP for policy issues. This committee is composed of the heads of participating HHS agencies including the Agency for Toxic Substances and Disease Registry (ATSDR), the Food and Drug Administration (FDA), National Center for Environmental Health of the CDC (NCEH/CDC), NCI/NIH, NIEHS/NIH, NIH, and NIOSH/CDC as well as the heads of several non-DHHS agencies that are concerned with human health including the Consumer Product Safety Commission (CPCS), the Environmental Protection Agency (EPA), and the Occupational Safety and Health Administration of the Department of Labor (OSHA).

The NTP Board of Scientific Counselors, which is composed of scientists from the public and private sectors, provides primary scientific oversight to the Program as well as to the NTP Center for the Evaluation of Risks to Human Reproduction. The Boards Technical Reports Review Subcommittee provides peer review of NTP long-term toxicity, carcinogenesis, and short-term toxicity study reports. The Report on Carcinogens Subcommittee provides external scientific evaluation of substances nominated for listing in or delisting from the *Report on Carcinogens.* The Advisory Committee on Alternative Toxicological Methods provides oversight to The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods.

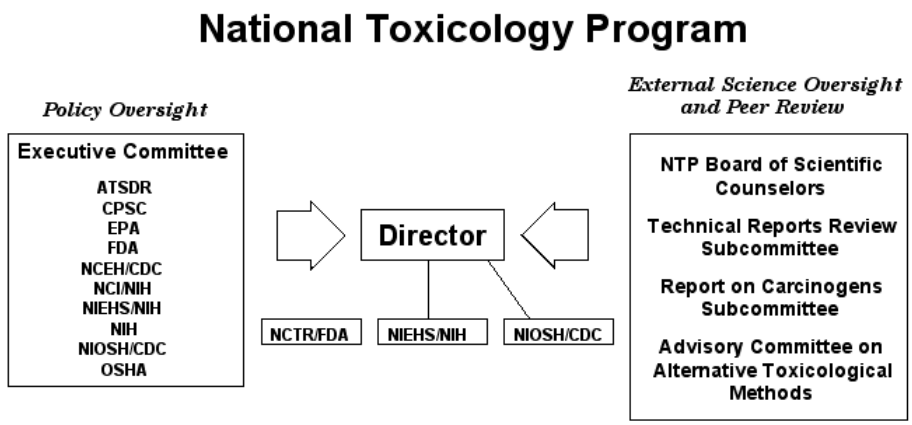

Figure 1. The National Toxicology Program, (NTP) is comprised of the relevant toxicology activities from three federal agencies - the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA), the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH), and the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC). The NTP is headquartered at the NIEHS/NIH and its director serves as director of the NTP. Oversight for policy issues is provided by the Executive Committee composed of the heads of key research and regulatory Federal agencies. Science oversight and peer review are provided through a mix of Federal, academic, industrial, and public interest science experts.

Role in Shaping Public Health Policy

Regulatory agencies make decisions for the protection of public health based on scientific information from multiple sources (Figure 2). The NTP plays a critical role in providing needed scientific data, interpretations, and guidance concerning the appropriate uses of these data to regulatory agencies as well as other groups involved in health-related research. The Program is committed to using the best science available in setting priorities for future studies and in designing, conducting, and interpreting the findings of those studies. The American people and government agencies at the State and Federal levels rely on the science base provided by the NTP in making credible decisions that will protect public health without unnecessarily increasing the regulatory burden on industry. Over the past two decades, the NTP has developed an increasingly interactive relationship with regulatory agencies and through this relationship has played an important, although indirect, role in shaping public health policy. The Program maintains an objective, science-based approach in dealing with critical issues in toxicology. Recognition of this fact by all participants in the public debate over the regulation of chemicals has afforded the NTP the status of an 'honest broker' in the continuing dialogue concerning the appropriate application of scientific advances to applied toxicology research and testing.

In recent years the NTP has placed a greater emphasis on providing human context to the interpretation and understanding of toxicologic information generated using animal or *in vitro* cell models. Examples of activities it covers include:

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

Some of the essential programs of the NTP that provide the foundation for the activities just described are outlined in this booklet.

Selected Reading

1. Fisher BE. Twenty years of toxicology. *Environmental Health Perspectives* 106(10):A480-487 (1998).
2. Lucier, GW, Barrett JC. Public health policy and the National Toxicology Program (editorial). *Environmental Health Perspectives* 106(10):A470-471 (1998).

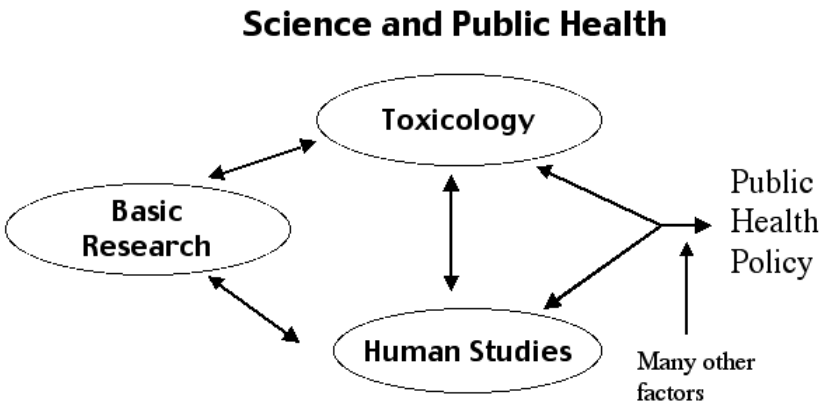

Figure 2: Scientific information comes from multiple sources and types of investigations: basic research, toxicology studies, and human studies. Each of these contributions to the science base upon which further study can be conducted. Regulatory agencies rely upon the availability of scientific data and its iinterpretation as well as other factors in making decisions about public health.

TOXICOLOGICAL EVALUATIONS

Nomination, Selection, Testing, and Review of Substances by the NTP

The nomination and selection process is integral to the effective operation and success of the NTP's testing program with respect to the testing of chemicals of greatest public health concern using current methodologies, the validation of new testing methodologies, and the evaluation of new concepts about potential mechanisms of toxicity. In keeping with its goal of providing up-to-date information about chemicals and potential toxicities, the NTP can initiate chronic bioassays (approximately eight per year) on only a small fraction of the thousands of chemicals for which there is little or no toxicological information. Many more chemicals are also studied to assess a variety of health-related effects, including but not limited to reproduction and development, immunotoxicity, neurotoxicity, genotoxicity, metabolism, toxicity to various organs, and carcinogenesis.

The NTP routinely solicits, accepts, and reviews for consideration nominations for toxicological studies to be undertaken by the Program on substances of potential human health concern. Particular assistance is sought for the nomination of studies that permit the testing of hypotheses to enhance the predictive ability of future NTP studies, address mechanisms of toxicity, or fill significant gaps in the knowledge of the toxicity of chemicals or classes of chemicals. The specific categories for which the NTP solicits nominations are given in Table 1. The nomination process is open and nominations are solicited from academia, Federal and State regulatory and health agencies, industry, and labor unions, as well as from environmental groups and the general public. Toxic release inventories and exposure surveys (e.g. National Health and Nutrition Examination Surveys (NHANES) and National Human Exposure Assessment Survey (NHEXAS) are also reviewed to identify chemicals of concern based upon the opportunity for identifying human exposure and body burden data.

