VALIDATION AND REGULATORY ACCEPTANCE OF TOXICOLOGICAL TEST METHODS

A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods

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ACRONYMS/ABBREVIATIONS

AAMI	Association for the Advancement of Medical Instrumentation
AOAC	Association of Official Analytical Chemists International
APHIS	Animal and Plant Health Inspection Service/USDA
ARS	Agricultural Research Service/USDA
ASTM	American Society for Testing and Materials
ATSDR	Agency for Toxic Substances and Disease Registry/DHHS
CBER	Center for Biologics Evaluation and Research/FDA
CDC	Centers for Disease Control and Prevention/DHHS
CDER	Center for Drug Evaluation and Research/FDA
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act (Superfund)/EPA
CFSAN	Center for Food Safety and Applied Nutrition/FDA
CPSC	Consumer Product Safety Commission
CRADA	Collaborative Research and Development Agreement
CVM	Center for Veterinary Medicine/FDA
DFS	Division of Food Safety/CFSAN/FDA
DHHS	Department of Health and Human Services
DLPAD/NIH	Division of Legislative Policy Analysis and Development
DMS	Division of Microbiological Studies/CFSAN/FDA
DOD	Department of Defense
DOE	Department of Energy
DOI	Department of the Interior
DOL	Department of Labor
DOT	Department of Transportation
DTR	Division of Toxicological Research/CFSAN/FDA
EEC	European Economic Council
EPA	Environmental Protection Agency
FDA	Food and Drug Administration/DHHS
FFDCA	Federal Food, Drug, and Cosmetics Act/FDA
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act/OPP/EPA
GLP	Good Laboratory Practice Regulations
ICCVAM	Ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ISO	International Organization for Standardization
IRAG	Ad hoc Interagency Regulatory Alternatives Group
MAD	Mutual Acceptance of Data Principles/OECD
NCI	National Cancer Institute/NIH
NCTR	National Center for Toxicological Research/FDA

NIEHS	National Institute of Environmental Health Sciences/NIH
NIH	National Institutes of Health/DHHS
NIOSH	National Institute for Occupational Safety and Health/CDC
NLM	National Library of Medicine/NIH
NRC	National Research Council
NTIS	National Technical Information Service
NTP	National Toxicology Program/DHHS
OECD	Organization for Economic Cooperation and Development
OLAR	Office of Laboratory Animal Research/NIH
OPPT	Office of Pollution Prevention and Toxics/EPA
OPP	Office of Pesticide Programs/EPA
ORD	Office of Research and Development/EPA
OSHA	Occupational Safety and Health Administration/DOL
OSRS	Office of Special Research Skills/CFSAN/FDA
SAB	Science Advisory Board/EPA
SAP	Scientific Advisory Panel/FIFRA/EPA
SOP	Standard Operating Procedures
TASARC	Tri-Agency Superfund Applied Research Committee
TSCA	Toxic Substances Control Act/OPPT/EPA
UN Transport	United Nations Committee of Experts on the Transport of Dangerous Goods
USDA	United States Department of Agriculture
USP	United States Pharmacopeia
3Rs	Refinement, Reduction, and Replacement (of Animal Use

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PREFACE

The National Institutes of Health Revitalization Act of 1993 (Public Law No. 103-43, Section 1301) directed the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/ NIH) to establish an Applied Toxicological Research and Testing Program which represents the NIEHSí component of the National Toxicology Program. The Act further directed the NIEHS to '(a) establish criteria for the validation and regulatory acceptance of alternative testing methods, and (b) recommend a process through which scientifically validated alternative methods can be accepted for regulatory use (Appendix F).

To fulfill this mandate, an ad hoc Inter-agency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) (the Committee) was established in 1994 by NIEHS to develop a report recommending criteria and processes for validation and regulatory acceptance of toxicological testing methods that would be useful to Federal agencies and the scientific community. The following Federal regulatory and research agencies and organizations participated in this effort:

Consumer Product Safety Commission

Department of Agriculture

- Agriculture Research Service
- Animal and Plant Health Inspection Service

Department of Defense

Department of Energy

Department of Health and Human Services

- Agency for Toxic Substances and Disease Registry
- Food and Drug Administration
- National Institute for Occupational Safety and Health/CDC
- National Institutes of Health
  - National Cancer Institute
  - National Institute of Environmental Health Sciences
  - National Library of Medicine
  - Office of Laboratory Animal Research

Department of the Interior

Department of Labor

- Occupational Safety and Health Administration

Department of Transportation

- Research and Special Programs Administration

Environmental Protection Agency

The Committee met initially in September 1994, and then monthly or bimonthly until completion of the report in December 1996. The Committee interpreted its charge as the development of general criteria and processes for the validation and regulatory acceptance of new and revised toxicological test methods.

The specific goals of this Report are to:

- communicate the criteria and procedures that Federal agencies should employ in considering new and revised test methods,
- encourage the development of new and revised test methods that will provide for improved assessment of the potential toxicity of agents to human health and other organisms in the environment,
- provide effective guidance for scientists for the validation and evaluation of new and revised test methods,
- contribute to the increased likelihood of regulatory acceptance of scientifically valid new and revised test methods,
- encourage the use of validated and accepted new and revised test methods,
- encourage, when scientifically feasible, the reduction and refinement of animal use in testing and the replacement of animal methods with non-animal methods or of animal species with phylogenetically lower species.

In developing the initial draft report, the Committee considered information obtained from the following sources: 1) a questionnaire completed by each agency on their criteria and processes for test method validation and acceptance, 2) public comments submitted in response to a *Federal Register* notice published December 7, 1994, requesting interested individuals and organizations to provide information for consideration by the Committee (Appendix G), 3) presentations from various government scientists, 4) review of pertinent available literature, and 5) comments and suggestions from Federal agencies.

>An NTP Workshop on Validation and Regulatory Acceptance of Alternative Test Methods was held on December 11-12, 1995, at the Crystal Gateway Marriott Hotel, Arlington, Virginia. The purpose of the workshop was to review the criteria and processes set forth in the draft report and accept comments and recommendations from workshop registrants and invited panelists, including representatives from industry, academe, public interest groups, and the international community. Written comments were also submitted in response to the *Federal Register* notice announcing availability of the draft report for public comment.

The draft report was also presented to participants at the Organization for Economic Cooperation and Development (OECD) Workshop on Harmonization of Validation and Acceptance Criteria for Alternative Test Methods held in Stockholm, Sweden, on January 22-24, 1996. Comments and recommendations generated by scientists from the OECD member countries were considered by the Committee. The Committee prepared a revised draft report for distribution to the participating agencies for comment and concurrence. This final Report will be published and circulated widely to interested parties. The Committee anticipates that this Report will facilitate the validation and regulatory acceptance of new and revised toxicological testing methods that will enhance the protection of human health and the environment, and also benefit animal welfare.

EXECUTIVE SUMMARY

EXECUTIVE SUMMARY

New and revised test methods to provide improved assessment of the potential toxic effects of chemicals and other agents on human health and the environment are being developed with increasing frequency. This includes the development of methods that evaluate new toxicity endpoints, incorporate current understanding of toxic mechanisms, improve test efficiency (reduction of time and expense), and further the goal to replace, reduce, and refine the use of animals in testing. These test methods are used to investigate the biologic mechanisms underlying toxicological processes, to assist the pre-market evaluation of new products, and to generate hazard identification and dose-response relationship information for health and environmental hazard classification and risk assessment purposes. Depending on the hazard classification and risk, industry and regulatory agencies may implement appropriate prevention and risk management practices to protect public health and the environment. Before a new or revised test method is used to generate information to support regulatory decisions, it must be (1) validated (its reliability and relevance for its proposed use must be determined) and (2) accepted, (one or more regulatory or research agencies must determine that it fills a specific need). This report describes recommended criteria and processes for the validation and regulatory acceptance of new and revised toxicological testing methods. In addition, it recommends ways to facilitate the development and adoption of new testing methodologies, both nationally and internationally.

The ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) determined that this Report should be applicable to all proposed toxicological testing methods, including those termed 'alternatives.' This decision was based on the premise that the validation and regulatory acceptance of alternative test methods should be no different than for other test methods. For purposes of this Report, alternative tests are those that incorporate replacement, reduction, or refinement of animal use. Replacement refers to the partial or total replacement of animals with non-animal systems, or the replacement of an animal species with a phylogenetically lower species (e.g., replacement of a mammal with an invertebrate). Reduction means reduction of the total number of animals required. Refinement refers to the incorporation of procedures to lessen or eliminate pain or distress to animals and enhance their well-being. Collectively, these are referred to as the 'three Rs' of alternatives.

Criteria to guide scientists in the development of new toxicological testing methods have not been readily available from Federal agencies. This Report provides guidance on the principles and processes that should be followed in the validation of a new method and clarifies the critical elements that should be addressed in the submission of a proposed method for agency approval. Such guidance should facilitate the evaluation of new methods by research and regulatory agencies and enhance the likelihood of acceptance of scientifically valid methods.

VALIDATION CRITERIA

For a new or revised test method to be considered validated for regulatory risk assessment purposes, it should generally meet the following criteria (the extent to which these criteria are met will vary with the method and its proposed use). However, there needs to be flexibility in assessing a method given its purpose and the supporting database (see Sections 2.3 and 2.4):

- The scientific and regulatory rationale for the test method, including a clear statement of its proposed use, should be available.
- The relationship of the test methodís endpoint(s) to the biologic effect of interest must be described. Although the relationship may be mechanistic or correlative, tests with biologic relevance to the toxic process being evaluated are preferred.
- A detailed protocol for the test method must be available and should include a description of the materials needed, a description of what is measured and how it is measured, acceptable test performance criteria (e.g., positive and negative control responses), a description of how data will be analyzed, a list of the species for which the test results are applicable, and a description of the known limitations of the test including a description of the classes of materials that the test can and cannot accurately assess.
- The extent of within-test variability, and the reproducibility of the test within and among laboratories must have been demonstrated. Data must be provided describing the level of intra- and interlaboratory reproducibility and how it varies over time. The degree to which biological variability affects this test reproducibility should be addressed.
- The test methodís performance must have been demonstrated using reference chemicals or test agents representative of the types of substances to which the test method will be applied, and should include both known positive and known negative agents. Unless it is hazardous to do so, chemicals or test agents should be tested under code to exclude bias.
- Sufficient data should be provided to permit a comparison of the performance of a proposed substitute test with that of the test it is designed to replace. Performance should be evaluated in relation to existing relevant toxicity testing data, and relevant toxicity information from the species of concern. Reference data from the comparable traditional test method should be available and of acceptable quality.
- The limitations of the method must be described; for example, in vitro or other non-animal test methods may not replicate all of the metabolic processes relevant to chemical toxicity that occur in vivo.
- Ideally, all data supporting the validity of a test method should be obtained and reported in accordance with Good Laboratory Practices (GLPs). Aspects of data collection not performed according to GLPs must be fully described, along with their potential impact.
- All data supporting the assessment of the validity of the test method must be available for review.
  - Detailed protocols should be readily available and in the public domain.

- The method(s) and results should be published or submitted for publication in an independent, peer-reviewed publication.
- The methodology and results should have been subjected to independent scientific review.

Because tests can be designed and used for different purposes by different organizations and for different categories of substances, the determination of whether a specific test method is considered by an agency to be useful for a specific purpose must be made on a case-by-case basis. Validation of a test method is a prerequisite for it to be considered for regulatory acceptance.

REGULATORY ACCEPTANCE CRITERIA

Validated methods are not automatically accepted by regulatory agencies; they need to fit into the regulatory structure. Flexibility is essential in determining the acceptability of methods to ensure that appropriate scientific information is considered in regulatory risk assessment. A test method proposed for regulatory acceptance generally should be supported by the following attributes (see Sections 3.4 and 3.5):

- The method should have undergone independent scientific peer review by disinterested persons who are experts in the field, knowledgeable in the method, and financially unencumbered by the outcome of the evaluation.
- There should be a detailed protocol with standard operating procedures (SOPs), a list of operating characteristics, and criteria for judging test performance and results.
- Data generated by the method should adequately measure or predict the endpoint of interest and demonstrate a linkage between either the new test and an existing test, or the new test and effects in the target species.
- There should be adequate test data for chemicals and products representative of those administered by the regulatory program or agency and for which the test is proposed.
- The method should generate data useful for risk assessment purposes, i.e., for hazard identification, dose-response assessment, and/or exposure assessment. Such methods may be useful alone or as part of a battery or tiered approach.
- The specific strengths and limitations of the test must be clearly identified and described.
- The test method must be robust (relatively insensitive to minor changes in protocol) and transferable among properly equipped and staffed laboratories.
- The method should be time and cost effective.
- The method should be one that can be harmonized with similar testing requirements of other agencies and international groups.
- The method should be suitable for international acceptance.
- The method must provide adequate consideration for the reduction, refinement, and replacement of animal use.

REGULATORY ACCEPTANCE PROCESS RECOMMENDATIONS

A Committee survey revealed that the way new methods are evaluated for regulatory acceptance varies among programs and agencies. There is no established process for coordinating the review of methods proposed to or by one Federal agency with other agencies that might find the method useful. The recommendations relating to regulatory acceptance that follow are directed at the development of a consistent process for evaluating new methods for regulatory acceptance. Due to rapid advances in science and technology, appropriate scientific expertise is essential for the evaluation of a new method. Without such expertise, acceptance of scientifically valid new methods could be delayed, or test methods could be inappropriately rejected or accepted. To increase the efficiency of reviews of proposed new and revised methods and to increase the likelihood of adequate scien-tific consideration of new methods, the following considerations should be incorporated into the processes leading to regulatory acceptance of new test methods (see Sec. 3.7).

Development and Validation

- Criteria for validation and regulatory acceptance must be taken into account in the planning and design stages of validation studies [see Validation Criteria (pp. 2-4) and Regulatory Acceptance Criteria (p. 4)].
- Development of novel and innovative test methods that will provide for improved risk assessment should be encouraged and funded. Federal regulatory agencies can and should help to drive innovation.
- Testing batteries and tiered testing strategies should be accommodated in regulatory testing requirements where appropriate, and new methods should be considered for incremental acceptance.
- While both correlative and mechanistic tests can be validated and accepted, mechanistically based methods relevant to the biological or health effects of concern should be encouraged.
- Given the continuing increase in the numbers and types of test methods being developed for varying purposes, the validation process should be flexible and adaptable.
- Test methods should be evaluated by consistent validation criteria and with the same degree of rigor regardless of whether the proposal derives from academe, industry, Federal government, or other nations.
- Individuals or organizations developing or proposing new or revised test methods should be in communication with the regulatory agencies that will be asked to review and accept the methods.
- Assessment of the validation status of a new test method should involve relevant Federal agencies.

Regulatory Review of New Methods

- An efficient and effective process leading to regulatory acceptance of alternative methods should involve regulators at all stages prior to regulatory acceptance: development, prevalidation, validation, and review.
- Current efforts to incorporate validated alternative test methods into regulatory testing strategies should be continued and expanded.
- Federal agencies should continue to hold workshops on validation and acceptance issues of concern.
- Federal agencies should establish internal central clearing systems for evaluation of new or revised methods submitted to the agency, and for the periodic review of methods recommended by the agency.
- Test methods should be periodically reviewed and, where appropriate, revised or replaced, in light

of scientific and policy developments. Considerations for such activities include the following: - animal and non-animal test methods that have the potential to support improved risk assessment and the potential to partially or fully replace existing toxicity tests for some or all of the products regulated should be reviewed and evaluated;

- frequency of review should be consistent with scientific activity orprogress in that discipline;

- the process should be efficient and expedient;

- the process should include outside stakeholders;

- the reviews and outcomes of the reviews should be made public;

- regulations, guidelines, or recommendations should be promulgated for validated and accepted toxicity tests or test batteries.

