
Chapter 4

The National Toxicology Program

CONTENTS

	<i>Page</i>
Background	147
The Need for Testing	147
History of Major Federal Efforts in Carcinogenicity Testing	148
NCI Testing Activities	148
Establishment of the National Toxicology Program	149
Organization of NTP	150
Structure	150
Resources	151
Nominating Chemicals for Testing	152
Selection	152
NTP Carcinogenicity Testing	154
Methods of Study and Analysis	154
Observations on the NTP Nomination and Selection Process	157
Criteria for Nomination and Selection	157
Number Source, and Disposition of Nominated Chemicals	160
Duration of Testing Process Stages	161
Case Study in Nomination and Selection: the Benzodiazepines	161
Mutagenicity Testing: Correlation With Animal Carcinogenicity	164
Relation of Chemical Carcinogenicity in Animals to Cancer in Humans.	166
Summary	167

Figures

<i>Figure No.</i>	<i>Page</i>
4-1. National Toxicology Program	150
4-2. NTP Chemical Nomination and Selection Process	153

Tables

<i>Table No.</i>	<i>Page</i>
4-1. Costs of NTP Studies of Fiscal Year 1986	152
4-2. NTP Chemical Selection Principles	158
4-3. Source of Nomination of Chemicals for Mutagenicity and Bioassay Testing by NTP by Year	159
4-4. Initial Response of Board of Scientific Counselors to Actions of the Chemical Evaluation Committee on 164 Chemicals, Fiscal Year 1982- Fiscal Year 1986	161
4-5. Time (in months) From Nomination of Chemicals Considered by the Chemical Evaluation Committee in Fiscal Year 1981 and Fiscal Year 1982 to Intermediate and Final Points in the Process of Approval for Testing by NTP	162
4-6. Status of Chemicals Approved for Testing by the NTP Executive Committee in Fiscal Year 1981 or Fiscal Year 1982 (Status as of January 1987)	162
4-7. History of Agency Priority Chemicals (Status as of April 1987)	162

The National Toxicology Program

BACKGROUND

In the 1960s, government agencies, especially the National Cancer Institute (NCI), used animal tests to predict carcinogenicity, though at first to learn more about the relation between chemical structure and carcinogenicity and not for regulatory purposes. In November of 1978, the Secretary of the then Department of Health, Education, and Welfare (DHEW) established the National Toxicology Program (NTP), aware of the need to test chemicals for carcinogenicity (and other toxic end points), the limited ability of existing programs to keep up with the demands of new legislation, and the lack of coordinated testing.

Cancers often develop more quickly in animals than in humans, although not in relation to life-span. Still, animal tests take time, 2 years of exposure for rodents, for example, and the tests are costly. In the 1970s, based primarily on the work of Bruce Ames, a high correlation was found between tests for mutagenicity of chemicals in microorganisms and carcinogenicity in animals (217). These genetic toxicology tests, and a second generation of short-term tests that followed, can be performed in days rather than years, and are much less costly than animal tests. The hope was expressed in DHEW that “by 1985 . . . better test systems will begin to replace the tedious and costly animal assay now required” (60). This

optimism has proved unfounded; the new tests have not proven superior to the original Ames test, which itself is an imperfect predictor of animal carcinogenicity.

Most animal carcinogenicity testing was transferred from NCI to NTP in 1981. In the first part of this chapter, the origins, support, and organization of NTP are described and the NTP process of selecting chemicals for testing is analyzed. A discussion follows of the relation of the results of the short-term tests to those of animal carcinogenicity studies, and finally the predictability of human carcinogenesis from animal tests. Some of these issues were discussed in chapter 2; they will be examined here only with regard to NTP.

Since this background paper focuses on the relation of carcinogen studies to regulatory decisions and the research activities of NTP, many of them conducted with the National Institute of Environmental Health Sciences (NIEHS) and the National Center for Toxicology Research (NCTR), receive scant consideration. NTP's goals include understanding the mechanisms by which cancers are initiated and propagated and developing better and quicker methods of determining chemicals' carcinogenicity.

THE NEED FOR TESTING

In 1980, NTP contracted with the National Research Council (NRC) to conduct a study, with a charge “to characterize the toxicity-testing needs for substances to which there is known or anticipated human exposure” (140). From approximately 5 million chemicals the Study committee compiled a list of 53,500 chemicals in 7 categories of human exposure. By systematic sampling of chemicals in each category, 675 chemicals were selected from this list. Multiple sources were examined to determine whether toxicity testing had

been conducted on these 675 chemicals. Extrapolating to the entire list, the committee estimated that there was no toxicity information on 38 percent of pesticides, 56 percent of cosmetic ingredients, 25 percent of drugs and excipients used in drug formulations, 46 percent of food additives, 78 percent of chemicals in commerce of which over 1 million pounds were produced in 1977, 76 percent of chemicals of which under 1 million pounds were produced, and 82 percent of chemicals whose production status was unknown or in-

determinable.¹ Tests for chronic toxicity were performed most frequently on drugs (39 percent) and least frequently on chemicals in commerce (3 to 4 percent). From the list of 675 chemicals, the committee selected 100 on which some toxicity information was available; 10, 15, or 20 were selected from each of the 7 categories to deter-

¹Chemicals that were environmental decomposition products, manufacturing contaminants, or natural substances were not systematically included.

mine the type and adequacy of testing. The report concluded, "Only about 8 percent of the tests met the standards of the reference protocol guidelines, and about another 19 percent were judged to be adequate." In discussions with OTA in 1986, Dr. Ernest McConnell, Director of the Toxicology Research and Testing Program, which is the principal NIEHS component of NTP, estimated that approximately 1,000 chemicals with high human exposure potential should be tested (120).

HISTORY OF MAJOR FEDERAL EFFORTS IN CARCINOGENICITY TESTING

NCI Testing Activities

NCI began animal testing of chemicals for carcinogenicity in 1961. Elizabeth Weisburger, one of NCI's charter researchers, described the aims of the project:

There was no mention of a program for large-scale bioassay of industrial or environmental materials. To quote, "first priority should be given to chemicals most likely to make a contribution to our knowledge of the etiology of cancer and deepen our understanding of their mode of action" (357).

In the late 1960s, NCI responded to demands for testing chemicals in the environment. In 1970, for instance, it initiated contracts for studies of 40 pesticides approved for use in the United States. Appropriations under the National Cancer Act of 1971 provided sufficient funds to initiate a greater number of long-term animal studies, which reached a peak of 200 in 1972. The increase was so rapid that the consequences were not fully appreciated. "Neither NCI nor the prime contractor had enough assistance in pathology to examine all the microscope slides which resulted" (357). The backlog of chemical studies was not eliminated until 1979.

Government laboratories could not accommodate the volume of testing. Moreover, these laboratories were designated primarily for basic research, not for the routine testing of chemicals for

toxicity.² Consequently, most of the animal tests were performed contractually by nongovernment laboratories. In 1973, NCI contracted with Tracer Jitco, Inc., to oversee the bioassay operations of the other contractors. Tracer Jitco also supplied data on chemicals being considered for testing to the Chemical Selection Working Group (CSWG) in NCI, which was responsible for the actual selection. A General Accounting Office report (201) found fault with this system. As a result, NCI instituted stricter monitoring of Tracer Jitco and its other contractors.

Despite concern over carcinogens in the environment, which contributed to the flood of testing in the 1970s, the process of notifying regulators was neither easy nor uncontroversial. Publications in the scientific literature indicating the carcinogenicity of 1,2-dibromoethane and 1,2-dibromo-3-chloropropane (in 1973) "led to no consternation or notice among regulatory agents [sic]" (357). To overcome this, NCI issued a "memorandum of alert" in 1975, when it became evident that trichloroethylene (TCE) was causing an increase of liver tumors with lung metastasis in some animals. According to Weisburger:

²In a congressional hearing in 1981, Dr. Vincent DeVita, director of NCI, commented that NCI "never developed this [animal testing] program to be a source of information for the regulatory agencies. Therefore, when suddenly there was pressure for us to provide routine information, we were not able nor properly constructed to do that" (213).

Criticism of the “memo of alert” was so great that this mechanism was not used again. Instead, the complete record of any bioassay was compiled in a Carcinogenesis Technical Report; draft versions of the reports were sent to the regulatory agencies for their information prior to release to the public (357).

The furor over the preliminary publication arose because the Food and Drug Administration (FDA) could no longer consider TCE as an acceptable solvent for decaffeination under the Delaney clause. According to Weisburger, NCI staff were unaware that it was used for that purpose.

Problems of communication generally intensified as more agencies became involved in regulating carcinogens. These agencies had the capability to perform tests for carcinogenicity or the authority to require industry to do so. No channels existed for agencies to communicate about the chemical tests that were in progress or recommended; duplicate testing sometimes resulted.

Sometimes testing was beyond the capability of an agency, yet information on the toxicity of a chemical would have been helpful in making regulatory decisions. No formal mechanism existed for regulatory agencies to request NCI testing. “The entire process [of test selection] was quite informal with discussion among [NCI] staff only” (357).

