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The National Toxicology Program and Immunological Toxicology*

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Introduction

THE NATIONAL Toxicology Program (NTP) was established in November 1978 (4) to study potentially toxic and hazardous chemicals and to develop scientific information that can be used for protecting the health of the American people and for the primary prevention of chemically induced disease. NTP centralizes and strengthens the Department of Health and Human Services (DHHS, formerly DHEW) activities in toxicology research, testing, and test development/validation efforts, and provides toxicological information needed by research and regulatory agencies (18, 20, 29). Specific goals are to: expand the toxicological profiles of the chemicals nominated, selected, and being tested; increase the number and rate of chemicals under test, as funding permits; develop, coordinate, and validate a series of tests/protocols more appropriate for regulatory needs; and communicate Program plans and results to governmental agencies, the medical and scientific communities, and the public.

Although chemical testing for toxicity, particularly for carcinogenicity and mutagenicity, remains a major Program focus, there has been a modest but significant increase in the proportion of resources committed to test method development and validation. Efforts to develop and validate protocols for assessing the effects of chemicals on immune response and host resistance are tangible examples of these types of efforts (23, 25, 32).

Several aspects of the National Toxicology Program have proven to be of general interest or have proven to be valuable mechanisms to ensure that NTP programs are responsive to current toxicological needs and represent a sound scientific basis.

1. Research and Testing Activities. The *Annual Plans* (20–24) and *Technical Bulletins* (9, 13) define current and planned research and testing activities. Through broad and timely distribution, all sectors of the scientific community may offer constructuve comments, which in a number of instances have prevented unnecessary duplication of effort or provided an opportunity to revise and enhance the significance of selected scientific initiatives (10, 11, 29, 30, 32, 33).

2. NTP Executive Committee. This committee brings together the research and regulatory agencies to ensure that toxicology research, testing, and test development carried out under the aegis of the NTP are responsive to the needs of those agencies and to the wants of the public (5, 25, 26, 31). The governmental offices and agencies that comprise the NTP Executive Committee are: Chairman, Consumer Product Safety Commission; Assistant Secretary for Health, Department of Health and Human Services; Administrator, Environmental Protection Agency; Commissioner, Food and Drug Administration; Director, National Cancer Institute; Director, National Institute for Occupational Safety and Health; Director, National Institute of Environmental Health Sciences; Director, National Institutes of Health; and Assistant Secretary of Labor, Occupational Safety and Health Administration. The Executive Committee reviews and approves the Annual Plan as well as selects and sets priorities on those chemicals selected to be tested.

3. NTP Board of Scientific Counselors. This Board provides scientific oversight of the NTP; advises the NTP Director and the NTP Executive Committee on scientific content, philosophy, and policy; and evaluates the merit and overall quality of the science conducted in the NTP components. Eight scientists initially appointed by the Secretary of the Department of Health and Human Services are (terms end during year shown in parentheses): Joseph C. Dunbar, Ph.D., Associate Professor of Physiology, Wayne State University School of Medicine (1982); Curtis Harper, Ph.D., Associate Professor of Pharmacology, University of North Carolina School of Medicine (1981); Margaret Hitchcock, Ph.D., Assistant Professor of Pharmacology, Yale University Medical School (1983); Majorie G. Horning, Ph.D., Professor of Biochemistry, Baylor College of Medicine (1983); Mortimer L. Mendelsohn, M.D., Ph.D., Director, Biochemical Sciences Division, Lawrence Livermore Laboratory, University of California (1982); Norton Nelson, Ph.D. (Chairperson), Professor, Environmental Medicine, New York University School of Medicine (1983); Thomas H. Shepard, M.D., Professor of Pediatrics and Head of Central Laboratory for Human Enbryology, University of Washington School of Medicine (1981); and Alice S. Whittemore, Ph.D., Adjunct Professor of Family, Community, and Preventive Medicine, Stanford University (1983).