- When evaluating the scientific acceptability of new or revised test methods, agencies should establish close links with the relevant scientific community to ensure continuing benefit from shared expertise.
- Concurrent submission of data from existing and proposed new methods will help facilitate regulatory acceptance of new methods and should be encouraged.
- Regulatory agency staff should be trained in the evaluation of data from newly accepted test methodologies.

Intra- and Interagency Coordination and Harmonization

- There should be interagency coordination of the evaluation of proposed test methods that are relevant to the needs of multiple agencies.
- A Federal interagency committee on test methods should be established to serve as a forum for the exchange of information, for the coordination of the review and evaluation of test methods, and for related activities. This committee should strive for interagency consistency in review and evaluation processes, and interagency and international acceptance of new and revised methods.
- Federal regulatory agencies should establish consistent processes and criteria for acceptance of new and revised toxicological test methods and should communicate them to interested parties.
- Federal regulatory programs should solicit input from other programs and agencies as they develop and modify test guidelines of general interest.
- Harmonization of hazard classification may be necessary before test guidelines can be harmonized.
- Proposed new or revised test methods relevant to the needs of more than one program or agency should be harmonized as appropriate.
- Interagency differences in test methods that purport to detect the same toxicological endpoints but differ unnecessarily in detail should be identified and harmonized.

Communication

- A consistent, coordinated process of involvement and communication among all stakeholders (e.g., researchers, developers, users, regulators, and the public) at all stages (development, prevalidation, validation, review, regulatory acceptance, and implementation) will facilitate the validation and acceptance of new test methods.
- Validation and regulatory acceptance should include the opportunity for input by interested

stakeholders inside and outside of government.

- The regulatory acceptance of new and revised test methods by agencies should be communicated to scientists and to various national and international organizations in journals, workshops, the *Federal Register*, and by other means.
- Agency regulations and guidelines should be readily available to the public.

International Harmonization

- U.S. agencies should attempt to harmonize guidelines through international organizations, such as the OECD, where appropriate.
- U.S. agencies should encourage harmonization of test guidelines across international organizations, e.g., between U.N. Transport and OECD, as appropriate.

IMPLEMENTATION

A standing interagency committee will be established to coordinate validation, acceptance, and national/international harmonization of toxicological test methods. The committee is designated as the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), and will replace the ad hoc ICCVAM. It will focus on toxicological test method issues for human and animal health and the environment that are common to multiple programs and agencies, without infringing on considerations unique to individual programs and agencies. It will recognize that regulatory acceptance is the purview of each Federal agency according to its mandates.

ICCVAM will seek to promote sound toxicological test methods that (1) enhance agenciesí ability to assess risks and make decisions, and (2) reduce animal use, refine procedures involving animals to make them less stressful, and replace animals in toxicological tests where scientifically feasible and practical (the 3Rs) (see Sec. 4).

1. INTRODUCTION

It has been estimated that over 80,000 chemicals are in use in the United States and that an average of over 2,000 new ones are introduced annually (NRC, 1984; OTA, 1995). While relatively few of these chemicals are likely to pose a significant risk to human health or the environment, the effects of most of them are unknown. The public and the environment may be exposed to these chemicals during or after their manufacture, distribution, use, and disposal. Exposure to a wide variety of chemicals and products, including industrial chemicals, pharmaceuticals, foods, personal care products, pesticides, and household chemical products may occur in the home and workplace. Exposure can also follow from mishaps in transport (such as spills) or from chemical pollutants in water, air, soil, and food.

Several Federal agencies have a responsibility to safeguard human health and the environment and to follow the fundamental public health precept of preventing unnecessary exposure to hazards. Federal agencies have developed and adopted testing methods to evaluate the potential hazardous effects of chemicals or to demonstrate their safety. These methods are used by scientists in government, industry, and academe to identify hazards and establish dose-response data to assess health and environmental risks. Federal agencies use the risk assessment principles and paradigm (Appendix E) described in the NRC publication (1983) 'Risk Assessment in the Federal Government: Managing the Process' and these principles are followed in this report. Risk assessment comprises (1) hazard identification ó the evaluation of the potential to produce adverse biological effects, (2) dose-response assessment & the determination of the influence of exposure levels on adverse effects, (3) exposure assessment ó the estimation of anticipated exposure to an agent, and (4) risk characterization & the description of the nature and often the magnitude of risk, including attendant uncertainty. The paradigm was recently extended to ecological risks (NRC, 1993) (Appendix E). The usefulness of the paradigm was confirmed in the report 'Science and Judgment in Risk Assessment î (NRC, 1994). Depending upon the assessment outcomes and other considerations, regulatory agencies may implement risk management and pollution prevention practices to protect public health and the environment.

New and revised toxicological test methods are being developed with increasing frequency. Scientists continue to seek methods that are less costly and time consuming, that incorporate new understanding of toxic mechanisms, that evaluate important endpoints not previously considered, and that improve prediction of the potential toxic effects of chemicals and other agents. Recent advances in molecular and cellular biology and new research technologies are being incorporated into these new testing methodologies.

The development of new test methods is driven by scientific, social, economic, and political factors. Currently, assessment of the potential adverse health and environmental effects of chemicals is accomplished largely by tests involving laboratory animals and plants. Public concern about animal use, however, has resulted in recent legislation requiring scientists to consider, prior to using animals, alternatives that do not use animals, that reduce the number used, or that minimize their pain and distress. These directives were included in the 1985 Animal Welfare Act Amendment (USC, 1985a) and the 1985 Health Research Extension Act (USC, 1985b). More recently, the 1993 NIH Revitalization Act (USC, 1993) (Appendix F) directed the NIEHS to develop and validate alternative methods that can reduce or eliminate the use of animals in acute or chronic safety testing. This Act also directed the NIEHS to develop criteria and processes for the validation and acceptance of test methods by regulatory agencies, and that mandate is the impetus for the development of this document. Because of similar concerns in

Europe, the European Union requires that an animal procedure shall not be performed if non-animal procedures are reasonably and practically available (EEC, 1986). As of January 1998, the European Union will prohibit the testing of cosmetics in animals if validated non-animal alternative methods exist (EEC, 1993).

Alternative toxicological tests are those that reduce the number of animals in a test, refine procedures to make them less painful or stressful, replace animals with non-animal systems, or replace one animal species with another that is lower on the phylogenetic scale. A number of useful alternative methods have been developed and accepted for the evaluation of the potential toxic effects of chemicals and products. For example, a well-known bacterial (Salmonella) assay (Zeiger, 1995a) that evaluates compounds for their ability to mutate DNA can be used to screen chemicals for potential mutagenicity and carcinogenicity and to investigate mechanisms of toxic action. The Limulus amebocyte lysate test, an in vitro method using blood cells from horseshoe crabs, has replaced rabbit pyrogenicity testing to detect endotoxins at considerable savings in time and cost (Flint, 1994). Reductions and, to some extent, refinements in animal testing have been incorporated into acute toxicity and skin and eye irritation test protocols, while in vitro measures of dermal corrosion potential for chemicals in selected classes have been approved for hazard classification of chemicals in transportation (DOT, 1995). While continued progress is expected in the development of alternative test methods, the complete elimination of in vivo tests is unlikely in the foreseeable future. On the other hand, phylogenetically lower organisms such as fish, invertebrates and algae are used for environmental effects testing to assess the ecotoxicity of chemicals. Such testing is conducted to predict the toxicity for a wide phylogenetic range of different organisms, from mammals, birds, fish, and higher plants, to invertebrates and algae.