Establishment of the National Toxicology Program

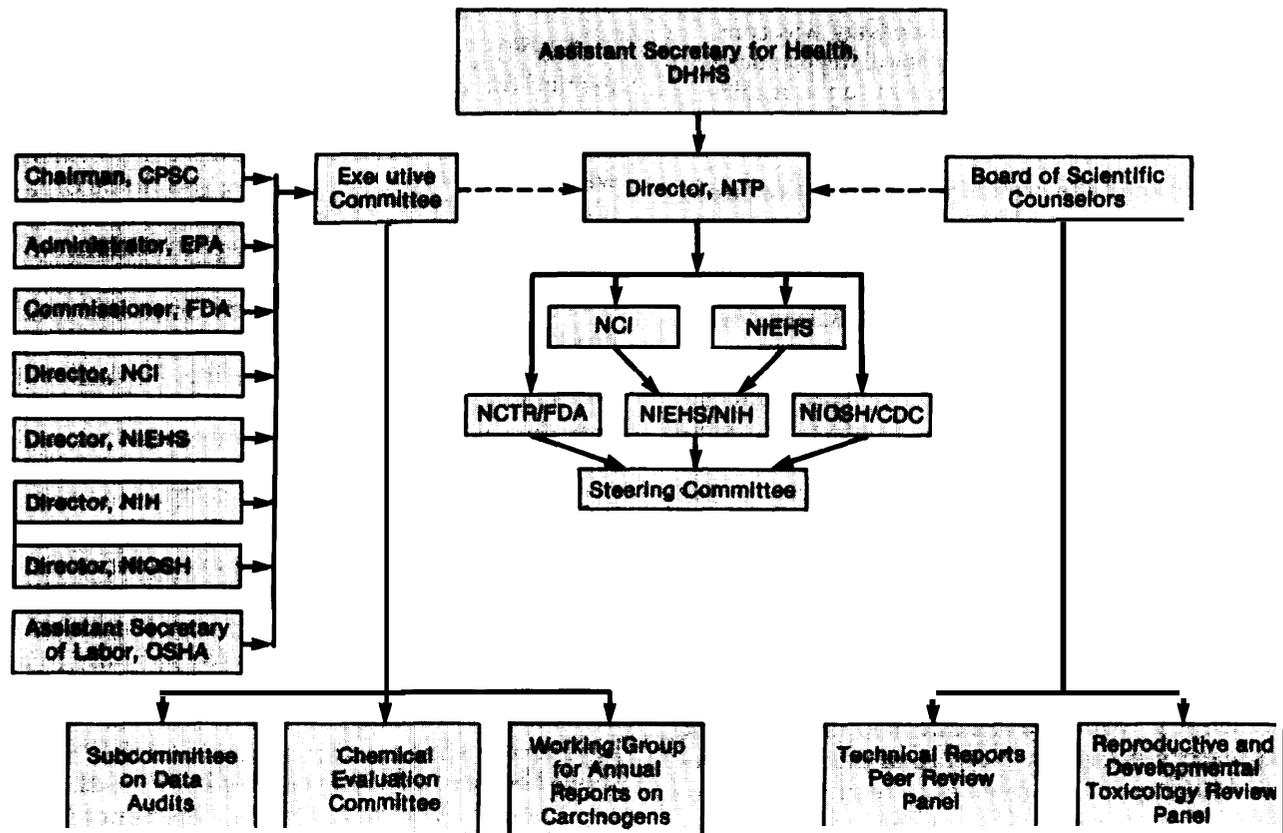
NTP was established in November 1978 by the Secretary of the then Department of Health, Education, and Welfare “to strengthen the Department’s activities in the testing of chemicals of public health concern, as well as in the development and validation of new and better integrated test methods” (268). To accomplish its goals, NTP was “comprised of the relevant [Public Health Service] activities” within FDA (namely, NCTR), NCI, Centers for Disease Control (CDC) (namely, the National Institute for Occupational Safety and Health (NIOSH)), and the NIEHS. Dr. David Rail, Director of NIEHS, was named Director of NTP, reporting to the Assistant Secretary for

Health. The organizational structure of NTP is shown in figure 4-1.

A 1981 paper prepared by NIEHS as background for a congressional hearing on NTP (90), commented that NTP was established as an interim measure because there was disagreement within DHEW as to how testing should be organized. The NTP Director was expected to coordinate the activities of various departmental components, but he could not “allocate resources, either funds or personnel, to areas of greater need and priority, except through agreement with the other agency heads.” At the hearing, Dr. Rail and Dr. Ronald Hart, director of NCTR, indicated that they were in frequent communication and that there was “a minimal amount of confusion” (213). Dr. Rail also delineated NTP’s role from that of the regulatory agencies. NTP’s responsibility was in risk identification and quantification, primarily in animals; the agencies’ responsibilities were determining human exposures and evaluating human risks and benefits. In response to a question from Congressman Albert Gore, Dr. Rail indicated that the allocation of so much of NTP’s budget on testing was not optimal and that more should be devoted to developing better methods. Dr. Hart, Dr. Vincent P. DeVita, Director of NCI, and Dr. Millar, Director of NIOSH, emphasized NTP’s role in testing chemicals.

In October 1981, the Secretary of the Department of Health and Human Services (DHHS) granted NTP permanent status. The funding arrangements remain voluntary. As stipulated in the original announcement, memoranda of understanding are signed by the head of each cooperating agency and the NTP Director, specifying the resources to be devoted to NTP, and identifying by organizational title the supporting elements of the participating agencies and their responsibilities (e.g., specific studies to be undertaken). With the transfer of the NCI Carcinogenesis Testing Program to NIEHS in July 1981, the vast majority of funds (87 percent of the NTP budget at the time) come from NIEHS. Dr. David Rail determines how much NIEHS will contribute to NTP, as the heads of NCTR and NIOSH determine their agencies’ contributions. At the present time, most

Figure 4-1.—National Toxicology Program (NTP)



SOURCE: National Toxicology Program, 1987.

of the staff assigned to NTP comes from NIEHS, consistent with the contribution NIEHS makes to NTP's budget. The memorandum of understanding between NIEHS and NTP indicates that the NIEHS Toxicology Research and Testing Program is "dedicated to the National Toxicology Pro-

gram." It lists the NIEHS program elements and key scientists committed to NTP, accepts responsibility for administration, and indicates its contributions to NTP in person-years, budget, and space (234).

ORGANIZATION OF NTP

Structure

The NTP Steering Committee was formed in 1980 to promote "cooperative working relationships" among the contributing DHHS agencies. The committee consists of the NTP Director and the heads of NIEHS, NCTR, and NIOSH. It meets three to four times yearly to review programs and

projects, resolve interagency problems, and make agency allocations for approved chemical toxicological studies (257).

The documentation of NTP activities and plans is accomplished through an annual plan, as stipulated by the Secretary of DHEW (268), who also specified the plan's contents: information on cur-

rent toxicology testing capacity and capacity in the coming year on plans for test development and validation, on the compounds to be tested, and on the regulatory and scientific opportunities that were considered in developing the plan. The Secretary established an Executive Committee to approve and monitor the annual plan. This committee consists of the heads of four regulatory agencies—the Environmental Protection Agency (EPA), FDA, the Occupational Safety and Health Administration (OSHA), and the Consumer Product Safety Commission (CPSC)—and the heads of the National Institute of Health (NIH), NCI, NIOSH, and NIEHS. On April 1, 1987, the Executive Committee voted to add the Agency for Toxic Substances and Diseases Registry to its membership. In addition, the Assistant Secretary for Health of DHHS is a nonvoting member and the Director of NCTR is a nonvoting consultant.

In testimony before Congress in 1980, Dr. Rail indicated that he had proposed that the Departments of Energy and Agriculture join the NTP Executive Committee (205). This has not happened. The composition of the Executive Committee provided the regulatory agencies outside of DHHS input to the planning and operation of NTP. These agencies, partly through the Executive Committee, have an important role in NTP activities, particularly in setting priorities for chemicals studies and in coordinating testing. In 1980, NTP reported that several chemicals recommended for industry testing by the Interagency Testing Committee (ITC) under the Toxic Substances Control Act (TSCA) were under test or scheduled for test by NTP (253). Since 1980, NTP has had a liaison representative with ITC to avoid redundancy of testing.

Resources

The number of chemicals tested depends primarily on the resources available. The budget for NTP activities (including NCI's contribution for testing) increased approximately 40 percent between 1979 and 1981. From fiscal year 1981 to 1987 the total NTP budget rose (including contributions from NCTR and NIOSH) from \$70.5 to \$77.9 million. After adjustment for inflation, this represents a small decline. The budget percentage devoted to testing fluctuated between 66

and 74 percent. Remaining funds were used for developing and validating testing methods and for management expenses. After NTP was established in 1978, participating agencies expected that they would receive larger appropriations to allocate to testing under NTP.

Since 1981, the inclusion of additional short-term tests, more detailed prechronic testing, and the use of three experimental doses and controls instead of two in chronic studies, which entails a greater number of animals, has increased the costs of testing a single substance. To resemble human exposures more closely, inhalation studies are being used more frequently than in the past. These studies entail special equipment and are the most costly of the chronic studies. Expenditures for analytical chemistry, a chemical repository, and auditing of data and laboratory practices have also increased.

The costs of various types of tests in fiscal year 1986 are shown in table 4-1. The prechronic study (to identify target organ toxicities and determine the doses to be used in the chronic phase) and the chronic study of a single chemical often cost over \$2 million. (The cost of the Salmonella assay, or, "Ames" test, is about \$3,300.) In fiscal year 1987 an estimated 43 chemicals will be in the prechronic phase of testing, including beginning studies ("starts") on an estimated 30, and 137 will be in the chronic phase, including starts on an estimated 7.