4. NTP Chemical Nomination and Selection. More chemicals are nominated for NTP consideration than can be selected for study. Early recognition of this pending asymmetry led the NTP Executive Committee to formulate a set of program guidelines (18, 25, 32). These resultant eight chemical selection criteria motivate an

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NTP matrix that operates throughout the NTP. All research, testing, and test development/validation efforts start here.

The NTP Executive Committee operates under the principle that industry will test chemicals for health and environmental effects as intended and mandated by Congress under legislative authorities. Therefore, the NTP, acting under its chemical selection principles, will test; (a) chemicals found in the environment that are not closely associated with commercial activities; (b) desirable substitutes for existing chemicals, particularly therapeutic agents, that might not be developed or tested without federal involvement; (c) chemicals that should be tested to improve scientific understanding of structure-activity relationships and thereby assist in defining groups of commercial chemicals that should be tested by industry; (d) certain chemicals tested by industry, or by others, the additional testing of which by the federal government is justified to verify the results; (e) previously tested chemicals for which other testing is desirable to cross-compare testing methods; (f) "old chemicals" with the potential for significant human exposure that are of social importance but which generate too little revenue to support an adequate testing program (some of these may be "grandfathered" under FDA laws); (g) two or more chemicals together, when combined human exposure occurs (such testing probably cannot be required of industry if the products of different companies are involved); and (h) in special situations, as determined by the Executive Committee, marketed chemicals that have potential for large-scale and/or intense human exposure, even if it may be possible to require industry to perform the testing.

Most chemicals are nominated and selected for testing because toxicological information is lacking and because the potential exists for human exposure. Other important criteria include production levels, physical and chemical properties, agency interests, and significance to society. The NTP toxicology testing strategy is to identify with assurance the major toxic effects for each chemical studied. This includes (in addition to identifying chemical mutagens and carcinogens) damage to critical target organs such as the lungs, liver, and nervous system.

Nominations of chemicals for toxicological testing are submitted by the NTP participating agencies as well as other government agencies, industry, labor, and the public. The nominating source is asked to submit the name of the chemical, the particular toxicological tests desired, the rationale for testing, and to provide the available background data on production, use, exposure, environmental occurrence, and toxic properties in a supporting summary document.

An initial examination determines which proposed chemicals have already been tested, are on test, are scheduled for test, or have been previously considered and rejected for testing by the NTP or its predecessors.

Literature containing relevant data are assessed and literature summaries are prepared for each chemical. Chemicals nominated for mutagenicity testing are only reviewed with respect to the available genetic toxicology information. Included in each literature summary are sections on: Chemical Identification, Surveillance Index, Human Exposure and Health Effects, Research Hypothesis to be Tested, Categories of Study, and Source of and Reason(s) for Nomination.

These summaries are reviewed and evaluated by the Chemical Evaluation Committee (CEC) (composed of representatives from CPSC, EPA, FDA, OSHA, NCI, NCTR, NIEHS, NIOSH, and NTP) who recommend the type(s) of testing to be considered. Any recommendations must satisfy at least one of the eight NTP principles of chemical selection.

Concurrently, announcements appear in the *Federal Register* and the *NTP Technical Bulletin* listing the chemicals and the recommended types of testing. The notice solicits comments as well as information on completed, ongoing, and planned testing in the private sector. These steps are taken to encourage others outside the immediate program to participate in the NTP evaluation and selection process. Revised summaries with additional public input are forwarded to the Board of Scientific Counselors for review. The Board evaluates the data and makes recommendations to the Executive Committee.

When the final summaries are submitted, the NTP Executive Committee decides whether to select, defer, or reject the chemicals for testing. Following Executive Committee action the chemicals are referred to one or more participating agencies in the NTP: NIEHS, NIOSH, NCTR. At this point certain approved chemicals may be identified as being inappropriate candidates for testing as a result of technical or budgetary reasons or in some cases public information describing ongoing testing may only have been submitted following Executive Committee decision. Such chemicals are then returned to the Executive Committee for reconsideration.

All chemicals selected are then tested as time and resources permit.