The development and acceptance activities for toxicological test methods in the U.S. are dispersed among various Federal research and regulatory programs and agencies, and a number of non-government organizations. These efforts range from basic to applied research and routine to specialized testing, a few of which are enumerated here (many other examples exist):

- The National Cancer Institute (NCI) funds the development of new, mechanistically based test methods for the detection of substances with anti-tumor effects
- The National Toxicology Program (NTP), which consists of the related research and testing activities of the NIEHS/NIH, NIOSH/CDC, and NCTR/FDA, has a responsibility to expand the number of chemicals tested, broaden the toxicological database on agents, develop new test methods, and communicate results to the public. Oversight of these activities is provided by the NTP Executive Committee, which consists of the heads of these agencies as well as those of the NCI, OSHA, CPSC, EPA, and ATSDR.
- The FDA publishes test guidelines that are used by industry to develop data on the safety of food additives.
- The Johns Hopkins University Center for Alternatives to Animal Testing (CAAT) supports investigators in developing in vitro test methods.
- Various established test protocols have been codified by the American Society for Testing and Materials (ASTM).

Toxicological test methods evolve through a series of steps from development of the method, to refinement of the test protocol, assurance of transferability among laboratories, and determination of the performance characteristics of the test (Curren et al., 1995). The method must then be validated to determine the reliability and relevance of the procedure for a given purpose (Balls et al., 1995a, Bruner et al., 1996). The acceptance of a test method by regulatory agencies builds upon, but is separate from, validation (Balls et al., 1990a). Recognizing that Federal agencies validate and accept test methods on a case-by-case basis with no established uniform procedures, this report recommends criteria and processes that can be consistently employed by agencies and test method developers. In addition, recommendations are provided to enhance cooperation on test method validation and evaluation throughout the Federal government within and across agencies and, possibly, internationally.

2. VALIDATION OF TEST METHODS

VALIDATION OF TEST METHODS

2.1 Background

This section focuses on the different approaches, criteria, and processes for test method development and validation that lead to scientific acceptance. Although it is difficult to view these factors apart from their impact on, or relevance to, regulatory agencies, the scientific validation processes and decisions are considered apart from the regulatory acceptance processes and requirements. The Committee did not view its purpose as that of establishing formal criteria for individual test methods or developing formal frameworks or mechanisms for validation of new or revised methods. Rather, it emphasized general scientific principles and processes to provide guidance to individuals or organizations developing test methods and/or submitting data from such methods to Federal agencies. The report is also designed to provide guidance for agencies considering the validity and acceptability of any new or revised method.

These general principles should guide validation and acceptance activities regardless of whether the principles are incorporated into a formal organizational framework or applied in an ad hoc manner.

An agency may need to determine the validity of a test method when (1) a test method being proposed by the agency will be required of organizations submitting or using data, (2) data on a new test method are first submitted to the agency, and
(3) the agency becomes aware of a new test method that may be used to develop data in a regulatory submission. In general, the procedures for assuring that a test method is validated should be the same for Federal agencies and non-Federal individuals or organizations.

The Committeeís survey of Federal agencies revealed no formal requirements for demonstrating the validity of a new or revised test method for a given purpose, i.e., there is no ëchecklist of steps that must be satisfied before an agency would consider a procedure valid for a specified purpose. The agencies indicated that validation of proposed test methods is determined on a case-by-case basis, taking various factors into consideration. Factors considered in this determination include formal recognition of a method by an organization such as ASTM, AOAC, USP, or OECD. Agencies also publish recommended testing methods to meet their requirements, such as the FDA 'Redbook' (FDA, 1993) or the various EPA test guidelines. At times, agencies have organized scientific symposia and workshops to obtain scientific consensus on the validation status of a method, or have directed interlaboratory evaluation efforts such as round-robin testing to validate specific methods. In addition, comments have been requested from the scientific community and the general public regarding the general acceptability of a proposed test method following publication in the *Federal Register*.

The Committee recognized at the outset that the issues being addressed (i.e., validation criteria and processes) are complex. They have been studied and addressed by numerous groups and organizations (AOAC, 1990a,b; ASTM 1992a,b; Balls et al., 1990b,c, 1995a; Bruner et al., 1996; Fentem et al., 1995; Frazier, 1990a,b, 1994; Goldberg et al., 1993; Green, 1993; Green et al., 1993; IRAG, 1993; OECD, 1990, 1996; Scala, 1987, 1995; Walum et al., 1994; Zeiger, 1995b), and such activities are continuing. Also, a number of organizations are engaged in the identification, development, and validation of new or revised test methods (AOAC, 1990b; ASTM, 1992a,b; Balls et al., 1990a,b, 1995a,b; Balls & Karcher, 1995; Fentem et al., 1995; Frazier 1990a,b, 1994; Goldberg et al., 1993; IRAG, 1993; OECD, 1990, 1996; Walum et al., 1994).

The Concept of Test Method Validation

Validation is the process by which the reliability and relevance of a test method are evaluated for the purpose of supporting a specific use (Balls et al., 1990b,c; OECD, 1990). The approaches and methods conform to scientific principles of objectivity and appropriate experimental design. The definitions and concepts used in this document closely follow previously published definitions (*Appendix A*).

A test is considered validated when its performance characteristics, advantages, and limitations have been adequately determined for a specific purpose. The measurement of a testís reliability and relevance are independent stages in the validation of a test method, and both are required. Reliability is an objective measure of a methodís intra- and interlaboratory reproducibility. If the test is not sufficiently reliable, it cannot be used for its intended purpose. Alternatively, if the test is not relevant, or of questionable relevance to the biological effect of interest, or if it is not an appropriate measure of the effect, its reliability is academic. The relevance of a test may be linked to the mechanism of the toxic effect it measures and to its proposed uses. Measures of the relevance of a test include the calculated operational characteristics (e.g., sensitivity, specificity, etc.) or statistically derived correlation coefficients, and determinations of the mechanistic association of the measured effects with the toxic events of interest.

There are no optimum or minimum levels of reproducibility or association with the event of interest that must be reached for a test to be considered 'validated'. The conditions under which the test will be used and the purposes to which its results will be applied will determine the levels of reliability and relevance that are needed (see Sec. 2.4.3).

2.3 Types and Uses of Test Methods

New tests can be designed as substitutes to replace, or be interchangeable with, currently accepted tests, or as tests that have no correlate with currently used tests or endpoints. Definitive tests provide data that are used to measure toxic effects or unequivocally identify hazardous substances and assess the risks posed by exposure to them. Screening methods are generally used to make preliminary hazard decisions (i.e., identify potential adverse effects), or to select chemicals or set priorities for other, more definitive tests. They often provide only a qualitative or semiquantitative response and are generally not designed to serve as definitive tests. In contrast, adjunct tests are used to increase the information base and/or aid in the interpretation of results from other, definitive methods. They are not used in isolation or as substitutes for definitive methods, but they often support the relevance of the definitive test method by providing information related to the mechanism of toxicity. For example, a test showing that the relevant metabolic pathways are similar in the test system and the species of interest supports the use of information from the definitive test system for hazard and risk assessments. These tests can be developed for newly identified endpoints or effects, or they can be used to replace existing adjunct methods.

Because the data from a substitute test will be used in lieu of a currently used test, its adoption requires evidence from validation studies that use of the method will provide a comparable or better level of protection of human health or the environment than current methods or approaches. Often, the test method being replaced is one that has been generally accepted by the scientific and regulatory communities. As a rule, these tests have been considered to be validated through their history of measuring the effects of concern as well as through the evolution of standardized protocols and data evaluation procedures. During the validation process, it is necessary to compare the performance of the substitute procedure against the accumulated information from the currently used test. Other tests will have been adopted following formal validation procedures; these will have available supporting validation documentation against which the new test method can be compared.

Often, new tests are developed that identify, or provide data, about toxicologic effects not addressed by existing test methods. These new tests should be based on specific biologic mechanisms or endpoints related to an effect of concern. The test itself often will help define the effect. When a new method is designed to measure an effect that is newly discovered or not well defined, there usually is no benchmark against which the usefulness or effectiveness of such a method can be judged.