Table 1
Categories for NTP Solicitations

- 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
- Orphan drugs or chemicals with significant exposure that generate too little revenue to support further evaluations
- Chemical agents first marketed prior to current testing requirements (e.g. 1977 Toxic Substances Control Act)
- 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
- Emergencies or other events that warrant immediate government evaluation of a chemical or agent

The nominations undergo several levels of review before toxicological studies are designed and implemented. A schematic of the chemical nomination and selection process is given in Figure 3. During the process, the NTP works actively with regulatory agencies and interested parties to supplement the information about nominated substances and to ensure that the nomination and selection process meets regulatory and public health needs. The NTP solicits public comment on the nominees throughout the process and comments are considered during review of the nominations. At the final step of formal review, the NTP Executive Committee reviews and evaluates the public comments and the testing recommendations for the nominations from the previous review groups. The Committee then makes recommendations about the nominations to the NTP to test, to defer testing for additional information, or to recommend not testing at this time. Information about nominating a substance for testing by the NTP is provided on the NTP web site (http://ntp-server.niehs.nih.gov).

The NTP has a broad mandate to provide toxicological characterizations for chemicals and agents of public health concern and strives to balance the selection of chemicals for study (e.g., occupational, environmental, food additives, agricultural chemicals, and pharmaceuticals). Substances may be

studied for a variety of health-related effects, including but not limited to reproductive and developmental toxicity, genotoxicity, immunotoxicity, metabolism and disposition, as well as carcinogenicity. In selecting chemicals for study, the NTP operates under the principle that industry will evaluate chemicals or other agents for health and environmental impacts as intended and mandated by Congress under legislative authorities. Chemicals are selected based upon two broad criteria: 1) those chemicals of greatest concern for public or occupational health and 2) chemicals for which toxicologic data is needed to fill major knowledge gaps, address mechanisms of toxicity, and reduce uncertainty in risk assessment. The selection of a substance by the Executive Committee does not automatically commit the NTP to its evaluation. The priority of the nominations and the

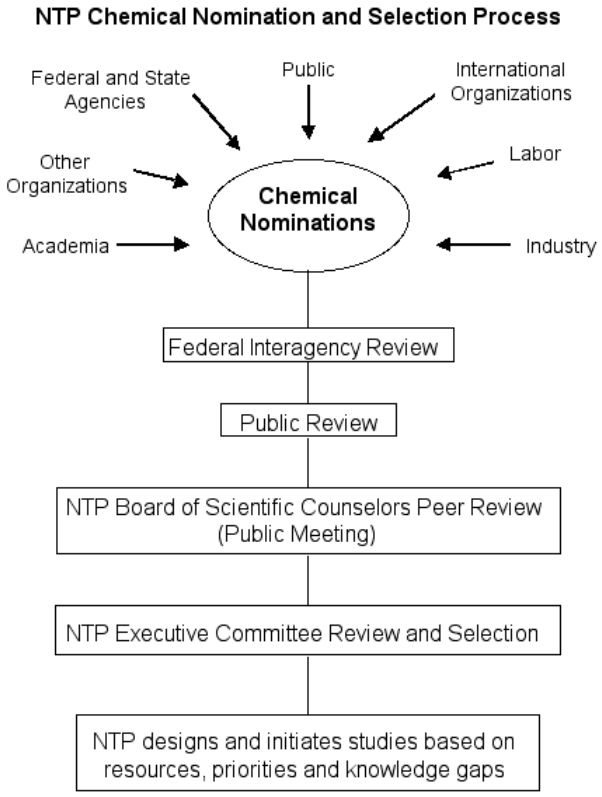

Figure 3. Schematic representation of the steps in nomination and selection of chemicals for study by the National Toxicology Program.

proposed studies are assessed during the nomination and selection process and reassessed during the study design process. During any of these stages, a chemical or study may be withdrawn if applicable research data or higher priority studies are identified or if a study proves impractical.

The chemicals recommended for study most recently by the NTP Executive Committee are given in Table 2. The NTP works on a broad range of high priority agents and issues. Examples of relatively new initiatives include the characterization of potential health effects from dietary supplements and herbal medicines and an assessment of the risk from exposure to the major disinfection by-products found in drinking water.

Table 2

Chemicals Approved December 2, 1999 for Study by the NTP Executive Committee

Aloe Vera Gel	Indole-3-carbinol
Ammonium Molybdate	Kava Kava Extract
5,6-Benzoflavone	Milk Thistle Extract
1,3-Dichloro-2-butene	3-Picoline
Ginseng and Ginsenosides	

For all selected chemicals, studies are initiated as time and resources permit. Prechronic, short-term studies generally are performed for two or 13 weeks. Long-term, chronic bioassays last up to two years. The NIEHS/NTP staff reviews the toxicology literature for each chemical and designs a comprehensive testing strategy. Project plans are reviewed by a NIEHS/NTP project review committee that evaluates the project plan (e.g. design,

methods, hypothesis, etc.) and proposes the vehicle for execution (e.g. grant, contract, etc). The NIEHS/NIH toxicokinetics faculty examines all chemicals under study and delineates any areas of chemical metabolism and kinetics for which sufficient data are lacking; studies are designed to address these deficiencies.

The results of toxicological studies undergo rigorous peer review. These findings are published in NTP Technical Reports and may also be published in peer reviewed scientific journals. The NTP Technical Reports Review Subcommittee, a standing subcommittee of the NTP Board of Scientific Counselors, evaluates the technical reports from carcinogenicity and toxicity studies in an open, public meeting. Candidates for peer review in 2000 are listed in Table 3. NTP Technical Reports are available to the public and other interested parties (see page 26).

Table 3 *Candidates to be Peer Reviewed in May 2000*
Chloral Hydrate (two-year study)
Chloral Hydrate (dietary restriction study)
p.p'-Dichlorodiphenyl Sulfone
Indium Phosphide
Naphthalene
Sodium Nitrite

Herbal Medicines

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

Due to increased advertisements and use of dietary supplements, the NTP has received numerous nominations for study of herbal medicines and other dietary supplements from both the public and Federal agencies. In order to focus NTP research on the most critical public health issues, the NTP with support of the NIH, the Department of Health and Human Services Office of Disease Prevention and Health Promotion, the FDA Office of Special Nutrition, and the Society for the Advancement of Women's Health held an international workshop in September 1998 to seek expert advice on the most relevant research needs. Based in part on input from the workshop, the NTP is currently planning and conducting research on several herbal medicines and other dietary supplements (Table 4). These studies will focus on characterization of potential adverse health effects including reproductive toxicity, neurotoxicity, and immunotoxicity as well as those associated with acute high dose exposure and chronic exposure to lower doses. In addition, special attention will be given to the potential for herb/herb and herb/drug interactions and the responses of sensitive subpopulations (e.g. pregnant women, the young, the developing fetus, the elderly, etc). Comments from the public and others regarding NTP research in this area are welcome and should be forwarded to the NTP Liaison and Scientific Review Office (see page 26).

Table 4 *Herbs and Active or Toxic Ingredient under Study by the NTP*	
Herb or Ingredient	Information
Berberine	An active ingredient in golden seal
Comfrey	Herb consumed in teas and as fresh leaves for salads; contains pyrrolizidine alkaloids, which are known to be toxic.
Echinacea	Most commonly used medicinal herb in the United States
Ginkgo Biloba Extract	Among the five or six most frequently used medicinal herbs
Golden Seal	Second or third most popular medicinal herb used in this country
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 worm wood
Other Herbs under Review at This Time	
Aloe Vera	Seventh most widely used herb; used as both a dietary supplement and component of cosmetics.

Ginseng and Gensenosides	Fourth most widely used medicinal herb; gensenosides are thought to be the active ingredients. Ginseng has been associated with various adverse health effects.
Kava Kava	Reported to be the fifth most widely used medicinal herb, has psychoactive properties and is sold as a calmative and antidepressant.