NCTR's budget allocated to NTP activities fell from \$6.1 million in fiscal year 1981 to \$4.3 million in 1986, with only \$1 million estimated for 1987. As a result of the budget reductions recently necessitated by the Gramm-Rudman-Hollings Act, NCTR discontinued long-term animal tests under NTP on one antihistamine and continued two others only when NTP through NIEHS agreed to fund their completion. NCTR is testing other chemicals through NTP. NIOSH's allocations to NTP have fluctuated considerably between 1981 and 1986: \$4.1 million in 1981 (the highest allocation), \$1.8 million in 1985 (the lowest), and \$3.7 million estimated for 1987. Not all of these funds are used for testing. Staff scientists who serve as chemical managers at NCTR and NIOSH continue to design protocols for test-

Table 4-1.—Costs of NTP Studies of Fiscal Year 1986

Type of study	Cost per study ^a	
Mutagenicity:		
Drosophila	\$ 11,083	
Salmonella	3,328	
Cytogenetics	12,932	
Mouse lymphoma	6,500	
Fertility & Reproduction:		
Fertility assessment	80,300	
Sperm morphology	5,300	
Teratology:		
Conventional	68,000	
Inhalation	350,000	
Prechronic Studies:^b		
	Low range ^c	High range ^c
Dosed feed/dosed water	440,000	730,000
Gavage	505,000	785,000
Skin paint	520,000	785,000
Inhalation	655,000	1,285,000
Chronic:^d		
Dosed feed/dosed water	1,210,000	1,860,000
Gavage	1,460,000	1,860,000
Skin paint	1,460,000	1,960,000
Inhalation	1,960,000	2,460,000

^aCosts include actual contract award, support contracts, plus in-house operating costs.

^bIncludes studies such as 14 day, 90 day, sperm morphology, vaginal cytology, clinical chemistry, urinalysis, hematology, chemical disposition, in-vivo short-term characterization.

^cCosts based on range of awards made in fiscal year 1986. Where awards were not made in some of the categories, estimates were prepared.

^dThree dose levels, one interim sacrifice, clinical chemistry and possibly hematology.

SOURCE: National Toxicology Program.

ing selected chemicals, but NIEHS provides most of the funds and staff for NTP testing.

Nominating Chemicals for Testing

NTP invites the nomination of chemicals for testing from any source. In fiscal year 1982, the NTP Executive Committee agreed to an FDA request that each participating agency could nominate one "priority" chemical per year for carcinogenicity testing. FDA wanted to ensure that more chemicals of concern be tested. The "agency priority" chemicals would be placed on a "fast track" for selection, as will be discussed further. If an agency fails to nominate a chemical in one year, it can still only nominate one the following year. Only five chemicals have been nominated by this fast-track route. They are D&C Yellow No. 11 (FDA), gallium arsenide (NIOSH), 2-butoxyethanol (CPSC), t-butylhydroquinone (FDA), and styrene (NIOSH). Nominating a chemical in this way does not preclude an agency from nominating other chemicals by the normal process in the same fiscal year. Any organization or indi-

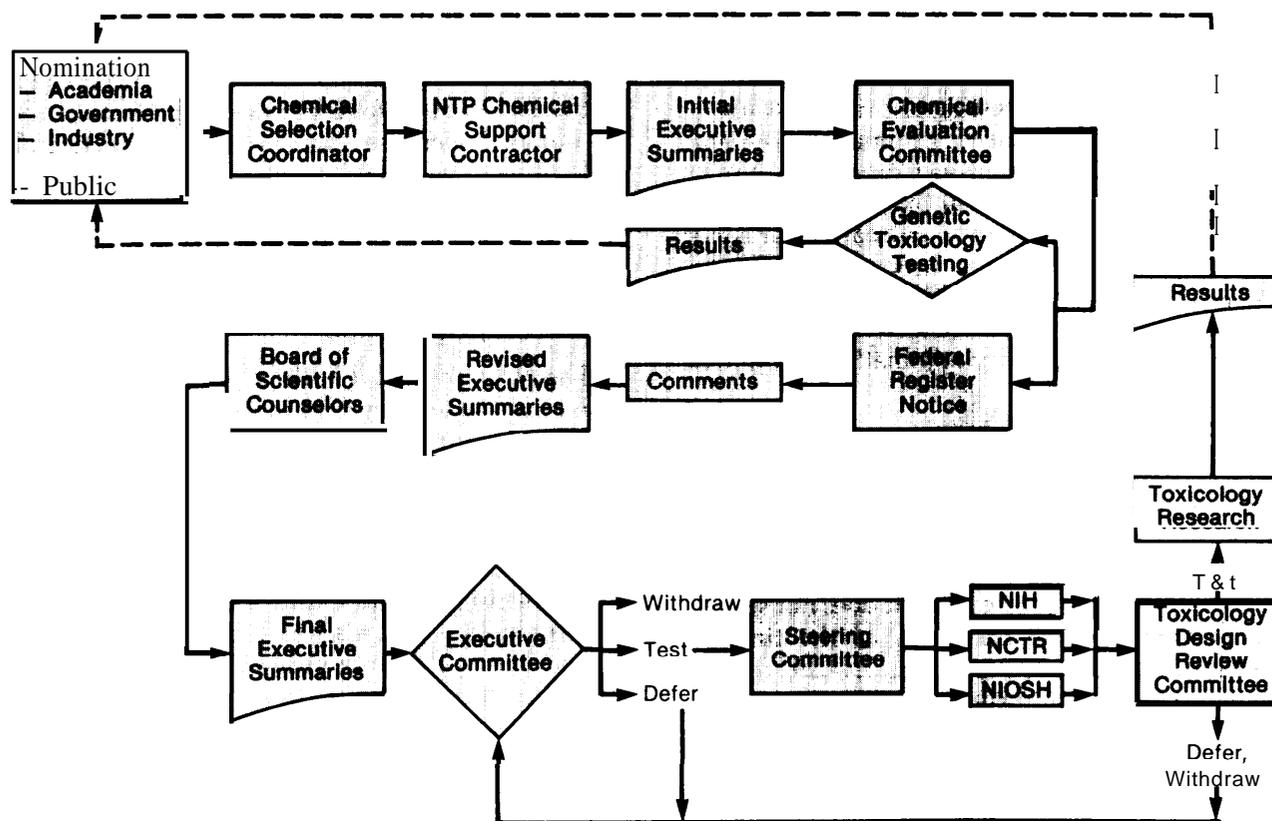
vidual can nominate as many chemicals as desired. The NTP Chemical Selection Coordinator and an Assistant to the Director of NTP remove from further processing chemicals that have already been or are being tested, or have been previously rejected for testing (although renominations that are submitted with additional information may be considered). "Draft executive summaries" are then prepared on the remaining chemicals, except those that have been nominated for genetic toxicology testing only. For these, the nominations are presented directly to the Chemical Evaluation Committee (CEC) with summary data on production levels.

Until 1984, the summaries were prepared by NCTR. In 1984, NTP contracted with Dynamac Corporation (Rockville, Maryland) to prepare the draft executive summaries. After selecting Dynamac as the successful bidder, one of the unsuccessful bidders objected to the award, claiming that Dynamac did not qualify as a small business. This delayed Dynamac's start and resulted in a backlog of nominations. The summaries prepared by Dynamac include information on chemical and physical properties, production, use, exposure, toxicology, and related regulatory activity (26). They are presented to the CEC, which makes the initial recommendation in selecting chemicals for testing. The nomination and selection process is diagramed in figure 4-2.

Selection

In congressional hearings for fiscal year 1981 appropriations, the directors of NCI and NTP proposed that funding be sufficient to begin 100 animal tests on chemicals in 1981. Dr. Rail proposed that this level of starts be maintained each year until 1984. At that time he said, "implementation of the Toxic Substances Control Act will allow us to begin to decrease the number of chemicals tested" (205). In commenting further on industry's role at the hearings, Dr. Donald Fredrickson, then NIH Director, said that it was impossible to attach commercial interests to chemicals that had been in the common domain for many years. He anticipated that when the Toxic Substances Control Act was "fully implemented, however, the NTP should be able to scale down"

Figure 4-2.—NTP Chemical Nomination and Selection Process



SOURCE: National Toxicology Program, 1987.

(205). The budget increase that would have supported 100 test starts annually never materialized and NTP had more nominated chemicals than it could study at that time (26).³ Under such circumstances, nominations of chemicals had to be considered carefully, and priorities for testing established.

The Executive Committee, composed of representatives from CPSC, EPA, FDA, OSHA, NCI, NIEHS, NIOSH, NCTR, and NTP, meets about

³After it became evident that funds would not be available to support as much testing as was anticipated, the participating agencies were each asked to designate the 10 highest *priority* chemicals from among the 140 chemicals that had already been recommended for testing by CEC by March 1980. Thirty-nine were chosen and approved by the NTP Executive Committee; for scientific reasons, not all were tested. Approximately 50 additional chemicals among the 140 were also approved by the NTP Executive Committee for testing by mid-1982.

four times a year to evaluate the drafts and recommend the types of testing, if any, to be performed along with their priorities. By having each member serve as a primary or secondary reviewer of each chemical nomination, NTP is assured of better participation from the other agencies. The CEC makes the final decision about nominated chemicals only for genetic toxicology testing. Approximately 2 months after a chemical is considered by the CEC, it is listed in the *Federal Register*, together with the Executive Committee recommendation. Its decisions on chemicals nominated only for genetic toxicology testing are not published in the *Federal Register*. Thirty days are given for responses but all responses are considered regardless of the date of receipt.

The executive summaries are revised to include public comments, and then submitted to NTP's Board of Scientific Counselors. The Board is com-

posed of eight nongovernmental scientists appointed by the Assistant Secretary for Health for staggered 4-year terms. It meets two or three times a year in public sessions. The Board's recommendations and suggested testing priorities, are incorporated into the executive summaries and submitted to the NTP Executive Committee. Notice of the one priority chemical that each regulatory agency is permitted to nominate each year is not sent to the Board, but goes directly to the Executive Committee from the CEC. Neither the CEC nor the Executive Committee have rejected any of the five agency priority nominations submitted to date.