Some toxicological test methods are considered mechanistic because they are based on specific biological processes that underlie toxicity. These tests can help define or categorize the mode of action of a toxic process, and can be useful for identifying classes of chemicals and products that act via similar biochemical pathways. For the purpose of risk assessment, it is important to link the measured effect with some relevant toxic or adverse effect. Methods with known mechanisms of action are generally easier to validate because they are directly relevant to the biological effect of concern, (e.g., a method to evaluate cholinesterase inhibition). The usefulness of such tests may extend beyond the classes of substances investigated because they are applicable to any class of substance that operates by that mechanism (Frazier, 1990a,b; 1994).

In contrast, tests may be developed where the mechanistic relationship of the test endpoint to the effect of interest may not be known or well defined, and the test may not provide insight into the nature of the biological response being measured. Acceptance of these methods, therefore, primarily depends upon the demonstration of a correlation between the new method and the response in the standard test. The applicability of such empirical tests to unstudied classes of compounds may not be appropriate because the compounds may cause toxicity by mechanisms not measured by the test.

Methods may be designed to stand alone, or as components of tiers, batteries, or hierarchical testing strategies (e.g., stepwise sequence of tests from simple to more complex). The process leading to the scientific validation of stand-alone tests, or tests that are to be used only as a component of a test battery, are similar.

Many test methods currently accepted by Federal agencies have been considered validated based on their history of use by the scientific community, even though their operational characteristics (e.g., reproducibility and predictivity) may not have been fully established at the time of adoption. Calculation of current performance using existing data is necessary so that the performance of new or revised methods can be compared to the existing method. Additionally, there may be legal or statutory constraints to the replacement of some well-established tests with tests measuring new or different effects, or with tests using different organisms.

2.4 The Process of Validation

Validation is a scientific process designed to characterize the operational characteristics, advantages, and limitations of a test method, and to demonstrate its reliability and relevance. The designation of a test as 'validated' or 'not validated' for a specific purpose is not irrevocable; subsequent data and experience with the test can lead to a loss or affirmation of its validation status. Also, a test method could be considered validated for a specific use, but not for other uses.

The criteria for validation of a test method are, to a limited extent, a function of the purpose for which the test method will be used. For example, the mechanisms of some effects are known or are relatively straightforward (e.g., skin corrosivity, estrogen receptor binding) while others (e.g., carcinogenicity, developmental toxicity) are complex and multi-faceted, or not well understood. The validation of tests for these different types of effects requires different approaches.

When validating new or revised test methods, hypotheses are developed regarding the effects measured and their relationships to the biological effects in the species of concern. The relationship between the new method and the effect it is designed to predict, or the procedure it is designed to replace, must be described. The definition of these relationships have been termed the prediction model (Bruner et al., 1996).

The relevance of a new method to the biological effect of interest, or to the procedure it is designed to replace, should be defined. The ideal definitive method measures an event that is mechanistically similar or related to the effect of interest, and the results correlate with the human health or ecological concern. The mechanistic relationship of the test endpoint to the toxic effect of concern should be established with a reasonable degree of rigor. In general, the closer the linkage between the effect measured and the toxicological effect of interest, the simpler the validation process will be. A test that measures an effect identical to the effect of concern increases confidence that the test will accurately predict or model the effect in the target species (e.g., humans or specific wildlife populations) of concern. For a test to replace one currently in use, it must be shown to reliably provide results at least equivalent to, or better than, the original method. This can be addressed in two ways. Where possible, the response in the target species should be the benchmark. This approach is usually limited by the availability of high-quality data in humans or other target species. In the other, more common approach, the results of the traditional method are used as the benchmark against which the candidate test is measured. For tests designed to be used primarily as adjuncts, there must be evidence that the results obtained are relevant to the definitive test or to the toxicological effect of interest.

Reproducibility and operational characteristics must be determined for test methods that measure new toxicological endpoints because data from these methods will define the expected range of responses and serve as the benchmark against which to compare future new tests. Test methods that measure new effects may produce data unfamiliar to regulatory reviewers. Before such data, or the test procedure itself, can be adequately evaluated, reviewers may have to obtain sufficient familiarity with the test from other organizations or individuals, or may need to develop the needed expertise.

Prior to performing a validation study, investigators must define the model being tested, the biological endpoint to be predicted, and the analytical procedure(s) and decision rules for evaluating the new test for its relevance. For example, if a test or battery of in vitro tests is designed for predicting eye irritation, the procedures by which the new test's results will be compared against the in vivo test results should be defined prior to performance of the test(s).

A validation study should be planned in advance of the distribution of chemicals and the beginning of testing. Establishment of a steering committee or management team to design and direct the validation study has been recommended (Balls et al., 1995a,b; Balls & Karcher, 1995; OECD, 1996). The responsibilities of such a committee would include the determination that there is sufficient information about the method to support a validation study (this can be determined by evaluating the information obtained during prevalidation). This committee would define the purpose of the study, assure that the test protocol is sufficiently developed and defined, develop recordkeeping procedures, select participating laboratories, select and code chemicals, and monitor laboratory and test performance. Following completion of the testing, this committee would review and evaluate the data or oversee its evaluation. It is important that a biostatistician be involved in the development of the validation study design and data collection formats and in the evaluation of the study results. Prevalidation and the steps in the validation process are outlined in Figure 1, and described in more detail, below.

2.4.1 Prevalidation

Critical to the validation process are a standardized test protocol and the ability of competent laboratories to perform the test. Prevalidation is the process by which testing laboratories are selected and demonstrate competence in performing the testing procedures, and during which the test protocols are standardized. It is important that this be established in advance of formal validation procedures. Having the laboratories perform the protocol with a small number of uncoded, well-defined substances will accomplish this objective. If a protocol can not be standardized or reproduced using known chemicals, it cannot be validated. After the test protocol is standardized and positive and negative control chemicals identified, information should be developed regarding the types of substances for which the test can be used. These preliminary steps have been referred to as 'prevalidation' (Curren et al., 1995) and 'test optimization' (OECD, 1996). Any deficiencies in laboratory performance or test protocol design can be addressed prior to the start of the validation study, and an "unqualified" laboratory can be eliminated from the study. This will ensure that the data obtained during the validation process will not be compromised by the inability of a participating laboratory to perform the test protocol, or by an inadequate protocol design. The data derived from the prevalidation procedure should be included with the data derived from the subsequent validation study, because it provides a record of the ability of the laboratories to perform the test procedure.

2.4.2 Validation Components

2.4.2.1 Formal, Detailed, and Robust Test Protocols

A prerequisite for the performance and evaluation of any validation study is a formal protocol (or procedural manual) that can be readily understood and followed by individuals in other laboratories, and by administrative and scientific review personnel. This protocol should clearly state the purpose of the test. It must include formal criteria for determining the doses or concentrations of test substances, for evaluation of the test results, and for the acceptance and rejection of data and experiments, and it must be sufficiently robust so as to be readily transferable among laboratories. Participants in the validation study must adhere to that protocol, and unavoidable deviations from it must be documented and their possible effects should be addressed. The procedures proposed for the routine use of the test after completion of the validation procedure should be the same as those used during the validation studies. When multiple laboratories participate in a study, the test protocol must be faithfully followed and the data recorded and analyzed using a common format.

2.4.2.2 Intra- and Interlaboratory Reproducibility

The reproducibility of a test can be measured in a nonquantitative manner or by a quantitative comparison of results. Reproducibility within a single laboratory and among different laboratories must be determined with qualified laboratories following the standardized protocol. The number of laboratories participating in a reproducibility assessment will vary according to the nature of the test. Other factors that could influence the number of participating laboratories are cost, the level of laboratory effort and commitment required, and the level of interest in the method. To enhance the credibility of the data and avoid investigator bias, the reproducibility trials should be performed using coded chemicals (see below), with the codes broken only after the trials are completed and the data compiled and evaluated. In some circumstances, safety considerations may preclude the coding of chemicals.