Selected Reading

1. Herbal Health. *Environmental Health Perspectives* 106(12): A590-592 (1998).
2. Medicinal Herbs: NTP Extracts the Facts. *Environmental Health Perspectives* 107(2): A604-605 (1999).

Safe Drinking Water Program

The Safe Water Drinking Program is of enormous, public health significance since it is estimated that more than 200 million Americans use treated drinking water. The availability of safe drinking water is a substantive health concern. Chlorination of our water supply is a standard treatment technique and is considered as one of the major, public health advances in the 20th century. Despite advances in expertise to purify and disinfect our water, chemical contaminants may still be found in finished water. These agents can be grouped into two broad categories, those that occur as a result of the disinfection process (disinfection by-products, DBPs) and those that occur naturally or by contamination in public water systems. The list of known or anticipated contaminants (Contaminant Candidate List, CCLs) was published by the EPA in the Federal Register on March 2, 1998 in response to a Congressional mandate under the Safe Drinking Water Act, amended in 1996.

Water chlorination reduces mortality and morbidity from infectious disease; therefore, any attempt to control DBP exposure must be balanced against increasing biological hazards. The current scientific data are insufficient to make policy decisions on how to balance chemical versus biological risk associated with drinking water. Thus, one of the most complex issues facing water utilities and the EPA is minimizing the potential for DBP-related health effects while still achieving effective control of waterborne microbial pathogens. The EPA is expected to set final water standards in 2002 or 2003, and the NTP is playing a critical role in providing data for that process.

The magnitude and complexity of the issue of safe drinking water exceeds the expertise and resources for any one agency. A toxicological approach to evaluating DBPs is not easy, since different disinfection processes result in different DBPs, and the source of water, time of year, and other factors can influence the presence and relative concentrations of different DBPs. Further, DBPs occur as complex mixtures in relatively low concentrations in drinking water making study difficult. The NTP is collaborating with the EPA (Office of Water and the National Health and Environmental Effects Research Laboratory) on a research program to assess the potential risks from human exposure to the major DBPs. The program includes a systematic, mechanism-based, toxicologic evaluation of DBPs that can help provide data for policy makers in setting drinking water standards. The selection of DBPs for study is based upon their presence in drinking water, their occurrence with different disinfection processes, their chemical structure, and representation from among the different DPB families: trihalomethanes, haloacetic acids, and haloacetonitriles. Research focuses on reproductive toxicity, immunotoxicity, and neurotoxicity as well as carcinogenesis, and research approaches include investigations using transgenic mouse models, standard rodent bioassays, and studies in fish. A list of substances under study by the NTP for the Safe Drinking Water Program is given in Table 5. NTP research is being conducted at NIEHS/NIH and also through agreements with the US Army for fish studies and EPA for immunotoxic investigations. The NTP is also involving the extramural research community through support of hypothesis-based mechanistic studies on DBPs and is working closely with the American Water Works Association Research Foundation (AWWARF) sharing protocols and research plans and making them aware of ongoing research activities. Some of the AWWARF's own research awards are being designed to complement activities of the NTP and EPA.

Table 5

Substances under Study for Safe Drinking Water Program

2(5H)-Furanone (MX)	Chloroform
Bromochloroacetic Acid	Dibromoacetic Acid
Bromodichloroacetic Acid	Dibromoacetonitrile
Bromodichloromethane	Dichloroacetic Acid
Chloramine	Sodium Bromate
Chlorate	Sodium Chlorite

DNA-Based Products

DNA-based therapies are currently being developed for the treatment of a wide range of human diseases. Examples include plasmid DNA encoding one or more antigenic proteins for vaccines against viral and bacterial pathogens, triplex forming synthetic oligonucleotides to modulate gene expression, and viral vectors for gene therapy. Though DNA-based products show significant promise, by their very nature they all pose a risk of interacting with the host genome or disrupting normal cellular processes in unexpected and unpredictable ways and with potentially adverse consequences. It is essential to identify hazards and potential risks associated with these therapies prior to widespread clinical application. Presently the FDA has only limited authority to require evaluation of non-acute, long-term safety risk associated with these therapies. In addition, the majority of the manufacturers of DNA-based products are generally small biotechnology companies and academic sponsors that lack the resources to perform long-term, large-scale studies on their products. The NTP and FDA are collaborating on an initiative to study the safety of DNA-based products that will address three major safety issues:

- The intracellular persistence and potential for integration into the host genome. Since certain of these products are intended for use in children (e.g., DNA vaccines), there is concern about life-long risk posed by integration.

- Their distribution to the gonads and the potential for integration and germ line transmission. DNA-based products may reach tissues in the body outside their presumably sequestered sites of administration (intradermal or intramuscular); therefore, there is concern about the potential for reproductive toxicity and/or transmission of altered genetic material to subsequent generations.
- Ectopic protein production and the potential for abnormal immune activation. Both viral vectors and DNA vaccines carry genes that stimulate host cells to secrete self and foreign proteins. There is concern about the potential for DNA-based products to promote development of autoimmune disease and disrupt immune homeostasis.

At this initiative gets underway, scientists from the Center for Biologic Evaluations and Research, FDA and the NTP are working together to design studies that address these important public health issues.

Human Exposure Assessment

The NTP increasingly recognizes the need for human data in its science base for decision-making. Information on human exposure to agents and their body burden is critical for a successful and scientifically sound approach to the evaluation of potential human health risks. However, the availability of human exposure assessment data is often the weakest component of risk assessment and limits the effective utilization of experimental data for making decisions about chemical exposures. Advances in analytical methodologies now enable the detection of environmental and occupational chemicals in small biological samples (e.g. blood, urine, and hair). Taking advantage of such advances, the NTP is leading a new interagency human exposure assessment initiative in collaboration with NCEH/CDC, NIOSH/CDC, and EPA to quantify the body burdens of chemicals released into the environment and workplace. Efforts being undertaken with this initiative should benefit public health and priority setting by:

- allowing the prioritizing of chemicals and chemical mixtures to be studied by the NTP to include their occurrence in human tissue,
- providing data documenting exposure to children,
- identifying potentially "sensitive" subpopulations,
- strengthening the interpretation and use of animal toxicity data in quantifying the amount of chemical and/or active metabolite in blood and target tissues,
- allowing for refinement of human risk assessments through construction of physiologically based pharmacokinetic/toxicokinetic models,
- examining relationships between health disparities and fiscal disparities,
- enhancing future epidemiological studies of exposure/disease relationships,
- allowing government agencies to determine the effectiveness of regulatory actions for environmental standards and clean-up sites,
- allowing better integration of human exposure information in other NTP activities such as the *Report on Carcinogens* and the NTP Center for the Evaluation of Risks to Human Reproduction, and
- linking these data with the NIEHS Environmental Genome Project to provide the science base essential for future, meaningful studies of gene/environment interactions in disease etiology. The Genome Project is a multi-center effort to identify systematically in the U.S. population the alleles of 200 or more environmental disease susceptibility genes. Information from the human exposure assessment initiative together with the Genome Project will be useful in the design of future, meaningful studies of gene/environment interactions in disease etiology.

The NIEHS/NIH organized a workshop 22-24 September 1999 to bring together scientists and policy-makers from government agencies, academia, industry, and community groups who have an interest in exposure assessment issues. The meeting was sponsored by the NIEHS/NIH, NTP, Office of Rare Diseases/NIH, NCI/NIH, NIOSH/CDC, NCEH/CDC, Office of Research and Development/EPA, and the American Industrial Health Council. This workshop provided information on disease-specific chemical exposure, highlighted knowledge gaps and research needs, and led to specific recommendations and opportunities for addressing those needs. Summary information about the workshop is available on the NTP web site (http://ntp-server.niehs.nih.gov) under the header, Meetings. Conference deliberations will be used as a foundation to guide and expand future research efforts on human exposure assessment.