The NTP Executive Committee makes the final decision on prechronic and chronic testing and testing priorities for those chemicals recommended by the CEC or Board. It has done this by selecting "priority chemicals" for testing each year commensurate with NTP resources. It does not set priorities among these chemicals at the time of selection. Notifications on chemicals CEC recommends for chemical disposition, genetic toxicology, or reproductive studies are not sent to the Executive Committee but to the program leaders in NTP-NIEHS responsible for corresponding areas; they make the decisions about testing. They also can select other chemicals for testing within their program subject to budgetary limitations.

Once chemicals are approved by the Executive Committee, the NTP Steering Committee refers them to one or more of the three constituent agencies of NTP (NIEHS, NCTR, and NIOSH) where, in turn, they are assigned to chemical managers.

NTP CARCINOGENICITY TESTING

Methods of Study and Analysis

Before a bioassay for carcinogenicity can be performed, preliminary information is needed. This is obtained by gathering data on chemical exposures and from studies done elsewhere. When there is some question about biological availability, chemical disposition and pharmacokinetic studies are conducted prior to prechronic and

These scientists develop testing protocols to submit to the Toxicology Design Review Committee (TDRC), a group of NTP scientists representing different disciplines. The chemical manager of the TDRC can also recommend that testing not be pursued, because, for instance, of technical difficulties, unavailability of chemicals, or adequate outside testing. This happens infrequently. Based on the studies under their supervision, the chemical managers can nominate additional chemicals for study or additional studies on chemicals they are already testing.

The *NTP Technical Bulletin* provided information on the Executive Committee's selections and plans for testing, and on the results of mutagenicity tests. More than 7,000 people received this bulletin, which was discontinued in 1983. According to NTP staff, a similar publication would be useful, to present the results of prechronic studies and plans for chronic studies and other information for public information and comment. NTP is considering a plan to publish the experimental design of chronic studies in the Federal *Register* to permit responses from interested readers. Beginning in 1986, NTP publicly named chemicals on which short-term toxicology studies had been completed, specifying the administration route, species, and duration for proposed prechronic studies on these chemicals; the names of the responsible chemical managers were also specified. Comments were invited on chemicals' current production, uses, exposure levels, and toxicology data, to help NTP decide whether additional studies, including long-term toxicology and carcinogenicity studies, are needed (254).

chronic studies. Based on information gathered before the prechronic studies, and also on budgetary constraints, chemical testing may be deferred or dropped. Such decisions have been made for 37 chemicals since 1982.

Chemical Disposition

Information must be obtained on how a chemical selected for testing is absorbed through vari-

ous administration routes, for example through gastrointestinal and respiratory tracts and skin. The route of administration usually selected for bioassay is the route through which humans will most likely be exposed, unless the compound cannot be absorbed by that route. Such a finding may also lead to a decision not to test.

Pharmacokinetic Studies

Determining the rates of absorption and conversion to other compounds at various doses helps in selecting the doses for prechronic and chronic studies. When several related compounds in a class are tested it is also important to know whether they are converted to a common metabolite; it may then be possible to test only one member of the class.

Prechronic Studies

In prechronic studies, animals (usually mice and rats) are administered various doses of the chemical first for 14 days and then for 13 weeks, to evaluate organ-specific pathological changes, body and organ weight changes, clinical signs, and other indicators of toxicity. From the evaluation, an estimated maximum tolerated dose (EMTD) is determined. The EMTD is usually the highest of three doses administered in the chronic studies that follow. The Ad Hoc Committee of the NTP Board of Scientific Counselors recommended that when results are nonlinear, additional intermediate doses should be used, and that consideration should be given to having the lowest dose in a chronic study in the range of human exposure (258). Such a dose is likely to yield a significant number of tumors only when larger doses cause tumors in a very high percentage of animals, as occurred in the original chronic inhalation studies on methylene chloride. Over half the male and female mice developed lung tumors at 2,000 ppm, and over half of them developed liver tumors at 4,000 ppm. NTP has decided to conduct additional studies in female mice, probably using doses of 2,000, 1,000, and 500 ppm to elucidate the chemical's mechanism of action. The OSHA maximum peak dose for 5 minutes in any 2-hour period is 2,000 ppm, and for an 8-hour time-weighted average is 4,500 ppm; its accept-

able ceiling is 1,000 ppm. NIOSH-recommended exposure limits are lower (131).⁴

Chronic Studies

In the chronic studies, the chemical is usually administered to both sexes of mice and rats for 2 years, at which time the surviving animals are sacrificed. Usually 60 animals of each sex and species receive each dose of the chemical for the duration of the study; an additional 60 of each sex and species serve as controls, receiving no chemical. Usually 10 of each experimental group (defined by species, sex, and dose) are sacrificed at 15 or 18 months to determine whether any tumors have already developed.

Evaluation of Results

The incidence of tumors in each group is determined, as are nontumorigenic effects, through necropsy and histopathologic examination. In the study of a single chemical, about 40,000 tissue selections may have to be examined (93). Omitting examination of certain sections succeeded in reducing costs. However, the calendar time required for testing was longer because of added review steps. Therefore, the attempt to reduce pathological studies is no longer made in standard studies.

A number of different statistical techniques are used to determine whether there is a significant increase in tumors associated with exposure to the chemical and, if so, whether there is a dose-response relationship (83). Data are also compared with those on tumor incidence in the NTP historical control database. The large number of

⁴The very high frequency of lung tumors (and also liver tumors in female mice) in methylene chloride tests, compared with controls, raised the possibility that the cancers reflected an acute toxic event causing high cell turnover. If so, the number of mutations per cell division need not be increased, but simply the number of cell divisions. Light microscopy provided no evidence for acute toxic effects in the lung or of increased cell turnover: more sensitive techniques will be used in the second study. It would be unlikely that increased cell turnover would occur at the lower doses, so that an excess of tumors would suggest a significant carcinogenic effect. The high frequency of chemically related liver tumors in female mice also affords an opportunity to determine whether these tumors have the same oncogene pattern as the background liver tumors. Such oncogene studies may provide added information on the significance of liver tumors.

these historical controls improves statistical power in determining whether rare tumors are in fact related to a chemical under study.

Classification of Carcinogenicity

The study in one sex of one species constitutes an "experiment." The NTP classifies carcinogenicity for each individual experiment. Based on statistical and biological significance, the results of each experiment are classified into one of five levels of evidence for carcinogenic activity:

1. clear evidence—a dose-related increase of malignant neoplasms or—a combination of benign and malignant neoplasms, or a marked increase of benign neoplasms that may progress to malignancy;
2. some evidence—the strength of the evidence for carcinogenicity is less than for the first category;
3. equivocal evidence—a marginal increase of neoplasms that may be chemically related;
4. no evidence; and
5. inadequate study—a major quantitative or qualitative limitation prevents interpretation (93).

Quality Assurance

Prechronic and chronic testing, necropsy, and histopathologic examination are performed primarily in contract laboratories. In May 1982, after testing had been transferred from NCI to NTP, Tracer Jitco ceased to provide oversight of contractor testing and NTP assumed greater responsibility for monitoring the tests. In 1983, NTP withdrew a draft report of carcinogenicity studies on methylene chloride administered by gavage because of a contractor's poor testing practices. As a result, NTP developed stringent quality assurance procedures (19,93). These include retrospective data audits of each step in testing, from analysis of the chemical to review of the histopathological sections. Contract laboratories are also visited at least once annually and must submit monthly progress reports. A report by the General Accounting Office in 1984 concluded that NTP's new auditing procedures greatly strengthened quality control of testing (201).

Histopathological sections are examined by two independent groups of pathologists. Although nei-

ther is blinded with regard to the dose the animal received or the gross lesions, the Chairman of the NTP Pathology Working Group (PWG) selects sections for additional examination by PWG members, who are not told whether the sections come from exposed or control animals.

Review and Publication

Following a retrospective audit of all study data and resolution of any discrepancies, a technical report is prepared by the chemical manager. It is first reviewed by NTP staff, and then submitted for peer review to the Technical Reports Review Subcommittee of the Board of Scientific Counselors. The public is informed when the results will be considered by the Peer Review Panel in open meeting. Since industry, labor, and academia are represented on the subcommittee, the classifications receive a full and candid critique from the principal parties concerned. Draft reports are made available to anyone on request.

Following approval by the Peer Review Panel, a final technical report is printed and distributed, usually within 9 months. When the preliminary histopathological evidence suggests that a chemical is highly carcinogenic, the agencies represented on the NTP Executive Committee are notified before the technical report is completed, as are manufacturers, trade associations, labor unions, public interest groups, and other groups monitoring carcinogenicity, such as the International Agency for Research on Cancer (IARC). Such a procedure was followed, for example, for the inhalation studies of methylene chloride and 1,3-butadiene. The notifications stated that the findings were preliminary.

NTP has an agreement with the National Library of Medicine to enter the results of NTP studies in TOXLINE, a computerized database to which the public has access, before printing and distribution of studies' technical reports. This will result in wider and earlier availability of the Summary results.

The results of genetic toxicity tests are published in peer-reviewed journals, for which there are frequently long delays between submission and publication. Results had been published in the NTP Technical *Bulletin* before its publication was terminated in 1983.

OBSERVATIONS ON THE NTP NOMINATION AND SELECTION PROCESS

Criteria for Nomination and Selection

Nominating sources are asked to submit a description of the chemical and its properties and any available information on:

1. production, uses, occurrences, and analysis;
2. toxicology;
3. disposition and structure-activity relationships;
4. ongoing toxicological and environmental studies; and
5. a rationale for the recommendation and suggested studies (257).