All test responses, regardless of whether they are in humans, animals, or cultured cells, contain a certain level of between-animal or between-culture variability (which may or may not be defined) that must be considered when evaluating the performance of the candidate method. This is related to, but not identical to, reproducibility. The variability of a method is a function of the range of responses obtained when the protocol is correctly performed. Generally, the variability of in vivo methods is greater than in vitro methods because of the wider degree of genetic and physiological diversity among whole animals. In addition, variation among humans and other organisms of concern is much greater than the variation among inbred or random-bred laboratory animals within the same strain. The sources of variability in a test should be identified and statistically defined. A test that exhibits a wide variability may be highly reproducible, but the variability will make the results among experimental trials or different laboratories difficult to evaluate, and may require more rigorous statistical treatment than less variable test results. In addition, procedures that exhibit wide variability may require larger numbers of test subjects and may thereby negate any advantage of a test procedure designed to reduce animal use or costs. The extent of variability that exists in the currently used test and in the new test can set limits on the maximum correlation that might exist between the two tests (Bruner et al., 1996).

2.4.2.3 Selection of Reference Substances

It is axiomatic that one can select a group of chemicals to yield any desired test result. For this reason, the chemical class or product-line representatives chosen for the prevalidation and validation procedures should be representative of the substances for which the test is designed, and should yield responses ranging from inactive to highly potent, to enable evaluation of the sensitivity of the new test. Also, because test method sensitivities will vary for different chemical classes, attempts should be made to understand the limitations of the test for specific chemicals or chemical classes (Lipnick et al., 1995) and to identify those chemicals or chemical classes that cannot be adequately evaluated by it. The results from the new test method are then compared to those from the standard test using the same chemicals. Because of these considerations, it is not possible to develop a single list of chemicals that can be used to evaluate the performance of different tests or different biological effects.

When evaluating a new method, there must be a sufficient number of chemicals to demonstrate the test's performance within a chemical class or among a range of chemical classes or products and among substances of differing reactivities. Other considerations are the cost and complexity of the method; for example, an in vitro test to identify estrogen receptor-binding chemicals would require less time and resources to perform than an in vivo rodent reproductive test. These cost and time considerations would determine the numbers of chemicals that could reasonably be tested in a validation exercise.

2.4.2.4 Reference Species

Evaluation of adverse consequences of chemical exposure is best determined in the species of interest. Therefore, where possible, the baseline reference for new test methods should be the response of the species of interest. This avoids the need to extrapolate between species, and any identified effects can be more reasonably judged as real and relevant. However, testing in the species of interest is not always possible or appropriate. For instance, although some toxic effects, such as dermal reactions from slight or mild irritants, can be evaluated directly in humans, more severe reactions cannot. Likewise, in evaluating effects on the ecosystem, it is not possible to investigate every inhabitant. Because of these limitations, test methods must use surrogate species or in vitro systems as the reference for the species of interest. For the purposes of risk assessment, there is a basic assumption that, in the absence of information to the contrary, the data from one animal species can be used to assess effects in another (EPA, 1982; Smrchek et al., 1993).

The quality of the correlations between the results from a new method and the results from testing in the species of interest or the currently used test method, is limited by the quality of the data against which the new method is being compared. If there are no adequate data against which to validate the new method, it may be necessary, for example, to develop in vivo data on the chemicals selected to be used for the validation study of an in vitro procedure.

2.4.2.5 Supporting Data and Data Quality

All of the data supporting a new method must be available, along with the detailed protocols under which the data were produced. Mathematically transformed data or summary conclusions alone are not sufficient; raw data should also be available for examination, as should supporting documentation, such as laboratory notebooks. It is generally helpful to consult in advance with the agency or agencies to which the data are to be submitted. Data accompanied by evidence of formal quality assurance or adherence to EPA, FDA, or OECD Good Laboratory Practices (GLPs) (EPA, 1983, 1994a,b; FDA, 1994; OECD, 1982, 1992) carry a higher level of assurance.

Many laboratories that develop or validate methods may not be familiar with GLPs or be organized in such a way as to perform studies under strict GLP guidelines. A component of GLPs that should be followed in all cases is that all protocols, experiment-related notes, and data entries must be detailed, accurate, and annotated with the names of the individuals keeping the records and the dates of the work.

2.4.2.6 Peer Review

One basic tenet of science is that test procedures, results, and conclusions should undergo critical peer review. Often, however, new test methods are proposed without evidence of such independent critical review. Peer reviewers should include individuals who will not be affected by the outcome of the results, but who are well-versed in the relevant experimental techniques and the specific method under review. Ideally, detailed test protocols and the results of the validation studies should be published in an independent peer-reviewed vehicle. If this is not available, other evidence of independent scientific review of the procedure and test results should be provided to the organization asked to determine the scientific validity of the test method.

2.4.3 Measurement of Test Performance

Tests may produce data of three general types: qualitative (yes-no), semiquantitative (rank order), and quantitative (numerical values). Qualitative data can be important indicators of the presence or absence of hazard potential, as in the use of a screen to identify an effect such as dermal corrosion. The demonstration of the severity of hazard potential generally requires at least semiquantitative information (e.g., an agent is either irritating, minimally irritating, or non-irritating to the eye), while evaluation of degree of hazard following exposure (i.e., risk) usually depends upon quantitative dose-response data (e.g., anything over a specific dose will cause an adverse effect).

A number of operational characteristics for qualitative data can be measured, such as sensitivity, specificity, positive and negative predictivity, and concordance (Cooper et al., 1979). Sensitivity is defined as the proportion of active substances that are correctly identified by the new test, and specificity is defined as the proportion of inactive substances that are correctly identified. Positive and negative predictivity are the frequencies of correct predictions obtained from the new test. Concordance is the overall agreement (positive and negative) between the new test results and the results from the method against which it is being compared. These measures are most useful for methods whose results can be categorized simply as 'positive' or 'negative' (i.e., are qualitative) and can be incorporated into a standard 2x2 table (*Appendix A*). These standard measures, however, may not provide an accurate representation of the performance of the test method if the results are quantitative or semiquantitative, and not easily converted to binary 'positive' or 'negative' responses. Other statistical evaluations would have to be performed for these data, such as probit models, calculations of confidence intervals or correlation coefficients, or stepwise analyses of variance (AOAC, 1990a,b; ASTM, 1992a,b; Balls et al., 1995a; Bruner et al., 1996; Diener et al., 1994). In all cases, the specific statistical procedure used will depend on the types of data obtained and the numerical ranges of the results (IRAG, 1993; OECD, 1990).

The performance of a test is highly dependent on the types of substances chosen for the validation procedure and the proportion of substances expected to yield positive responses (prevalence) (Scala, 1987). For this reason, the proportion of active chemicals in a validation study should reflect the proportion of active chemicals expected among the substances for which the test will be used. A test that is highly sensitive and tends to yield positive responses will appear highly effective when tested against a population containing a high proportion of true positives but ineffective against a population with few true positives. Conversely, a relatively insensitive test will appear highly effective against a population that contains a very large proportion of inactive substances, such as may be found in the environment.

Measurements of performance describe how often the test produces 'false positives' or 'false negatives.' False positives are obtained when the test errs on the side of safety and leads to the characterization of hazard where no hazard exists. A false negative will understate a substancesí potential hazard. The false positive and false negative rates of a test may affect its usefulness for specific purposes. For example, a screening test for skin corrosivity that has a low false positive and high false negative rate may be useful to identify some corrosive industrial chemicals. There would be a high degree of confidence in the positive, but not in the negative results. Chemicals with negative results that may be widely used by the general population would need to proceed to the next level of testing to confirm their safety (i.e., noncorrosiveness) or hazard.