Selected Reading

1. Gibb, S. NIEHS launches interagency effort to bolster exposure analysis. *Risk Policy Report* 6(10):3-5 (1999).
2. Lucier GW, Schecter A. Human exposure assessment and the National Toxicology Program. *Environmental Health Perspectives* 106(10):623-627 (1998).

Assessment of Endocrine Disruptors

The NIEHS/NIH and NCEH/CDC are collaborating on a pilot project for quantifying approximately 70 chemicals found in either human blood or urine that are considered to be endocrine disruptors. The biological samples are from the National Health and Nutrition Examination Surveys (NHANES), which covers both men and women from a range of age, socio-economic, and ethnic groups. These data will be used to estimate human exposure to endocrine disrupting agents within the U.S. population and to identify the ones of greatest public health concern. Phytoestrogens and phthalate esters are two classes of compounds for which studies are in progress. Continued development of this interagency exposure initiative will focus on other NTP priority exposures, such as herbal medicines and drinking water disinfection by-products, to facilitate sound, scientific evaluations of agents of priority concern for public health.

Upcoming Meeting: Low Dose Issues for Endocrine Disruptors

The EPA is implementing an Endocrine Disruptor Screening Program as required by the 1996 Food Quality Protection Act. As part of this program, the EPA is in the process of choosing appropriate assays to use for screening those agents and developing standardized, validated protocols for those assays. A critical aspect of the protocol development is dose-setting, especially since in this instance hormonally active agents may cause effects at doses lower than those normally selected for toxicological testing. The EPA has asked the NTP to establish an independent, scientific panel to review the evidence related to low-dose effects and to consider their implications for the development, validation, and interpretation of test protocols. The scientific panel meeting is tentatively scheduled for fall 2000. As plans are finalized, information will be posted on the NTP web site (http://ntp-server.niehs.nih.gov) or can be obtained by contacting the NTP Liaison and Scientific Review Office (see page 26).

Occupational Health Exposures

The NTP is coordinating an interagency effort between NIEHS/NIH and NIOSH/CDC to better characterize worker exposures and to use this

information both for worker education and to identify occupational health research gaps. This project involves NIOSH-wide participation and should impact the health agenda of both NIOSH/CDC and NTP by focusing NTP resources on obtaining 'real world' information about worker practices, exposures, and possibly related health effects. Such information is needed to better identify areas for research and to design better laboratory studies on the health effects of chemicals, mixtures, and exposure circumstances encountered in the workplace. NIOSH/CDC will work together with the NTP in nominating agents for study and in designing laboratory studies and will undertake its own research projects under this agreement.

NIOSH/CDC is currently planning to conduct a national, cross-sectional, on-site survey of establishments and workers analogous to the 1972-1974 National Occupational Hazard Survey (NOHS), the 1981-1983 National Occupational Exposure Survey (NOES), and the 1984-1989 National Occupational Health Survey of Mining (NOHSM). The new survey will include all industry sectors covered by the OSHA and Mine Safety and Health Administration. It will gather nationally representative data on chemical, physical, and biological agents to which workers are potentially exposed, as well as data on exposure controls and health and safety practices. This survey should provide information for targeting research in areas with the highest likelihood of reducing workplace illness and promoting occupational health and safety.

One area currently under evaluation is worker exposure to cellulose insulation. Cellulose insulation has been nominated to the NTP for general toxicologic evaluation; however, currently no data regarding actual worker practices, exposures, and possible health effects caused by its use have been identified. Workers and work sites are being assessed and exposure data collected for analysis. Samples of the potentially inhalable cellulose insulation dust are also being collected for analysis. These data should provide background information to NIOSH/CDC relative to any future recommendations about use of cellulose insulation and worker education. It will also provide the NTP information to develop protocols for laboratory toxicologic studies on cellulose insulation.

Another project is examining the physical and chemical characteristics of asphalt fumes generated in a laboratory setting under simulated road paving conditions. Asphalt fumes generated with road paving are associated with acute irritation of both mucous membranes and skin; cancer risk associated with this operation is not established. Detailed physical and chemical analysis of the fumes and development of a standard, well-characterized fume generator are two of the major obstacles to laboratory studies in this area. One goal of this project is to evaluate fully and to provide specific operating conditions of a generator in order to be able to reproduce environmental exposures equivalent to those encountered by road paving crews. Another is to more precisely define the physical and chemical compositions of the asphalt fumes under well-defined, environmental conditions. The information from this study should facilitate development of protocols for future research studies on this agent.

EMERGING RESEARCH STRATEGIES

A large number of chemicals (>80,000) are currently in use. The NTP continually faces the task of determining which ones should be studied with the limited resources available for toxicological testing. As a consequence, the NTP must set its priorities and develop strategies that best use its resources in ways that strengthen the science base used by regulatory agencies in making decisions for public health policy. It is clear that regulatory agencies, such as the EPA, are committed to using all relevant information in their assessments. Implementing new strategies, which provide additional or more accurate information, can strengthen the science base on which regulatory decisions are based. New methods, especially those that provide mechanistic data, may also allow for more rapid screening and decreased dependence on chronic two-year rodent bioassays.

Scientists at the NIEHS/NIH, NCTR/FDA, and NIOSH/CDC are involved in development and validation of a wide range of testing methods for toxicological research including transgenic animals for carcinogenicity testing and genetically engineered cell systems to measure endocrine disruptor activity. University-based researchers are also involved in this effort through the NIEHS/NIH extramural grants program. Model systems under development include non-mammalian species, transgenic species, genetically engineered *in vitro* cell systems, microchip array technology, and computer-based predictive toxicology models.

Mechanism-Based Toxicology

As a leader in the design, conduct, and evaluation of toxicological data for use in making public health decisions, the NTP's testing program is committed to the use of mechanism-based toxicology studies as an enhancement to traditional approaches. This field uses molecular biology tools to characterize interactions of chemicals with critical target genes. Examples of mechanism-based toxicology include identification of receptor-mediated toxicants, molecular screening strategies, use of transgenic animal models, and the development of alternative or complementary *in vivo* tests to use with rodent bioassays. Inclusion of such strategies can provide insight into the molecular and biological events associated with a chemical's toxic effect and provide mechanistic information that is useful in assessing human risk. Such information can also lead to the development of more specific and sensitive (and often less expensive) tests for use in risk assessment. A strong linkage exists between mechanism-based toxicology and the development of more biologically based risk assessment models. Such models are useful in clarifying dose-response relationships, making species comparisons, and identifying sources of inter-individual variability.

Transgenic Animals

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

The use of these genetically altered mouse models holds promise in carcinogenesis research and testing and clearly is more rapid and less expensive than traditional NTP two-year bioassay studies. Over the past few years, the NIEHS/NIH and NTP have been actively evaluating transgenic strains in toxicological testing strategies. The models with greatest potential usefulness at this time are the $p53^{def}$ (*p53+/-*heterozygous) and Tg.AC (*v-Ha-ras*

transgene). These strains show specificity in their abilities to discriminate between genotoxic carcinogens and noncarcinogens ($p53^{def}$) and between both genotoxic and nongenotoxic carcinogens and noncarcinogens (Tg.AC). Evaluation of the specificity and usefulness of these two transgenic models is continuing and other models are also being examined.