The Ad Hoc Panel charged by NTP's Board of Scientific Counselors to examine NTP's testing and evaluation program criticized the lack of emphasis on human exposure, either its magnitude or frequency, in the process of selection (258). In its reply to the Ad Hoc Panel, NTP maintained that exposure was considered in selecting chemicals for tests, and that it would obtain current information from manufacturers on production volumes and exposures during production and use (259). NTP now communicates both with manufacturers and trade associations. It also uses information on potential worker exposures from the National Occupational Hazard Survey and the National Occupational Exposure Survey conducted by NIOSH. The NRC report earlier cited also emphasized the importance of considering exposure along with "suspicion of toxic activity," in planning toxicity tests (138).

There are a number of problems, however, in emphasizing exposure in this way. The first is inadequacy of information. The NRC committee found that, of all types of information needed for health hazard assessments, the least information was available on exposure. For 36 chemicals in its subsample of 100 tested chemicals "no data were available from which the committee could determine the extent of exposure, and, for 75 of the substances in the subsample, no information

was available from which trends in exposure could be estimated."⁵

A second problem relates to the second point that the NRC committee suggested should provide a basis for selecting chemicals, "suspicion of toxic activity." Despite strides in understanding the chemical substituents that may cause toxicity, great ignorance remains. Again, the original intent of the NCI program was to learn more about the structure-activity relationships of carcinogenicity, and this focus was carried over into the NTP. What has become increasingly apparent, however, is the inability to predict with certainty the carcinogenicity of a chemical from its structure. This unpredictability creates a dilemma in setting policy for testing. On the one hand, priority could be given to chemicals whose testing could reveal more about the relation of chemical properties to carcinogenicity. On the other, it could be given to chemicals for which potential or actual human exposure (or exposure of other components of the ecosystem) is great, or which is suspected of being a human carcinogen. Not all chemicals fit into both categories. (Nor is it always possible to predict or determine exposure.) A toxicologist with the Environmental Defense Fund commented to OTA:

It is much more likely for a substance with strong structural resemblance to a known carcinogen or mutagen to be nominated for testing, rather than a compound for which there is quasi-epidemiologic reason to suspect carcinogenicity (186).

⁵The NRC report gave several reasons for the lack of exposure data: 1) There are few reporting requirements. "Even data on production volumes of substances and numbers of people involved in manufacture, distribution, use, and waste disposal are limited." 2) There is little incentive for voluntary reporting. 3) Monitoring for compliance of standards focuses on specific substances, few of which were included in its subsample. "Furthermore, data collected for compliance monitoring may be of limited value in evaluating population exposures." 4) "Little is known about physical processes and procedures that affect the exposure potential for uses other than those intended. For example, the intensity of occupational exposure is strongly influenced by the choice of process and control equipment, and the intensity of environmental exposure is strongly influenced by the selection of waste-disposal technique, chemical reactivity, and degree of biodegradability" (138).

A third problem in emphasizing exposure is duplication of NTP efforts with efforts by manufacturers or processors required by regulatory agencies. Section 4 of TSCA established ITC to "designate" or "recommend" chemicals in commerce, as defined by TSCA, to be tested for certain health or environmental effects. EPA can then require manufacturers and processors to test these chemicals. (See ch. 3.) As mentioned earlier, several chemicals recommended for industry testing under TSCA were under test or scheduled for test by NTP in 1980 (117).

At the suggestion of EPA officials, who were concerned about NTP's activities overlapping with those of TSCA, NTP established eight "chemical selection principles," drafted in 1979 by EPA staff members (table 4-2). The introduction to the principles recognizes that industry has responsibility for testing chemicals under the authority of agencies created by Congress, although principle 8 indicates that there may be special situations in which NTP would test chemicals that "have potential for large-scale and/or intense human exposure," even if industry could be required to test them. Principle 3 recognizes improving the understanding of structure-activity relationships as a criterion for selection. Principle 5, permitting testing of previously tested chemicals "to cross-compare testing methods," follows from NTP's goal of developing and validating new tests. The remaining principles implicitly recognize the importance of human exposure as a basis for selection, but for those chemicals that industry cannot be required to test. These include chemicals in the environment not associated with commercial activities (principle 1), old chemicals whose manufacturers derive "too little revenue to support an adequate testing program" (principle 6), and groups of chemicals manufactured by different companies for which the companies "probably cannot be required" to test (principle 7).

Duplication of NTP and regulatory agency testing is also avoided through liaison between NTP and ITC; the NIEHS is a voting member of ITC, and EPA, under whose authority ITC operates, is represented on CEC. Before conducting a detailed review, ITC asks NTP for information. At the present time, ITC and NTP use the same contractor, Dynamac, to prepare the documen-

table 4-2.—NTP Chemical Selection Principles^a

1. Chemicals found in the environment that are not closely associated with commercial activities (1 1);
2. Desirable substitutes for existing chemicals, particularly therapeutic agents, that might not be developed or tested without Federal involvement (1);
3. 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 (91);
4. Certain chemicals tested by industry, or by others, the additional testing of which by the Federal Government is justified to verify the results (27);
5. Previously tested chemicals for which other testing is desirable to cross-compare testing methods (8);
6. "Old chemicals" with the potential for significant human exposure which 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) (15);
7. 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) (1); and
8. In special situations, as determined by the Executive Committee, marketed chemicals which have potential for large-scale and/or intense human exposure, even if it may be possible to require industry to perform the testing (39).

^aNumbers in parentheses indicate the number of times the principle was used to support a CEC recommendation for testing in animals.

SOURCE: National Toxicology Program

tation for their reviews (18). Liaison reduces the likelihood of duplicate testing under the NTP principle 8. ITC has nominated chemicals for NTP testing to decide whether to recommend chemicals for more extensive industry testing (principle 3). Most chemicals nominated by ITC have been for short-term genetic toxicology testing (see table 4-3).

In table 4-2, the number of times CEC cited each principle to support a recommendation for extensive animal testing between fiscal year 1981 and 1986 is shown in parentheses following each principle for 123 chemicals; more than 1 or even 2 principles were used to justify the testing of some chemicals. Of the 193 citations of principles, the most frequent was of principle 3 (referring to structure-activity relationships), a total of 91 times. Although principle 7, focusing on combinations of chemicals, has only been invoked once, NTP has other initiatives under way on mixtures that are not part of the chemical nomination and selection process. These include a contract with the National Academy of Sciences to develop

Table 4.3.—Source of Nomination of Chemicals for Mutagenicity and Bioassay Testing by NTP by Year

Source	Year of nomination															
	1979		1980		1981		1982		1983		1984		1985		1986	
	Mut	Bio	Mut	Bio	Mut	Bio	Mut	Bio	Mut	Bio	Mut	Bio	Mut	Bio	Mut	Bio
Government:																
CPSC								1	34 ^b			1 ^a				
EPA										1	19	28		1		
FDA									1		1	1		7		5 ^c
ITC							2		5	1	1			1		1
NCI	176		56		48		14		6		4		15	5 ^d	24 ^e	
NIEHS					136		195		62		30	15	121	3		6
NIOSH					92			2								4
OSHA									1					1		
State agencies			1		1		5						1			
Nongovernment:																
Individuals								1	7	3		4	1	2	1	3
Industry					1											
National Academy of Sciences										2						
Professional associations														1		
Unions							9				5		1			1
Totals	NA	303	3009	60	228	50	197	32	108	14	50	58	122	33	6	44

^a2-butoxyethanol also nominated by UAW international Union; only shown under CPSC.

^bAlkyl epoxides nominated jointly by EPA and NIEHS; only shown under EPA.

^cIncludes 4 benzodiazepines nominated by NIEHS in 1984.

^dIncludes 8 submitted for reconsideration and one (oxymetholone) also nominated by NIEHS (shown only under NCI).

^eIncludes 6 resubmissions by NCI and one nominated by NIEHS in 1984.

^fNTP could not provide information on the nominators for bioassay testing for fiscal years 1979, 1980, and 1981 other than from NCI.

^gNominations came from EPA, NIOSH and FDA (NCTR). Breakdown not available. The EPA nominations were submitted in response to ITC designations of chemical classes for possible industry-required testing and to aid in the pre-manufacture notification program.

^hAdded in.

KEY: Mut—mutagenicity tests only, Bio—bioassay (extensive testing in animals); NA—not available; EPA—Environmental Protection Agency, FDA—Food and Drug Administration, ITC—interagency Testing Committee; NCI—National Cancer Institute; NIEHS—National Institute of Environmental Health Sciences, NIOSH—National Institute for Occupational Safety and Health; OSHA—Occupational Safety and Health Administration

NOTE: Data on sources of nominations for mutagenicity tests is complete only after 1981. In prior years, blank spaces mean that data was not available, not that nominations from the particular source were not made. For the same reason, the same applies to blank spaces for bioassay nominations prior to 1982

SOURCE: National Toxicology Program

guidelines for studying complex mixtures; chemical disposition studies of well-defined mixtures; and a Superfund-sponsored effort to characterize the toxic potential of chemicals and mixtures found at waste dump sites.'