2.4.4 Validation of Computational Systems

Different validation criteria are needed when computer-based computational systems (i.e., those that predict responses on the basis of computer algorithms) are proposed for use. It may not be appropriate to evaluate the intra- or interlaboratory variability of those systems whose performance is controlled by a computer algorithm. Also, because the structure of the test chemical has to be entered into the program, it is not possible to test coded chemicals in the same manner as in biologically based tests. When validating computational test systems, the chemicals used in the initial development of the algorithm must not be among the chemicals used to validate the test. Despite these limitations, results obtained from computational systems must still be compared with the results obtained using the biological or chemical systems that are being predicted.

2.4.5 Validation by Retrospective Analysis

A variant of an existing method may sometimes be validated by retrospective analysis of an existing database. This has the advantage of not requiring additional laboratory resources, while enabling a direct comparison of the results of the new procedure with those of the original procedure. If the original method was shown to be reproducible, then the new method should also be reproducible. For example, in a retrospective statistical analysis of existing Draize eye test databases that were developed using six animals per dose, it was shown that similar conclusions would have been reached if only two or three animals per dose had been used (Springer et al., 1993).

2.4.6 Validation of Test Batteries

For the prediction of complex events such as eye irritation, carcinogenicity, or teratogenicity, batteries of tests that measure different effects may have to be used. Validation of such batteries depends on their configurations and the intended uses of the data. The most effective test batteries are those in which each component test measures an effect related to the overall biological effect of interest, but where there is little or no overlap (complementarity) among the individual tests. Before the usefulness of test batteries for specific endpoints can be evaluated, the individual component tests must be validated. Only after this step can the predictivity of the assembled battery of tests can be measured against the event of interest.

2.4.7 Summary

In summary, the specific goals of the validation study and the hypotheses to be tested must be clearly defined. The test method must be shown to be reproducible and understandable in the context of the science and, for substitute tests, the procedure should offer an advantage over the currently accepted procedures. In addition, the known limitations of the procedure must be presented, along with supporting data. The untransformed test data and results must be available, and they must have been peer-reviewed or be available for review by the knowledgeable scientific community. Because tests can be designed and used for different purposes (e.g., as substitutes or screens) by different organizations, and with varying categories of substances, the test validation process should be highly flexible and adapted to the specific test and its proposed use. Despite this need for flexibility, all the various factors that make up a validation process must be included. The determination of whether a procedure is considered to be scientifically validated must be made on a case-by-case basis, and can only be made in the context of the proposed use(s) of the test. The criteria for validation of a new or revised text are summarized below.

2.5 Validation Criteria

For a new or revised test method to be considered validated for regulatory risk assessment purposes, generally it should meet the following criteria (the extent to which they are met will vary with the method and its proposed use). However, there needs to be flexibility in assessing a method given its purpose and the supporting database (see Sections 2.3 and 2.4):

- The scientific and regulatory rationale for the test method, including a clear statement of its proposed use, should be available.
- The relationship of the test methodís endpoint(s) to the biologic effect of interest must be described. While the relationship may be mechanistic or correlative, tests with biologic relevance to the toxic process being evaluated are preferred.
- A detailed protocol for the test method must be available and should include a description of the materials needed, a description of what is measured and how it is measured, acceptable test performance criteria (e.g., positive and negative control responses), a description of how data will be analyzed, a list of the species for which the test results are applicable, a description of the known limitations of the test, and a description of the classes of materials that the test can and cannot accurately assess.
- The extent of within-test variability and the reproducibility of the test within and among laboratories must have been evaluated. Data must be provided describing the level of intra- and interlaboratory reproducibility and how it varies over time. The degree to which biological variability affects this overall test reproducibility should be addressed.
- The test methodís performance must have been demonstrated using reference chemicals or test agents representative of the types of substances to which the test method will be applied, and should include known positive and known negative agents. Unless it is potentially hazardous to do so, chemicals or test agents should be tested under code to exclude bias.
- Sufficient data should be provided to permit a comparison of the performance of a proposed substitute test to that of the test it is designed to replace. Performance should be evaluated in relation to existing relevant toxicity testing data and relevant toxicity information from the species of concern. Reference data from the comparable traditional test method should be available and of acceptable quality.

- The limitations of the method must be described; for example, in vitro or other non-animal test methods may not replicate all of the metabolic processes relevant to chemical toxicity that occur in vivo.
- Ideally, all data supporting the validity of a test method should be obtained and reported in accordance with Good Laboratory Practices (GLPs). Aspects of data collection not performed according to GLPs must be fully described, along with their potential impact.
- All data supporting the assessment of the validity of the test method must be available for review.

- Detailed protocols should be readily available in the public domain.
- The method(s) and results should be published in an independent, peer reviewed publication.
- Methodology and results should have been subject to independent scientific review.

Because tests can be designed and used for different purposes by different organizations and for different categories of substances, the determination of whether a specific test method is considered by an agency to be useful for a specific purpose must be made on a case-by-case basis. Validation of a test method is a prerequisite for it to be considered for regulatory acceptance.

Figure 2.1
VALIDATION PROCESS

I. Test Development

II. Prevalidation/Test Optimization
A. Preliminary planning
1. Define basis and purpose of test
2. Develop protocol
3. Develop control values
4. Develop data/outcome prediction model

B. Activities
1. Qualify and train laboratories
2. Measure intra- and interlaboratory reproducibility
3. Identify limitations of test

III. Determine Readiness for Validation
A. Analyze test development and prevalidation data
B. Standardize protocol

IV. Test Validation
A. Form steering committee/ management team
Define purpose of validation study
Design study
Select participating laboratories
Establish management evaluation and oversight procedures

B. Pretest procedures
Implement data recordkeeping procedures
Select reference chemicals

Code and distribute reference chemicals

C. Test coded chemicals
Measure interlaboratory performance
Compile and evaluate data

D. Evaluate test
Analyze and summarize test results
Challenge data with prediction model
Peer review of protocol and data
Accept, revise, or reject model

V. Submission of Test for Regulatory Approval
A. Prepare report
B. Make supporting data available
C. Prepare results for publication

3. REGULATORY ACCEPTANCE OF TOXICOLOGICAL TEST METHODS

3. REGULATORY ACCEPTANCE OF TOXICOLOGICAL TEST METHODS

3.1 Background

Regulatory agencies are mandated to protect human and animal health and the environment. Decision-making about hazards and risks requires data that usually include toxicological test results. Research and regulatory agencies develop or adopt test methods or strategies to ensure that toxicological data are scientifically sound, consistent, and usable in the risk assessment process.

The testing mandates of Federal regulatory agencies vary with their legislative authority. In a few cases, testing procedures are found in legislation (e.g., USC, 1960). More often, however, recommendations and requirements for toxicity testing are described in regulations (e.g., packaging of hazardous materials [DOT, 1990]), policy documents (e.g., acute toxicity testing positions published in the *Federal Register* by CPSC [1984], and FDA [1988]), published testing guidelines (e.g., FDA [1993] 'Redbook' of toxicological principles; the EPA [1988], CPSC [1992]) and unpublished guidance. Publications by non-Federal organizations such as the ICH, USP, ASTM, AAMI, OECD, and the UN Transport also serve as sources for testing procedures. Agencies and programs without regulatory authority (e.g., NIEHS, NIOSH, and DOD) also generate toxicological data for use in human health and/or ecological risk assessments, and they too must determine test method acceptability. For the purposes of this report, 'regulatory acceptance' refers to acceptance of a method to generate information for risk assessment, whether or not the method requires regulatory agency approval.

Regulatory agencies and programs have vastly different requirements for scientific dataówhether it is to determine safe exposures for consumers or workers, or the toxic effect on humans, animals, and the environment that may follow from exposure to industrial chemicals, pesticides, biologicals, human or veterinary drugs, cosmetics, consumer products, or chemicals in transport (Table 3.1). In some cases, as with certain authorities under CPSC, DOT, and OSHA, industry uses agency or other guidelines to evaluate and then appropriately label products as to their potential hazards; the scientific data supporting the labeling are not submitted to these agencies. In other cases, such as certain authorities under the EPA and FDA, industry uses agency guidance to generate extensive data that must be submitted for the agencies to evaluate risks to human health or organisms in the environment.