As part of this evaluation process, the NTP is participating in an industry/government consortium of Federal agencies and 25 pharmaceutical companies to study the utility of a number of alternative assays designed to augment or replace the two-year rodent bioassay. The International Life Sciences Institute/Health and Environmental Sciences Institute (ILSI/HESI), a non-profit foundation, is coordinating this effort. A set of about a dozen chemicals is being evaluated in five, short-term model systems including three, genetically altered mouse models; the newborn mouse assay; and the Syrian hamster embryo cell transformation assay. The NTP is testing six chemicals in the Tg.AC model by two routes of administration. An ILSI/HESI organized meeting is scheduled for November 2000 in the Washington, DC area to discuss and evaluate the effort's findings. The results will contribute to an NTP evaluation of genetically engineered mouse models by the Interagency Coordinating Committee on the Validation of Alternative Methods that is scheduled for 2001.

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2. Eastin, WC, Haseman JK, Mahler JF, Bucher JR. The National Toxicology Program Evaluation of Genetically altered Mice as Predictive Models for Identifying Carcinogens. *Toxicologic Pathology* 26(4):461-473 (1998).
3. Tennant RW, Stasiewicz S, Mennear J, French JE, Spalding JW. Genetically altered mouse models for identifying carcinogens. In: The Use of Short- and Medium-term Tests for Carcinogens and Data on Genetic Effects in Carcinogenic Hazard Evalaution. (McGregor, DB, Rice JM, Venitt S, eds). IARC Scientific Publications No. 146, Lyon:International Agency for Research on Cancer, 1999;123-150.

Toxicogenomics

The NTP plays a critical role in providing needed scientific information, interpretations, and guidance to agencies involved in health-related research. Regulatory and public health agencies recognize the importance of mechanistic/molecular information in decision-making. With the advent of new genomic technologies, the NTP is moving forward into this arena, acknowledging that effective priority-setting strategies will be needed to guide its efforts in prioritizing investigations of exposures in order to determine which gene expression patterns are most likely linked to environmental causes of human disease.

cDNA Microarray Technology

cDNA microarray technology has emerged as a gene expression tool by which scientists can detect genome-wide differential expression of thousands of genes and offers a methodological advancement for environmental health research. The application of a large number of genes or expressed sequence tags in a condensed array on a glass slide comprises a cDNA microarray. Microarray technology is used to characterize exposures through the direct comparison of the expression of thousands of genes simultaneously in control and exposed samples. By comparing gene expression patterns from various exposures, one can potentially identify 'signature' patterns that may serve as biomarkers of potential exposure or potential disease. This approach may be useful to identify toxicities of individual substances or mixtures, to determine dose-response relationships, to identify susceptible tissues and cell types, and to extrapolate information between species; all of this information would contribute to the risk assessment process. The identification of expression patterns associated with specific cellular signals and processes could also provide insights into mechanism of actions by toxicants.

The NIEHS/NIH has developed a human cDNA chip, ToxChip, which contains copies or clones of about 2,000 of the approximate 80,000 genes in the human genome in a grid pattern on a glass slide, and a human Discovery Chip containing 12,000 clones. The NIEHS/NIH is evaluating known toxicants and building a database of expression information in order to determine the typical genetic changes or 'signature' profiles that they produce. Microarrays are also being made from common test animals and organisms including mice, rats, frog (*Xenopus*), and yeast. As this technology continues to be used and improved, it will assist NTP scientists in evaluating and comparing compounds under study. Such information will contribute greatly to enhancing current efforts in mechanism-based toxicology and provide valuable information to the science base used in risk assessment.

Selected Reading

1. Paules RS, Tennant RW, Barrett, JC, Lucier, GW. Bringing genomics into risk analysis: The promises and problems. *Risk Policy Report*. September 17, 1999.
2. Nuwaysir, EF, Bittner M, Trent J, Barrett JC, Afshari CA. Microarrays and toxicology: The advent of toxicogenomics. *Molecular Carcinogenesis* 24:153-159 (1999).
3. Afshari CA, Nuwaysir EF, Barrett JC. Application of cDNA microarray technology to carcinogen identification, toxicology, and drug safety evaluation. *Cancer Research* 59:4759-4760 (1999).

Real Time and Quantitative PCR (RTAQ-PCR) Technology

Quantitative RTAQ-PCR is used to measure specific levels of gene products within a sample obtained from either cells or tissues. Real-time, fluorescence detection of PCR products is a recent advancement in this technology that allows for quantitative analyses to be conducted more rapidly and on more samples at a time thereby making this method favorable for large studies on gene expression. In addition, RTAQ-PCR affords a high throughput method for the analysis of DNA polymorphisms, such as those being identified in the NIEHS Environmental Genome Project. The quantitative aspect of this technique is especially attractive for investigations of the molecular effects of environmental toxicants on gene expression. This analysis provides a useful tool with which to assess the effect(s) of toxicant exposure on cellular function at the molecular level, to determine dose-response relationships, and to evaluate differences among species in response to toxicants. It also allows for the inclusion of mechanism-based endpoints in human epidemiological studies by permitting the evaluation of potential molecular biomarkers of exposure and/or response. Efforts are ongoing at the NIEHS/NIH and NTP to develop the use of RTAQ-PCR for the analysis of 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD)-inducible, peroxisome proliferator-inducible, and DNA damage-inducible genes. This method is planned for use in gene expression analysis of tissues obtained from animals treated with dioxin-like compounds.

Laser Capture Microdissection Technology

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

ability to expand its use of molecular biology tools to characterize interactions of chemicals with critical target genes.

Risk Assessment Methodologies

Risk assessment involves the synthesis of large amounts of diverse data to arrive at a decision concerning the plausibility and magnitude of hazards posed by environmental agents. The NTP's effort in risk assessment is closely tied to its growing initiatives in mechanism-based toxicology. This linkage provides opportunities to improve priority setting, use mechanistic information to estimate risk from environmental or occupational exposures, clarify dose-response relationships in the 'low dose' range, select the most appropriate experimental systems for estimating risk, and develop science-based models for specific subpopulations (e.g., age, gender, genetic predisposition, ethnicity, etc.). The NTP's initiative on methodology for risk assessment includes the development of a strong linkage between laboratory methods and the development and use of mechanism-based mathematical models. These models are used to quantify the sequence of events that start with chemical exposure and end with overt toxicity. Models, whose designs are biologically based, allow researchers to link a broad array of experimental findings in ways that are both biologically logical and useful in risk assessment for defining dose-response relationships, making species comparisons, and assessing inter-individual variability. The development of biologically realistic models is an iterative process that builds on data related to the biological behavior of the agent in tissue samples, animal models, and/or humans and on the rapidly expanding knowledge of the physiological, cellular, and molecular events contributing to various disease processes. NTP initiatives, such as the human exposure assessment and the new toxicogenomic technologies being developed at NIEHS/NIH, should improve the availability of human and animal mechanistic data for use in development of risk assessment models and the quality of those models.

The complete dosimetry of a chemical or physical agent describes its absorption, distribution, metabolism, and elimination (ADME) at differing levels of exposure over all ages, via multiple routes of exposure, and under varying genetic backgrounds in human and animal models. Modern computing resources make it possible to expand traditional analysis using compartmental models to analyze chemical disposition using physiologically based pharmacokinetic/toxicokinetic (PBPK) models. Such models have an improved and realistic description of key physiological processes and biochemical activities that affect both the ADME of the parent compound and the precursors to toxicity. PBPK models are being used increasingly to extrapolate animal doses to human doses, because they allow full consideration of species differences in physiology, biochemistry, and anatomy. Models have been developed or are under development for multiple cancer endpoints, developmental endpoints, and reproductive toxicity (Table 6).