5. Test Development and Validation. The strategy for test method development and validation examines existing and emerging methodologies to identify those that may be adequately sensitive and reproducible (25, 26). Those offering improvements over older methods will be selected for validation. When basic research findings suggest new areas of toxicological testing, NTP will undertake the appropriate method development and validation. Existing methodologies that are being examined for modification include techniques used to detect impaired liver or kidney function and neurobehavioral toxicity; and new areas undergoing method development and validation include behavioral teratology, immunotoxicology, short-term tests for presumptive carcinogenic potential, and fertility and reproductive toxicology.

Test method validation signals a two-stage process: 1) Does the procedure(s) yield test results that are reproducible within and between laboratories? 2) Does the test(s) predict for toxic potential in humans? The latter

demands that NTP keep abreast of and examine closely any results from human epidemiological studies that correlate or contrast with experimental test data. The NTP approach to testing emphasizes developing new and better test methods. This overture does not imply flaws in traditional toxicology and regulatory test requirements, but reflects rapid advancements in testing methodology and expanding boundaries of scientific knowledge. Thus, NTP plans to validate possible alternatives that may be performed more reliably, yield new toxicological data, give results relevant to human disease, and develop a testing approach that produces equivalent results in a faster, more economical manner. Often, testing results affect regulatory or public health issues, and the NTP will meld these innovative techniques with "standard" methods to ensure results that are germane and of utility to regulatory and public health needs. When standard methods are used, the NTP will attempt to incorporate those standards presently advocated by regulatory agencies, such as the life-time rodent bioassay.

6. Toxicology Research and Testing. Toxicology research and testing within NTP is divided into three major disciplines: genetic toxicology, general toxicology, and carcinogenesis (12, 18, 23, 24, 25, 26, 32). Other program areas have been arbitrarily assigned to one of these three, even though much interchange occurs; for example, interprogram utility of *Salmonella*/microsome assays within the genetic toxicology and the carcinogenesis programs.

The following section gives a brief yet highlighted overview of the immunological toxicology program of the NTP. (For more details about this activity see J. H. Dean et al., "Procedures Available to Examine the Immunotoxicity of Chemicals and Drugs," in this workshop pages 137–148.)

7. Immunotoxicology. The primary goals of the Immunotoxicology Program of the NTP are to select and validate a simple yet selective panel of assays to precisely and reproducibly assess alterations of immune function and host resistance after exposure to immunotoxic chemicals or drugs. The objectives of this program are being accomplished through an in-house research effort at the National Institute of Environmental Health Sciences and through research and development contracts that allow interlaboratory validation of new methods with chemicals of known immunotoxic potency. Presently under development, standardization, or routine application in rodents are methods for assessing delayed hypersensitivity responses to novel antigens; quantification of thymus-dependent lymphocyte (T cell) function and numbers; assessment of bursal-equivalent (B cell) responses to T-dependent and independent antigens; parameters of macrophage function; quantification of bone marrow cellularity and colony-forming cell units; and resistance to bacterial, virus, parasite, and tumor cell transplant challenge. Study protocols encompass both adult exposure and prenatal exposure during lymphoid organogenesis, which appears to be a period of maximal sensitivity to immunotoxic chemicals or drugs. Another major emphasis is to gain an understanding of the nature and magnitude of immune alteration predisposing to altered resistance to bacteria, viruses, parasites, or neoplastically transformed cells.