OTA obtained from NTP a list of the chemicals for which principle 3 was invoked to recommend either genetic toxicology or animal studies. Eighty-eight chemicals on the list were unequivocally positive in one or more short-term genetic toxicology tests, but have not been tested further by NTP. OTA asked ITC and the EPA Office of Toxic Substances whether they had required, or considered requiring, industry to test any of these chemicals in accord with principle 3. Of the 77 chemicals that were in the TSCA inventory, ITC

has considered 61 chemicals and conducted detailed reviews on 19 that might present the greatest human health hazards. Of the 19, it has deferred further consideration of 12 and recommended 7 for testing to EPA. Deferral is based on low production volumes, low exposure potential, or adequate knowledge of closely related chemicals. Three of the seven recommended chemicals were tested before NTP was created. In 1983 EPA issued an Advance Notice of Proposed Rule Making on one of these, bisphenol A diglycidyl ether (320). Although EPA noted the mutagenicity of several glycidyl compounds, it did not cite NTP studies among its sources. EPA has also issued a Notice of Proposed Rule Making on two of the other chemicals recommended by ITC after NTP was established, namely, meta- and ortho-phenylenediamine (321). The mutagenicity of these compounds was reported before the creation of NTP, and neither the ITC recommendation nor the pro-

^hIn general, NTP strives to test single, pure chemicals. Seldom, however, are humans exposed to isolated pure chemicals.

posed rule cites the NTP genetic toxicology results. The other two reports on chemicals ITC recommended for testing do not mention a positive NTP mutagenicity result. Of the 12 chemicals whose consideration ITC deferred, 4 were reviewed before NTP was organized. Five of the remaining eight (including four xylidines) mention a positive NTP genetic toxicology result (18).

Thus, a small proportion of the chemicals studied under principle 3 that have positive genetic toxicology results have been recommended or proposed for industry testing. Many of the chemicals studied under principle 3 were of little interest to ITC or EPA, however, usually because of low production or low human exposure potential. An NTP official told OTA that many of the chemicals selected under principle 3 represented chemical classes nominated solely for Salmonella testing, to examine the effects of structural modifications on the genotoxic potential of the class and to ascertain the usefulness and predictivity of the assay for the different classes of chemicals. In these cases, the principle was not used to propose chemical candidates for further industry testing (26). A rewording of principle 3, or perhaps dividing it into two principles, one focusing on structure-activity relationships and the other on defining groups of chemicals for industry testing, might clarify the situation.

Number, Source, and Disposition of Nominated Chemicals

Table 4-3 indicates the sources of nominations submitted each year to NTP for mutagenicity testing and bioassays (animal testing). From 1980 to 1986, 1,011 chemicals have been nominated only for mutagenicity testing. There has been a steady downward trend in the number nominated for mutagenicity tests, except in 1985. In that year, NIEHS nominated 121 dump site chemicals. Since 1981, 54 percent of all nominations for mutagenicity tests have been made by NIEHS; its nomination of fewer chemicals accounts for most of the decline. In 1981 and 1982, most of the NIEHS nominations requested examining structure-activity relations, which has not been the case more recently.

A total of 594 chemicals have been nominated for bioassays from 1979 to 1986. In January 1979, shortly after NTP was created, all participating agencies were asked for their nominations and several hundred were received. Thereafter the numbers were considerably smaller. Since 1980, the number of chemicals nominated for animal testing has fluctuated between 14 and 60 per year without any discernible trend.

Of the 942 nominations for all types of testing between 1981 and 1986, 42 came from nongovernment sources; unions nominated chemicals most frequently, a total of 16.

As a result of positive mutagenicity tests, some chemicals were nominated for more extensive testing. New information about a chemical can also lead to renomination and recommendations for additional testing. For instance, methyl isocyanate was originally selected only for genetic toxicology tests, because extended human exposure was considered unlikely (26). After the disaster at the Union Carbide plant in Bhopal, India, in which methyl isocyanate was accidentally released, studies on the delayed effects of a single exposure were undertaken (257).

Of 186 chemicals nominated for more than mutagenicity testing and reviewed by CEC between 1981 and 1986, 114 were recommended for testing (61 percent). CEC recommended animal testing of 59 percent of chemicals nominated by government agencies and of 72 percent of those nominated by nongovernment sources. It did not recommend testing of more than half of the chemicals nominated by FDA and about half of the chemicals nominated by NIEHS. In 1986, CEC did not recommend 53 percent of chemicals for testing, a greater proportion than ever before.

Examination of the data provided by NTP indicated the initial response of the Board of Scientific Counselors to 164 chemicals acted on by CEC (table 4-4). Regarding 130 chemicals (79 percent), CEC and the Board agreed in their recommendations. For eight (5 percent), CEC recommended testing and the Board recommended that testing not be done. For four (2.4 percent), CEC did not recommend testing while the Board recommended testing. These four chemicals were all reconsidered by the Board after 1984.

The number of chemicals selected for testing is consistent with the NTP budget. Because of budgetary cutbacks in fiscal year 1986, priorities were recently set for chemicals selected for testing, but for which studies had not yet begun. Chemicals given low priority may not be tested unless new information about their toxicities or exposures raises their rank or additional funding becomes available.

Duration of Testing Process Stages

OTA obtained information from NTP on the time from nomination to NTP Executive Committee action for every chemical nominated for bioassay reviewed by CEC in fiscal year 1981 or 1982 (table 4-5). For those chemicals in this "cohort" whose bioassays the Executive Committee approved, OTA obtained the time from Executive Committee action to testing status as of January 1987 (table 4-6). For most chemicals, it took over 2 years from the time of nomination to action by the Executive Committee. It took approximately half of this time before CEC acted. These times do not appear to have decreased appreciably in more recent years. Action by the Board of Scientific Counselors added another 3 to 8 months. For 7 chemicals for which CEC recommended no testing and for 13 for which it recommended testing (see table 4-4) the Board deferred action; it also deferred action on one chemical as had CEC. These deferrals add further time. Of these 21 chemicals, 6 have been rereviewed by the Board. In each case, on rereview the Board agreed with CEC's original recommendation. It should be recalled that Board review is omitted for any regulatory agency's priority chemical.

No chemical approved for animal testing in either fiscal year 1981 or 1982 has yet passed through the entire process. Of the 30 chemicals approved for testing in those 2 years, only four have reached the stage of chronic testing. Twenty-two are being tested in the prechronic phase. Testing of four has been deferred or withdrawn because of budget constraints. Of these four chemicals, **one presented unusual technical** difficulties (benzoyl chloride), one was no longer produced (m-chloroaniline), one had been tested for carcinogenicity by industry and found by NTP disposition studies not to be absorbed from the gas-

Table 4-4.—initial Response of Board of Scientific Counselors to Actions of the Chemical Evaluation Committee (CEC) on 164 Chemicals, Fiscal Year 1982 - Fiscal Year 1986

CEC action	Board's recommendation (number of chemicals)		
	No test	Defer	Test
No test	35	7	4
Defer.	0	1	1
Test.	8	13	95

SOURCE: National Toxicology Program.

trointestinal tract (CI Vat Blue No. 1), and one was found not to metabolize to the suspected carcinogen (1,3 -dichloro-5,5-dimethylhydantoin). Considering the time to develop protocols, announce, accept, and negotiate contracts for testing, perform chemical disposition studies to determine the extent of absorption through various administration routes, and conduct prechronic studies, it becomes clear that the 2 years of the chronic phase itself greatly underrepresents the total time needed for carcinogenicity bioassays.

Table 4-7 lists the five priority chemicals nominated by the regulatory agencies for "fast track" analysis. This process took no longer than 5 months for any of the chemicals from the time of nomination as an agency priority chemical to NTP Executive Committee selection, a time shorter than for any nonpriority chemical by 3 months. The shorter time is due to rapid preparation of the executive summaries by NTP, rather than by a contractor, and to omitting consideration by the Board of Scientific Counselors. Although none of the agency priority chemicals have reached the stage of chronic testing yet, the earliest was selected in 1984. Thus, while the early processing for the chemicals has been prompt, too little time has elapsed to conclude whether their testing will be completed more rapidly.

Case Study in Nomination and Selection: the Benzodiazepines

From documents obtained from NTP and interviews with some of those involved, OTA tracked the nomination and selection of five widely used benzodiazepine (BDZ) drugs that were recently approved conditionally for NTP testing. OTA cannot say whether the process has been similar

Table 4-5.—Time (in months) From Nomination of Chemicals Considered by the Chemical Evaluation Committee (CEC) in Fiscal Year 1981 and Fiscal Year 1982 to Intermediate and Final Points in the Process of Approval for Testing by NTP

Year of CEC review	From time of nomination to:										
	CEC recommendation (months)			Action by Board of Scientific Counselors (months)				Action by Executive Committee (months)			
	<13	13-18	19-24	<13	13-18	19-24	>24	<19	19-24	25-30	>30
Fiscal year 1981:											
Number of chemicals	33	12	1	9	23	14	0	1	3	19	0
Fiscal year 1982:											
Number of chemicals	28	18	1	0	16	30	1	0	0	4	8

^a23 nominated chemicals were not recommended for prechronic and/or carcinogenicity testing and were not, therefore, referred to the Executive Committee.
^b35 nominated chemicals were not recommended for prechronic and/or carcinogenicity testing and were not, therefore, referred to the Executive Committee.

SOURCE: National Toxicology Program.

Table 4.6.—Status of Chemicals Approved for Testing by the NTP Executive Committee in Fiscal Year 1981 or Fiscal Year 1982 (status as of January 1987)

Year of executive committee approval	Deferred or withdrawn	Prechronic phase				Chronic phase	
		TDRC ^a approval	Contract awarded	Testing initiated	TDRC ^a review	Contract awarded	Necropsy complete-d
Fiscal year 1981:							
Number of chemicals	4 ^b	2	0	8	0	3 ^c	1
Fiscal year 1982:							
Number of chemicals	0	2	1	8	1	0	0

^aToxicology Design Review Committee.