In recent years, the NTP has expanded its efforts in toxicokinetics to provide a better understanding of the behavior of chemicals under study in test animal species. It is generally believed that internal markers of tissue dosimetry, including tissue concentrations of the test agent or its metabolites, are better measures of effective dose than is external exposure. Inclusion of PBPK models in NTP technical reports is becoming routine, and such information should give guidance to regulatory agencies interested in extrapolation of data across species.

Table 6

Physiologically Based Toxicokinetic Modeling

Chemical	Route of Exposure
Anthraquinone	Oral in feed
Butadiene [a]	Inhalation
p-p'-Dichlorodiphenylsulfone [b]	Oral in feed
Isoprene	Inhalation
Melatonin [a]	Endogenous
Mercury (pregnant rat) [a]	Inhalation
Methyleugenol	Oral by gavage
Naphthalene [b]	Inhalation
Polychlorinated Biphenyls (209 congeners) [a]	
Primidone	Oral in feed
Sodium Nitrite [b]	Oral in drinking water
2,3,7,8-Tetrachloro-dibenzo-*p*-dioxin (TCDD)[a]	Oral and dermal

[a]ongoing PBPK modeling research
[b]to be presented at the May 2000 NTP peer review

For many chemicals or classes of chemicals, there is emerging data related to gene expression, protein levels, receptor binding and interaction, and cellular protein changes that should allow development of biochemical models more complex than simple ADME models. Such models can provide mechanistic insights into the origin of biological changes at the cellular and molecular levels, resulting from a particular exposure, and improve risk assessment. Mechanistic models have been constructed to characterize Ah receptor-dependent transcriptional activation of dioxin-responsive genes, enzyme induction in TCDD-treated rats, and the physiological and biochemical processes involved in renal accumulation of $a_2$$\Box$-globulin and in the deposition of $a_2$$\Box$-globulin-binding ligands in male rats.

Upcoming Workshop: Of Mice, Humans and Models: Future Research Directions for Improving Risk Assessment Methods

As part of its efforts to expand and improve risk assessment methods and their application, the NIEHS/NIH and NTP are sponsoring a workshop in cooperation with NIOSH/CDC, EPA, the Chemical Manufacturers Association, and the United Auto Workers. The meeting is scheduled for 16-18 August 2000 in Aspen, Colorado. The goals for this workshop include developing a national agenda and support for research on risk assessment methods, identifying and overcoming problems with current methods, and developing partnerships for increasing stakeholder and community input. Following the workshop proceedings will be available.

REPORT ON CARCINOGENS

The *Report on Carcinogens* (RoC), previously called the *Annual Report on Carcinogens* is prepared in response to Section 301 of the Public Health Service Act, as amended. This law stipulates that the Secretary, HHS shall publish a report which contains a list of all substances (i) which 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, HHS has delegated responsibility for preparation of the Report to the Director of the NTP. The RoC is prepared by the NTP with the assistance of other Federal health research and regulatory agencies and non-government institutions. 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 Report is not intended to constitute a risk assessment; it is a hazard identification document only.

The NTP solicits and encourages the broadest participation from interested individuals or parties in nominating agents, substances, mixtures, or exposure circumstances for listing in or delisting from the RoC. Nominations are requested to contain a rationale for listing or delisting. Appropriate supporting background documents (e.g., journal articles, NTP Technical Reports, International Agency for Research on Cancer listings, exposure surveys, release inventories, etc.) that support a nomination should be provided or referenced when possible. Anyone may nominate a substance to be considered for listing in or delisting from the RoC. The nominations should be submitted to Dr. C.W. Jameson, NTP, Report on Carcinogens, NIEHS/NIH, P.O. Box 12233, MD EC-14, Research Triangle Park, NC 27709, T: 919-541-4096, F: 919-541-0947, e-mail: jameson@niehs.nih.gov. The review of agents, substances, mixtures, or exposure circumstances for listing in or delisting from the RoC involves a multi-phased, peer review process. Specific criteria are used to assess whether or not a substance should be listed in the Report. An outline of the review process is provided in Figure 4. Guidelines for submission of nominations and the specific criteria used for their review are given on the NTP web site (http://ntp-server.niehs.nih.gov).

In 1994, the NTP Director initiated a public review of the RoC to 1) broaden the input to preparation of the Report, 2) broaden the scope of scientific review associated with the RoC, and 3) provide review of the criteria used for listing substances in the RoC. In 1996, the Secretary, HHS approved revised criteria, which allow for listing decisions to be made with consideration given to all relevant information including mechanism of action. The revised process and criteria were used in the preparation of the 8th RoC published in 1998 and were used in the review of nominations for the 9th Report. During the preparation and review of the 8th and 9th RoCs, the NTP received comments from interested stakeholders on proposed listings, the process and procedures used in the review, and the criteria for listing/delisting. The NTP has been deliberate in its efforts to solicit public input and to understand the concerns of stakeholders. In October 1999, the NTP held a public meeting in an effort to obtain the broadest base of input and to provide all interested parties an opportunity to express their views about the review process and/or the evaluation criteria. Stakeholders' advice has been extremely important in guiding the NTP about ways to strengthen and improve the procedures used for the RoC's preparation and review while maintaining a scientific, open, and transparent process. The NTP has evaluated all of the recommendations about the RoC received over the past three years and is considering some changes that should be announced in the near future.

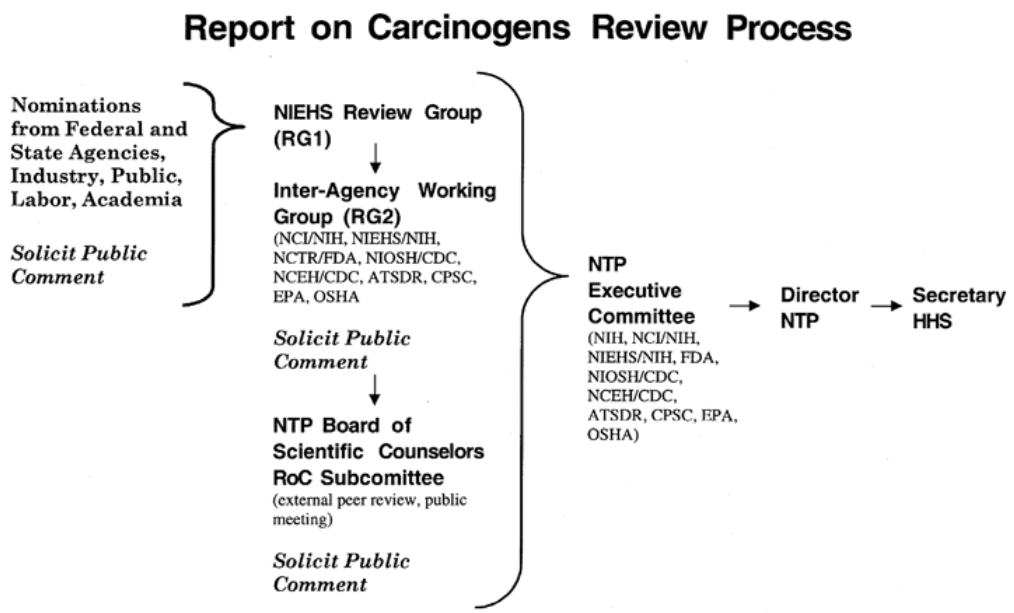

Figure 4. A diagram of the process for review of nominations for the *Report on Carcinogens* (RoC)

The 8th RoC is available on the NTP home page

(see above) or may be obtained by contacting the Environmental Health Information Service (EHIS, see page 26). The 9th Report is scheduled for publication in 2000. A list of the nominations under consideration for listing in or delisting from the 9th RoC is given in Table 7 and nominations under consideration for the 10th Report are given in Table 8. The preparation and review process for the RoC extends over approximately a two-year period; publication of the 10th Edition is anticipated for 2001.