Immune deficiency diseases or cytoreductive chemotherapy are associated with a higher incidence of infectious diseases and secondary malignancy. The evidence for increased bacterial, viral, fungal, and parasitic diseases in patients on chronic immunosuppressive therapy has been well documented (1). Infections are also a major cause of postsurgical complication believed due to transient postoperative immunosuppression caused by stress or halogenated hydrocarbon gas anesthesia. McKhann (17) observed that the incidence of secondary cancer in renal transplant recipients on prolonged immunosuppressive chemotherapy was 4.6 to 61 times higher than observed in the general population. In McKhann's study when only lymphoreticular cancers are considered, the incidence of tumor development was 333% higher than in the general population. Squamous cell carcinoma of the skin was observed in 14% of patients on prolonged chemotherapy following renal transplants. Penn and Starzl (27) found the incidence of malignant tumors in transplant recipients approximately 80 times greater than an equivalent control population. Likewise, Gatti and Good (8) observed a significantly higher frequency of lymphoreticular neoplasia in patients with primary immunodeficiency diseases and suggested that most of these individuals died of bacterial or fungal infections before they were old enough to express solid tumors. Studies in laboratory animals (6, 15, 19), support these clinical observations and demonstrate an enhanced incidence of ultraviolet-induced or benzopyrene-induced cancer in mice treated with immunosuppressive agents. The mechanistic relationship between carcinogenesis and immune alterations is complex and poorly understood, although these data support the hypothesis that immune dysfunction may serve as a cocarcinogen in the etiology of some tumors (2, 16, 28). Unfortunately, little insight has been gained about which of the various effector mechanisms of the immune response is essential for host resistance to infectious agents since the discovery almost 20 years ago of the distinct thymus-dependent and bursal equivalent derived lymphocyte systems. Recently, the use of immunotoxic chemicals and drugs as probes has begun to help provide a new understanding into the complex relationship that exists between immune function and host resistance. Additionally, the advent of new monoclonal reagents that allow cell subset identification and selection should also facilitate this understanding.

Immune dysfunction as evidenced by depressed antibody-mediated immunity and/or cell-mediated immunity (CMI) has been observed in rodents exposed to sublethal levels of chemicals of environmental concern. Chemicals that produced immune alterations in rodents include 2,3,7,8-tetrachlorodibenzo-*p* dioxin (TCDD); polychlorinated biphenyls (PCB); polybrominated bi-

phenyls (PBB); gallic acid; hexachlorobenzene (HCB); orthophenylphenol; organometals; and heavy metals [see reviews (7, 14, 34)]. In some studies exposure to immunotoxic chemicals also alters resistance to bacteria, viruses, parasites, and transplantable tumor cell challenge [see reviews (7, 34)]. In addition, exposure of humans to polybrominated biphenyls has been associated with immune dysfunctions similar to that observed in rodent studies (3).

Data provided by NTP's special rodent immunology studies should provide insight into mechanisms of potential immunotoxicity in humans. Furthermore, a comprehensive assessment of the immunological safety of a suspect agent should reduce the potential risk to humans of newly manufactured chemicals or drugs.

8. Information Generation and Dissemination. The National Toxicology Program must ascertain the toxicology of selected chemicals and assure that results will have scientific and regulatory significance. The end product is information—scientific information necessary in deciding social issues relative to public health and the environment. To provide that information, the NTP identified two important aspects: first, information must be disseminated to other scientists so that peer review and feedback assure scientific quality; second, since the scientific product helps society evaluate identified toxicological risks, information must be disseminated to not only the regulators responsible for protecting against potentially hazardous risks, but also to those exposed to the risks. Thus, the NTP has established and uses a coordinated communications network to disseminate toxicological information.

The value of information arising from NTP depends in part on the quality and timeliness of information received into the program. The NTP therefore actively seeks information from all sources: federal, state, and local governments; trade associations, industry, and labor; academia; professional societies and public interest groups; the press; individuals; other countries; and all other interested parties. Information received includes nominations of chemicals to be tested; critique and questions about scientific procedures, policies, priorities, and resource allocations; and any other suggestions for program improvement. To encourage multiple communication, NTP program materials are and must be disseminated widely and rapidly, and questions answered in a timely manner.

NTP Publications. In addition to the NTP technical reports, journal articles, and other research documentation, the NTP makes available four publications: *NTP Annual Plans, NTP Annual Reviews of Current DHHS, DOE, and EPA Research Related to Toxicology, NTP Quarterly Technical Bulletins,* and *NTP Annual Reports on Carcinogens.* Copies of these reports and other information about the NTP can be obtained by writing to: NTP Public Information Office, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709.

Additionally, NTP welcomes questions, comments, and suggestions about the National Toxicology Program.

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