^bThree chemicals were deferred in July 1986 because of budget constraints. One chemical was withdrawn by the Executive Committee in October, 1984.

^cChronic testing initiated for one chemical in December 1986. Contract awards anticipated for the other two by March, 1987.

SOURCE: National Toxicology Program.

Table 4-7.—History of Agency Priority Chemicals (status as of April 1987)

Chemical	Nominating agency	Date of priority nomination	NTP Executive Committee		Status, April 1987
			selection date	selection date	
D&C Yellow No. 11	FDA	9/27/83	11/27/84	11/27/84	Prechronic testing
Gallium arsenide ^a	NIOSH	7/16/84		8/31/84	Contracted for prechronic testing
2-Butoxyethanol	CPSC	9/27/84		3/07/85	Out for bid (prechronic)
t-Butyl-hydroquinone	FDA	7/12/85		12/19/85	Out for bid (prechronic)
Styrene	NIOSH	12/13/85		2/13/86	Protocol for prechronic in preparation

^aOriginally nominated Dec. 8, 1983.

SOURCE: National Toxicology Program.

for other chemicals. Questions on studies of classes of chemicals and on industry's role in testing were also raised in NTP's consideration of these drugs for testing.

Four of the BDZs that were nominated are frequently used to relieve anxiety: diazepam (Valium), chlordiazepoxide (Librium) and clorazepate (Tranxene), and oxazepam (Serax). The fifth, flurazepam (Dalmane), is used as a hypnotic (sleep inducer). The first three are metabolized to oxazepam. These drugs were marketed prior to 1968, when FDA began to require carcinogenicity test-

ing. They are still extensively used, with over 2.5 million prescriptions written for the least frequently prescribed (oxazepam), and over 25 million for the most frequently prescribed (diazepam) in 1983. Diazepam ranked third in "new prescriptions" in 1985 and fourth for "new and refilled" prescriptions.⁷

⁷This and the following information on toxicity studies was obtained from the draft executive summaries submitted by Dynamac to NTP on October 28, 1986. Citations are to the original sources.

In 1980, an NTP senior toxicologist, Dr. James Huff, sent a memo to the Deputy Director of NTP, in which he suggested that diazepam, chlordiazepoxide, and oxazepam "be tested first in the Genetic Toxicology Component and at the same time be nominated for long-term carcinogenesis bioassay." His concern was based on a report of liver cell adenomas in mice receiving oxazepam. Huff considered the study inadequate because of the small number of animals used and the short duration (94). No nomination was submitted to the NTP office responsible for processing nominations. In 1984 Huff sent to the Director of Toxicology Research and Testing Program and the assistant to the director of NTP, who are responsible for processing nominations, a new report of liver tumors in male mice administered large doses of another BDZ, ripazepam, together with a copy of his earlier memo. Shortly thereafter, the three, as well as clorazepate, were nominated for study by NIEHS (25).

The FDA joined in the nomination on March 31, 1986, after NTP requested that it provide unpublished data on toxicologic testing of the drugs that could be included in the executive summaries (26). At that time, FDA also nominated flurazepam for study. FDA based its nominations on

- the extensive use of these drugs,
- the inadequacy or absence of carcinogenicity studies on them,
- an increased incidence of liver tumors in mice and benign thyroid tumors in rats given some newer BDZs that were required to undergo extensive carcinogenicity testing prior to marketing,⁹ and
- the need to determine whether the types of tumors observed in the newer drugs were characteristic effects of the class of BDZs or specific for each chemical (361).

In fact, FDA has not approved new drug applications for some of the newer BDZs because of the finding of tumors in animals (193).

⁹The 1968 FDA guidelines for carcinogenicity testing called for an 18-month study in rats only. Manufacturers now submit data on both sexes of rats and mice in their new drug applications, although there is no formal requirement for them to do so.

The nominations were not reviewed by CEC until September 1986, after their nomination by FDA. The delay between the time of initial nomination and CEC review was due largely to a backlog that had accumulated while NTP was attempting to get a new contractor to prepare the summaries for the CEC. CEC recommended the drugs for bioassay (prechronic and chronic testing two with high priority (diazepam and flurazepam) and one (oxazepam) with moderately high priority. (The recommendations were based on principles 3 and 4; see table 4-2.) It did not recommend clorazepate and chlordiazepoxide for testing because the three related BDZs were recommended. The summaries prepared for CEC do not make clear, however, that oxazepam is the major metabolite of both drugs.⁹ Additional reasons for not recommending these drugs were that previous chronic studies indicated minimal toxicity of clorazepate, although the reviewer noted that "no carcinogenicity studies were done," and that the use of chlordiazepoxide "was declining" (261).

The Board of Scientific Counselors reviewed the five BDZs in November 1986. One of the questions raised by a counselor at the meeting was whether the results for one drug could be extrapolated to others that have the same metabolite. The answer given by NTP staff was "no." In fact, one of the problems of doing "class" studies is that when one or more members of a class is found to be carcinogenic, a new member of the class, which may differ by only a single substituent, must be tested in order to establish its carcinogenicity. It is for this reason that NTP has tested several derivatives of benzidine and several phthalates. The difficulty arises because of the imperfect ability to predict carcinogenicity from structure.

⁹Data in the summary prepared for CEC does not indicate oxazepam in the blood or urine following chlordiazepoxide administration to human volunteers. The summary on clorazepate does not indicate the urinary metabolites following administration of the drug. Desmethyldiazepam, an immediate precursor of oxazepam, "is the major metabolite of clorazepate in the blood." Diazepam is also converted to desmethyldiazepam. In one study, only 16 percent of the administered dose was excreted as a conjugate of oxazepam, the major form of oxazepam excretion.

The Board voted unanimously to recommend that NTP test all five BDZs subject to an extensive review of existing studies “to determine sex/species combination in which to test individual chemicals” (260). The NTP Executive Committee accepted the recommendation on December 18, 1986, giving highest priority to the three drugs originally recommended for testing by the CEC. Because of budgetary constraints, it is not yet clear how many of the five drugs will be tested in animals.

The only BDZ selected by NTP for genetic toxicology testing prior to review by CEC was diazepam, and this occurred in December 1985. Following action of the CEC in September 1986, NTP genetic toxicology staff added flurazepam and oxazepam. To OTA’s knowledge these tests have not yet been conducted. When the CEC was considering the BDZs the question was asked why FDA did not require industry to test the compounds. Although the patents on the older BDZs had expired, FDA could still have required companies to test drugs that they are marketing. (They did get Wyeth and Hoffmann-LaRoche to perform

carcinogenicity studies in rats on oxazepam and diazepam, respectively, after they had been marketed but while they were still under patent. In view of the finding on the newer BDZs, FDA is now interested in mouse studies.) FDA apparently chose not to require such testing in 1986 for at least two reasons. First, they would have to get several companies to collaborate in carrying out a suitable protocol; since the drugs were no longer under patent, several companies were marketing each BDZ. (There is precedent, however, for FDA’s requiring manufacturers to agree on a common protocol and to contribute to testing of pharmaceutical agents (193).) Second, FDA had limited leverage on the companies. Without evidence of these drugs’ human carcinogenicity, regardless of their widespread use, it is not likely that FDA would prevail if it sought to remove the drugs from the market. FDA could require that the drug label state that the drug has not been tested adequately for carcinogenicity (if that was the case), but an FDA spokesperson doubted that most clinicians would be affected greatly by such a statement (193).

MUTAGENICITY TESTING: CORRELATION WITH ANIMAL CARCINOGENICITY

The initiation of cancer may involve the mutation of particular nucleotides that form the backbone of DNA. Evidence that chemicals cause mutation or combine with DNA or affect its function can be obtained rapidly by a variety of *in vitro* and *in vivo* methods. The recent discovery that certain genes (proto-oncogenes) can be converted to oncogenes by known chemical carcinogens (chemicals associated with the presence of tumors) extends this work and promises better short-term methods to determine carcinogenicity (115). Work on developing such methods is being conducted at NTP and NCTR.

At the present time, the “Ames” test, which measures mutant frequencies in one or more strains of *Salmonella* bacteria, is the most extensively used test for genetic toxicity. At NTP, such results are considered in deciding how to proceed

with bioassays for carcinogenicity in rats and mice. NTP results of *Salmonella* mutagenesis tests have been published on 775 chemicals. These tests were performed in one or more of three contract laboratories. The laboratories did not know the identity of the chemicals. Multiple doses were each tested in triplicate. Positive and negative control chemicals (that is, known mutagens and known nonmutagens) were also used in each experiment. Reproducibility within and between laboratories was documented in most cases. Of the 775 chemicals tested, 194 (25 percent) were clearly mutagenic, and 49 (6 percent) gave questionable results (in one laboratory) or different results in different laboratories. The remaining 532 (69 percent) were negative (87,133,367).