Table 7
*Summary of the Agents, Substances, Mixtures, or Exposure Circumstances Reviewed for Possible Listing in or Delisting from the 9th Report on Carcinogen**

Alcoholic Beverage Consumption	Nickel compounds
1,3-Butadiene	Phenolphthalein
Benzidine-based Dyes as a class	Saccharin
Boot and shoe manufacture and repair	Silica, crystalline (respirable size)
Cadmium and Cadmium compounds	Smokeless tobacco
Chloroprene	Solar radiation and exposure to sunlamps and sunbeds
Diesel exhaust particulates	Sulfuric acid mist
Environmental tobacco smoke	Tamoxifen
Ethyl acrylate	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCCD)
Ethylene oxide	Tetrafluoroethylene
Isoprene	Tobacco smoking
Methyl-*t*-butyl ether	Trichloroethylene

*These agents were reviewed by three groups, the NIEHS/NIH Review Group (RG1), Inter-Agency Working Group (RG2), and the NTP Board of Scientific Counselors RoC Subcommittee. Actions by each of the groups can be found at the NTP web-site, http://ntp-server.niehs.nih.gov.

Table 8
Summary of the Agents, Substances, Mixtures, or Exposure Circumstances Nominated for Possible Listing in or Delisting from the 10th

Report on Carcinogen	
Reviewed in 1999/2000*	For Review in 2000/2001
2-Amino-3-methylimidzol[4,5-f]quinoline (IQ)	Chloramphenicol
Beryllium and Beryllium Compounds	Human Papillomaviruses (HPVs)
2,2-bis-(Bromomethyl)-1,3-propanediol	Lead and Lead Compounds
2,3-Dibromo-1-propanol	Methyleugenol
Dyes metabolized to 3,3'-Dimethoxybenzidine (Dimethoxybenzidine Dyes as a class)	Nickel (metallic) and Nickel Alloys
Dyes metabolized to 3,3'-Dimethylbenzidine (Dimethylbenzidine Dyes as a class)	Estrogens
Styrene-7,8-oxide	Talc (with and without asbestiform fibers)
Vinyl Bromide	Trichloroethylene
Vinyl Flouride	UV Radiation, Broad Spectrum (UVA, UVB, UVC)
	Wood Dust

*These agents were reviewed by three groups, the NIEHS/NIH Review Group (RG1), Inter-Agency Working Group (RG2), and the NTP Board of Scientific Counselors RoC Subcommittee.

NTP CENTERS

NTP Interagency Center for the Evaluation of Alternative Toxicological Methods

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) was established in 1997 in response to the 1993 NIH Revitalization Act to reduce, refine, or replace the use of animals in research and testing. ICCVAM has membership from 14 Federal agencies and programs (ATSDR; CPSC; Departments of Defense, Energy, Interior, and Transportation; EPA; FDA; NCI/NIH; NIEHS/NIH; NIH; NIOSH/CDC; National Library of Medicine/NIH; and OSHA). The Committee functions to provide cross-agency communication and coordination on issues relating to validation, acceptance, and national/international harmonization of toxicological testing methods.

The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) was established in 1998 to provide operational support for ICCVAM and to coordinate committee-related review panels and workshops. ICCVAM and NICEATM work to promote the validation and regulatory acceptance of toxicological test methods that are more predictive of human and ecological effects than those currently available and to communicate with stakeholders and the public. The desired outcomes from these new methods are an improvement in agencies abilities to assess risk and make regulatory decisions and the refinement, reduction, and replacement of animals in toxicological testing. The Advisory Committee on Alternative Toxicological Methods established by DHHS meets biannually to provide ICCVAM and NICEATM advice on activities and priorities. It is composed of knowledgeable representatives from academia, industry, public interest and animal welfare organizations, other agencies, and the international community.

Workshops are held, as needed, for evaluation of the adequacy of existing methods, identification of areas needing alternative methods, and evaluation of proposed validation studies. A formal, scientific review process is in place for evaluation of the validation status of proposed alternative testing methods. Briefly,

- ICCVAM considers whether a proposed alternative method should be evaluated.
- Peer review panels are convened and charged with development of a scientific consensus on the usefulness, limitations, and validation status of the proposed test method.
- ICCVAM reviews the panels document for scientific validity and potential acceptability of a test method and forwards the report to regulatory agencies. Each agency decides the regulatory acceptability of a new method according to its own mandates.

The murine Local Lymph Node Assay (LLNA), a method for assessing allergic contact dermatitis of chemicals, was reviewed in September 1998, and the final peer review report was made available in February 1999. The Peer Review Panel concluded that the LLNA is a valid alternative to currently accepted guinea pig test methods and also provides for the refinement and reduction of animal use. Additional recommendations on the usefulness and limitations of the LLNA are provided in the peer review report available from the Center or accessed on the web at http://iccvam.niehs.nih.gov /llnarep.htm. Unlike traditional guinea pig test methods, this mechanistic test quantifies the proliferation of lymphocytes in the regional lymph nodes in response to a chemical and can be used to evaluate its relative potency. From an operational and economic viewpoint, the LLNA also appears to be more efficient, quicker, cost less, use fewer animals, and subject the animals to less pain and distress. This was the first alternative test method to be reviewed using the ICCVAM process. The panel's conclusions were forwarded to the various ICCVAM agencies with a request for response and comments on implementation of LLNA. Agency response has been overwhelmingly favorable and the CPSC, EPA, OSHA, and FDA concurred with the Expert Panel and announced their acceptance of LLNA in October 1999.

In January 1999, a peer review panel reviewed the *in vitro* method, Corrositex^{R}, which is used for assessing the dermal corrosivity potential of chemicals. The Panel concluded that the method could be used for assessing the corrosivity of certain chemical classes and used in a tiered testing approach for some other classes. When used in this manner, the method provides for the refinement, reduction, and partial replacement of animal use.

The final peer review report was published in June 1999 and forwarded to agencies for their consideration. Copies of the report may be requested from the Center or accessed on the web at http://iccvam.niehs.nih.gov/corprrep.htm.

The Center and ICCVAM are currently coordinating an expert panel meeting on FETAX, the Frog Embryo Teratogenesis Assay in *Xenopus*. This test is proposed as a screening method to evaluate the developmental toxicity potential of chemicals. The panel will address the validation status of this method, current and potential applications, and provide recommendations regarding further research, development, and validation activities. The peer review of a revised Up and Down Procedure for Acute Oral Toxicity (OECD Test Guideline 425) is planned for mid-2000. ICCVAM also anticipates future review of selected endocrine disruptor screening and testing methods, new test methods proposed for evaluation of chemicals in the EPA High Production Volume Testing Program, and transgenic mouse models for carcinogenicity testing.

Information about ICCVAM and NICEATM is found at http://iccvam.niehs.nih.gov or contact: NICEATM, NIEHS/NIH, P.O. Box 12233, MD EC-17, Research Triangle Park, NC 27709, T: 919-541-3398, F: 919-541-0947, e-mail: iccvam@niehs.nih.gov. For specific questions about ICCVAM and the Center or regarding nominations of alternative testing methods contact:

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Selected Reading

1. NTP. Validation and Regulatory Acceptance of Toxicological Test Methods, A Report on the Ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods. NIH Publication No. 97-3981. Research Triangle Park: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NTP, 1997.
2. Chhabra RS, Bucher JR, Stokes WS. US National Toxicology Program strategies for use of alternate test systems. In: Animal Alternatives, Welfare and Ethics, (van Zutphen LFM, Balls M. eds) Elsevier Science, B.V.1997;607-615.

NTP Center for the Evaluation of Risks to Human Reproduction

The NTP and the NIEHS/NIH established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June 1998 in response to growing awareness, both public and scientific, that the ability of humans to conceive offspring and the normal, healthy development of children during pregnancy and childhood maybe adversely affected by environmental exposures. The primary purpose of the Center is to provide scientifically rigorous, unbiased, and timely evaluations of human and experimental evidence for adverse effects on reproduction, including development, caused by agents to which humans may be exposed. The goals of the individual assessments are to:

- interpret for and provide to the general public information about the strength of scientific evidence that a given exposure or exposure circumstance poses a hazard to reproduction and the health and welfare of children;
- provide regulatory agencies with objective and scientifically credible assessments of 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 welcome throughout and it can occur through 1) nomination of chemicals for evaluation, 2) submission of comments on the prioritizing and selecting of nominated chemicals, 3) submission of comments on the evaluation of a particular chemical at the time of an expert panel meeting, 4) nomination of scientists for the expert panels, and 5) submission of comments on the expert panel report. Nominations can come from interested individuals, Federal and State agencies, NTP staff, labor, and industry. A Core Committee, composed of representatives from NTP-participating agencies, reviews nominations and recommends candidate chemicals to the NTP. Subsequently, the Core Committee reviews dossiers prepared on the candidates by the Center and the public comments and submits a list of prioritized chemicals to the NTP for final selection. Chemicals for review are selected based upon several factors including production volume, extent of human exposures, public concern about chemical hazard, published evidence of reproductive or developmental toxicity, and evaluation of any additional information. An expert panel meets in a public session, reviews the scientific evidence on the chemical(s) under review, receives public comments, and then prepares a report on the chemical(s) that is published in *Environmental Health Perspectives* (EHP). NTP staff prepares a final NTP Center Report on the evaluated chemical(s) that integrates background information on the chemical(s), findings of the expert panel, a summary of the public comments, and a discussion of any additional, recent studies. This final report is made publicly available, distributed to Federal and State agencies, and published in EHP.

The Center's first Expert Panel was formed in summer 1999 to evaluate the scientific 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. Phthalates were chosen based on their high production volume, extent of human exposures, use in children's products, and/or published evidence of reproductive or developmental toxicity. The Expert Panel has met twice and expects to complete its review in the summer 2000. The Expert Panel's report, once completed, will be published in EHP and available on the Centers web site.

The nomination of chemicals for review or scientists for an expert panel can be made to the Center (NTP Center for the Evaluation of Risks to Human Reproduction, Attn: Dr. John A. Moore, 1800 Diagonal Road, Suite 500, Alexandria, VA 22314-2808, T: 703-838-9440, F: 703-684-2223). Additional

information about the Center can be obtained from its homepage or by contacting Dr. Michael D. Shelby, Director CERHR, NIEHS/NTP, P.O. Box 12233, MD B3-09, Research Triangle Park, NC 27709, T: 919-541-3455, F: 919-541-4634, e-mail: shelby@niehs.nih.gov.

NTP Center for Phototoxicology

The FDA has had an interest in the phototoxicity and photocarcinogenicity of therapeutics, cosmetics, devices, and food supplements and additives. Concurrently, the NIEHS/NIH developed a basic photobiology research program and through the NTP has considered nominations that required phototoxicology testing. To further promote research and testing in this area, the new FDA-NIEHS Phototoxicology Research and Testing Laboratory has been established and designated an NTP Center of Phototoxicology. The facility is located at the NCTR/FDA in Jefferson, Arkansas. The laboratory is designed for testing the effects of drugs, chemicals used in cosmetic preparations, and/or other agents (e.g., sun block additives, tanning enhancers, skin colorants, and tattoo inks) on UV radiation or simulated solar light-induced toxicity and cancer.

Studies conducted in the new laboratory will be planned to meet the rigors of scientific scrutiny and peer review. A standing committee (Toxicology Study Selection and Review Committee), composed of scientists with expertise in this area from FDA, NTP, NIEHS/NIH, other Federal agencies, and academia, review the design of protocols and progress on studies. Such studies should generate critically important scientific data for use in determining potential human health risks from the effects of therapeutic agents, chemicals used in cosmetics, device materials, food additives and supplements, tanning enhancers, etc. on light-induced skin toxicity and skin cancer. With the increasing exposure of the public to UV radiation or sunlight through more frequent use of tanning booths and leisure/pleasure time spent in sunlight-oriented activities (e.g., beach, swimming pool), research in this area is becoming increasingly important. This new center should have a significant impact on the quality of public health through the generation of scientific data that can facilitate accurate and mechanistically based determinations of human cancer or other health risks from combinations of drugs or compounds with sunlight.

Interest in developing a jointly operated phototoxicology research and testing laboratory was heightened with FDA's nomination of a-hydroxy acids to the NTP. These compounds are primarily used as dermatological chemoexfoliants, and two consequences of chemoexfoliation are an increased proliferation of epidermal epithelial cells and deeper penetration of electromagnetic radiation into the skin. A facility for conducting phototoxicity, photoimmunotoxicity, and photocarcinogenicity studies using UV light or simulated solar light did not exist at FDA or NIEHS/NIH, thus a need was created for the FDA-NIEHS Phototoxicology Research and Testing Laboratory. Through an interagency agreement between FDA and NIEHS/NIH, a study is underway to investigate the effect of a-hydroxy acids and a-hydroxy acid treatment on the induction of mouse skin cancer by simulated solar light or fluorescent UV radiation. This study uses the SKH-1 hairless mouse as the primary test animal, which is the recognized model for such studies. Additional test animals (e.g., transgenics models) may be included in subsequent proposals on this and other compounds/treatments.

COMMUNICATION AND PUBLIC OUTREACH

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

On-line, searchable access and printed copies of NTP publications including the *Report on Carcinogens*, NTP Technical Reports, NTP Toxicology Reports, and other NTP documents, are available through the Environmental Health Information Service (EHIS) at http://ehis.niehs.nih.gov. Subscription packages to the EHIS include access to NTP publications as well as *Environmental Health Perspectives* (primary issues and supplements), the Rodent Historical Controls Database, the Chemical Health and Safety Database, and a variety of other services. To purchase single copies or to subscribe on-line, visit the EHIS at http://ehis.niehs.nih.gov, e-mail: ehis@niehs.nih.gov, or call 1-800-315-3010.

The Central Data Management Office oversees distribution (upon 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, and summaries of minutes from NTP meetings (Board of Scientific Counselors and its subcommittees). Requests for these documents should be directed to: Central Data Management, NIEHS/NIH, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709, T: 919-541-3419, F: 919-541-3687, e-mail: cdm@niehs.nih.gov.

The NTP is interested in and welcomes stakeholder input into its programs and priorities. Nominations, inquiries, and comments from the public and other interested parties are welcome at any time. The NTP Liaison and Scientific Review Office serves as the focal point for receiving input to the Program and for overseeing the distribution of information about programs, workshops, initiatives, etc. Questions regarding the program, comments, and input can be submitted to: NTP Liaison and Scientific Review Office, NIEHS/NIH, P.O. Box 12233, Research Triangle Park, NC 27709, T: 919-541-0530, F: 919-541-0295, e-mail: liaison@starbase.niehs.nih.gov.

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