At the request of the Board of Scientific Counselors, NTP compared the results of short-term tests for genetic toxicity with those of animal tests

for carcinogenicity (194). It performed Salmonella mutagenicity tests on 44 chemicals that were carcinogenic in NTP tests in at least 1 animal experiment (an experiment is defined as the test in 1 sex of 1 species), on 20 chemicals that were negative in all animal experiments (both sexes of 2 species, usually mice and rats), and on 9 chemicals that gave equivocal results in the animal studies. Except for 10 chemicals that could not be tested for technical reasons, these 73 chemicals were all of those tested in NTP long-term studies of carcinogenicity in which the animals were sacrificed in 1977 or later and on which the conclusions had been approved by peer review before 1985. Counting the equivocal animal tests as negative, 20 of the 24 chemicals that were mutagenic in the Salmonella test had proved to be carcinogenic in animals (83 percent, predictive value positive (PVP)), and 25 of the 49 chemicals that were non-mutagenic had proved to be noncarcinogenic (51 percent, predictive value negative (PVN)). The chance that an animal carcinogen would give a positive Salmonella test (the test's sensitivity) was 45 percent, and the chance that a noncarcinogen would give a negative test (the test's specificity) was 86 percent. There is some increase in the PVN when the following factors are taken into consideration: carcinogenic potency (the lowest dose producing tumors in animals), malignancy of animal tumors, number of animal experiments that gave positive results, number of organ sites with tumors, and exclusion of liver tumors. But there are decreases in the PVP when each of these factors is considered. With 224 chemicals in the entire NCI-NTP database on which Salmonella and carcinogenicity testing had been done, the PVP was 69 percent; the PVN, 45 percent; the sensitivity, 54 percent; and the specificity, 70 percent. The range of concordance—agreement between Salmonella and animal test results (positive

and positive, negative and negative) —when each of these factors is examined separately, varies between 62 and 74 percent.

NTP performs additional short-term tests on most chemicals in the genetic toxicology program. These include the mouse lymphoma (ML) mutagenesis assay, tests for chromosome aberrations (CA) in Chinese hamster ovary (CHO) cells, and sister chromatid exchanges (SCE) in CHO cells. These tests are all performed in vitro. NTP also examined the predictivity of these tests for animal carcinogenicity alone and in combination with each other or the Salmonella test. The PVP of each was less than the Salmonella test (ML 66 percent, CA 73 percent, and SCE 67 percent), and the PVN was about the same (50 to 52 percent). There was no combination of two, three or all of these tests that gave much higher concordance with animal test results than the Salmonella test alone (66 percent v. 62 percent). Nor were chemicals that caused positive responses at lower doses in any of these genetic tests more likely to be carcinogenic in animal bioassays than those for which higher doses were needed. Nor did a sequential approach—in which chemicals negative in one genetic test were then subject to the other three short-term tests—significantly improve the ability to distinguish animal carcinogens from noncarcinogens. NTP found no combination of tests or other test to represent a substantial improvement over the Salmonella test.

NTP performed 2 other short-term genetic tests on some of the 73 chemicals: the test for unscheduled DNA synthesis in rat primary hepatocytes on 44, and the sex-linked recessive lethal assay in *Drosophila* on 27. The specificity and PVP were both high (with a specificity of 0.93 and 1.0 respectively, and a PVP of 0.86 and 1.0 respectively), but sensitivity and PVN were much lower for these than for the other tests.

Some short-term tests involving mammalian cells can be conducted in vivo. The chemical is administered to the animal, and tests are performed on cells obtained either from liver or bone marrow. In vivo tests for unscheduled DNA synthesis in rodent liver cells were generally negative for animal carcinogens and noncarcinogens, in tests of 16 chemicals. Two short-term tests, SCE

¹⁰This and the following information was obtained at the NTP Board of Counselors meeting, November 25, 1986.

¹¹This was not true for all chemicals. For instance, very low doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin ("dioxin") and polybrominated biphenyl (PBB) mixtures were positive in all animal experiments, as was reserpine in three of four animal experiments. Yet all three chemicals gave negative results in the Salmonella and other tests for genetic toxicity (described below). Dioxin and PBBs are known to act as tumor promoters under certain circumstances.

and CA, were performed on the same 16 chemicals in vivo on mouse bone marrow cells. Of the eight that were known animal carcinogens, all gave positive SCE results in vitro; only six gave positive results in vivo. Seven of the animal noncarcinogens gave positive SCES in vitro; only four gave positive results in vivo. The carcinogens also gave fewer positive in vivo CA responses than positive responses in vitro (four compared to six); the noncarcinogens gave fewer positive in vivo responses (one compared to four in vitro). When in vivo tests on mouse bone marrow were performed on seven carcinogen-noncarcinogen pairs of structural analogs, the CA test correctly identified the carcinogenic member of five of the chemicals without giving any false positives. In the SCE test, four of the carcinogens were positive, but five of the noncarcinogens were also positive. NTP will perform in vivo assays of additional chemicals. It will also incorporate them into prechronic tests. It is possible that nonmutagenic chemicals do not act as initiators, that is, do not cause mutations in DNA, but instead act as pro-

motors, whose mechanism of action is poorly understood. Animals administered promoters sometimes show regression of preneoplastic or neoplastic lesions after the administration of the chemical is stopped. For some chemicals selected for carcinogenicity testing, but which are negative in short-term tests, NTP conducts "stop" studies, in which administration is discontinued in some animals but not in others (115); regression or absence of tumors in the first set, but not in the second would be consistent with the observations made so far for several promoters.

Although the results of these studies show a fair degree of consistency among the different tests, suggesting that not all studies need be performed, they fail to show very good agreement with the results of animal testing. In discussing the results, NTP staff concluded that the short-term tests could not be used as surrogates for long-term rodent studies, but could be helpful in assessing carcinogenic potential (260).

RELATION OF CHEMICAL CARCINOGENICITY IN ANIMALS TO CANCER IN HUMANS

IARC investigators recently compiled data on the ability of animal tests to predict that a chemical is a human carcinogen.

Of 30 exposures (to chemicals) for which there is sufficient evidence of carcinogenicity to humans, the animal data provide sufficient evidence for 19. . . limited evidence for seven . . . and inadequate evidence or no data for four . . . Of the 14 exposures for which there is limited evidence of carcinogenicity to humans, the experimental data provide sufficient evidence for eight . . . limited evidence for three . . . and inadequate evidence or no data for three.

. . . The four exposures for which there is sufficient evidence of carcinogenicity to humans that have not been adequately tested in experimental animals are: certain combined chemotherapy regimens including MOPP (mechlorethamine [nitrogen mustard] oncovine [vincristine], procarbazine, prednisone), conjugated oestrogens, smokeless tobacco products and treosulphan. However, for some individual components

of MOPP—nitrogen mustard and procarbazine—there is sufficient evidence of carcinogenicity in experimental animals . . . Further, it is reasonable to believe that conjugated oestrogens would react similarly to other oestrogens in experimental animals . . . ; for some oestrogens there is sufficient evidence of carcinogenicity to animals (360).

For 37 of the 44 chemicals considered to be carcinogenic in humans, there was evidence of carcinogenicity in animals; in these cases, the evidence was "sufficient" for 27 and "limited" for 10. '2 (The remaining seven chemicals were not studied adequately.) Further strengthening this association was the finding that for every chemi-

²IARC'S definition of "sufficient evidence" in animals includes increased incidence of malignant tumors in multiple species or strains. The definition of "limited evidence" includes studies involving a single species or strain, inadequate dosage levels or period of followup, too few animals, and high rate of spontaneous tumors.

cal supported by sufficient evidence of carcinogenicity for both humans and animals, the same organ was involved in both; the types of tumor were often identical or similar. Some chemicals that subsequently proved to be human carcinogens were first demonstrated to be carcinogens in experimental animals (4-aminobiphenyl, diethylstilbesterol, melphalan, methoxsalen with ultraviolet A, mustard gas, and vinyl chloride).

It does not follow from these studies that all chemicals carcinogenic in animals will prove to

be carcinogenic in humans. It would be helpful to demonstrate that chemicals that are not carcinogenic in humans are also noncarcinogenic in animals. This would be an expensive undertaking. Moreover, no universally accepted list of human noncarcinogens exists. Although FDA requires that all new drugs for long-term or widespread use be tested, most other chemicals are selected for testing because it is suspected they cause cancer in humans.

SUMMARY

The establishment of NTP has improved coordination of testing within the government. NTP performs a valuable role in developing and evaluating new tests. It continues to elucidate structure-activity relationships in chemical carcinogenesis.

In most cases, NTP's process of evaluating nominated chemicals gathers available information to permit informed decisions on selection. It is not clear that the chemicals to which humans are significantly exposed are being selected adequately, in part because relatively few chemicals are nominated. NTP does not have direct control of nominations, other than by publishing information on the nominations process. It does consider human exposures in recommending chemicals for study.

Regardless of whether the chemicals that pose the greatest threat to humans are being nominated, more chemicals are nominated than can be tested given current budgets. It is possible that industry could perform more tests, as the BDZ case study suggests. There is little evidence to suggest that chemicals tested under NTP to "assist in defining groups of commercial chemicals that should be tested by industry" (see table 4-2, principle 3) are subsequently being tested by industry.

The time from nomination to selection is over 2 years for most chemicals. This time could be shorter. Whether the testing process itself can be shortened is problematic. The performance of

chemical disposition and prechronic tests is necessary, and eliminating them would reduce the validity of the chronic bioassays. Testing had been completed by January 1987 on only one of the chemicals selected in fiscal year 1981 and 1982. Developing protocols, awarding contracts, and performing chemical disposition and prechronic and chronic tests takes at least 5 years; the evaluation of organs and microscopic sections adds at least an additional year; and preparation of the report, review, and publication add still more time. The time required is so intrinsically long that some chemicals presenting significant exposures may no longer do so by the time testing is completed. There should be a mechanism by which NTP is promptly informed of changes in the production status of chemicals, or of the substitution of analogs, so it can modify testing schedules and protocols accordingly. This aim may be accomplished to some extent by NTP's announcement of the completion of prechronic studies with a request for submission of relevant data. Chemical managers also attempt to obtain data on current production levels.

It is a grave oversimplification to maintain that animal testing takes 2 years. The current research by NTP and NCTR toward finding better biological markers of carcinogenicity may lead to better and more rapid means of detecting carcinogens in both humans and animals.