3.2 National and International Practices

Federal regulatory agencies have different approaches for approving toxicological test methods, and procedures differ among programs within the same agency. Some testing programs are involved with international organizations that agree upon test methodologies for particular chemicals, products, and chemical exposures. These agreements significantly reduce the need for repeat testing by similar authorities in different countries and result in a saving of industrial resources and a reduction in laboratory animal use. For examples of recent test method activities in Federal agencies and international bodies, see Table 3.2.

3.2.1 U.S. Regulatory Agencies

The ICCVAM asked each regulatory agency what criteria and processes it used to evaluate new and revised toxicological test methods. There were many similarities, but also significant differences.

- Most Federal agencies agree that in addition to the specific requirements of the agency, new methods must meet certain minimum standards for validation. Also, the methods must be reviewed and commented upon by the interested public. See Table 3.2 for examples of test methods approved or being considered for approval.
- Federal agencies differ widely in the procedures they use for determining whether a new or revised method is ready for regulatory use and have no established uniform process for exchanging information about proposed new or revised testing guidelines, although most agencies publish notices in the *Federal Register* for comment at some stage of the approval process.
- With few exceptions (e.g., IRAG), there has been no attempt across U.S. agencies to harmonize guidance for validation or regulatory acceptance of new or revised test methods.
- International test guidelines adopted by one Federal regulatory program or agency are not necessarily used or accepted by others. For example, while the Federal government is an active member of the OECD, which is working toward international harmonization of testing methods for all chemicals, products, and exposures. Testing done in accordance with OECD guidelines is not

always acceptable to all agencies.

To illustrate some of the procedures that are in place in Federal agencies for accepting test methods, *Appendix B* compares the approval processes for one research institution (ATSDR) and three regulatory agency programs (EPA OPP, EPA OPPT, and FDA CFSAN). Given that evaluation processes differ markedly, there would be merit in adopting a single Federal process and uniform criteria that would promote cooperation and consistency among programs and agencies.

3.2.2 International Organizations

Federal regulatory agencies currently participate in international organizations dealing with toxicological testing, e.g., DOT with the UN Transport, EPA with OECD, and FDA with ICH, (see *Appendix C*). Each organization deals with specific categories of chemicals and exposures. Presently there is no formal process for harmonization of test guidelines across these international authorities, although OECD is leading an effort to harmonize international classification criteria for hazardous materials of all types.

The OECD comprises representatives from the governments of 28 major industrialized countries in Europe, the Pacific Basin, and North America. In the past, its testing program focused on industrial chemicals; more recently, however, it has expanded to include pesticides. The goal of OECD is to establish universal testing guidelines applicable to all chemicals and exposures. The U.S. solicits input from Federal agencies, industry, and public interest groups to develop a U.S. position on proposed OECD testing guidelines. OECD uses member country consensus to gain agreement on test guidelines. Once a method has been approved by the OECD, agencies in member countries are expected to accept data generated in accordance with the test guideline. The ICH draws together regulatory agencies and industry organizations from the European Union, Japan, and the U.S. to deliberate on matters associated with the approval of human pharmaceuticals and biologicals. The U.S. approval process for ICH guidelines includes publication in the *Federal Register* for comment. The UN Transport deals with hazardous substances in transport and is the only international body dealing with testing that affects essentially all countries in the world.

International regulatory requirements can be a significant barrier to the introduction of new methods. When a traditional method is accepted internationally but a new method is not yet accepted everywhere a product is to be marketed, it is likely that the traditional method will continue to be used. In addition to the acceptance of validated alternatives to traditional methods by international organizations such as OECD, international discussion of the goals to be achieved by the introduction of new methods would facilitate the acceptance process. Even if agreement is not reached, the positions of the parties would enable study sponsors to make informed choices among older and newer methods.

3.3 Approaches to the Use of Test Methods

3.3.1 Tests for Specific Chemical Products and Classes

Tests need not be validated for the universe of chemicals prior to being used by regulatory agencies. The validation of tests may proceed in a stepwise fashion for various classes of chemicals as testing experience accrues (Goldberg et al., 1995). For instance, a test may be accepted for some, but not all, classes of chemicals or product lines. Simultaneous submission of data from both the new and the traditional test is another way of accumulating practical experience in a stepwise fashion (Balls et al., 1990b). Similarly, for some hazard judgments, new methods may be used with chemicals of unknown activity when close structural analogues have been tested both by the traditional and the new method. The same approach may be followed to evaluate the effects of changes in formulations where toxicological data exist on formulations containing the same ingredients in different proportions (Green et al., 1993).

3.3.2 Evaluation of Test Performance

The goal of risk assessment is to accurately estimate hazards and risks for humans or other species, and toxicological test data provide essential information for this process. Testing strategies are sought that minimize both false positive and false negative test outcomes. It is not reasonable to define acceptable ranges for these 'false' responses. The acceptable balance between false positives and false negatives will depend on the proposed uses of the test and the effect being measured. For example, if a test is proposed as a screen to set priorities for definitive testing, it may be acceptable to adopt one that gives a relatively high rate of false positives and a low rate of false negatives. Alternatively, if a test is to be used to label chemicals as possible human developmental toxicants or carcinogens, a test with a high false negative rate might allow many potentially hazardous chemicals into the environment whereas one with a high false positive rate might wrongly label many potentially useful chemicals as hazards. Ideally, it is preferable to use a test with low false positive and low false negative rates.

In the face of uncertainty, inferences are needed to link the information that is available. In these cases, risk-averse science policy positions may be adopted. Thus, depending upon the test method and the consequences of making an error in judgment, it may be better to accept methods that somewhat over-predict hazard in order to minimize undetected hazard.

From a technical perspective, the regulatory process consists of protocol(s) and study designs that provide the data for risk assessment. In principle, a revised test method can consist of a simple modification of an accepted study design or involve a significant change in the protocol. Proposers of new and revised methods should explain how the method fits into the risk assessment process and what additional modifications may be needed to enhance its strengths and accommodate its limitations. To assist this process, all test guidelines should state the experimental and risk assessment objective, i.e., precisely what purpose does this study serve in the overall risk assessment process.

3.3.3 Use of Replacement Alternatives

Given that non-animal replacement test methods measure one or a limited number of responses, they are poor surrogates for the myriad of chemical interactions that occur in vivo. It is important to recognize that in vitro tests are simplified models for processes that occur in vivo. Submission of an in vitro test method should fully disclose the shortcomings of the method in assessing the effects determined in a related in vivo test method (e.g., metabolism of the material; endpoints of concern). In vitro alternative tests might be useful as screens or adjuncts to detect a specific biological effect (e.g., a specific reproductive toxicity parameter).

Tiered approaches to testing or test batteries in which two or more tests are used to replace or reduce the use of animal methods should be given due consideration as alternatives to traditional test methods. The search for methods that reduce or replace animal usage should not be limited to biological ones. Computer modeling and the use of structure activity paradigms to predict toxicity should also be considered.

3.3.4 Need for Hazard Classification Harmonization

If a new test method is to be used for hazard classification and labeling purposes (e.g., acute oral toxicity or dermal irritation), it may be useful to harmonize existing hazard criteria among organizations before the new method is approved. It is also conceivable that a new test may not relate to a traditional method; it may use new fundamental observations (e.g., use of a human test in lieu of an established animal test). In such cases, a new hazard classification system may be warranted.

3.4 Information Needed for Consideration of Test Methods

Regulatory agencies do not readily accept new and revised test methods; many different checkpoints must be crossed along the way (Clark, 1994; Fielder, 1994). A desire by technical staff and management to amend test methods when it is desirable and feasible is essential (Fentem & Balls, 1994). Hurdles that must be overcome are lack of valid methods, bias on the part of scientists and managers both inside and outside of regulatory agencies, fear of litigation due to purported absence of sensitivity of new methods, and the work involved to change guidelines, regulations, or statutes.

In addition to the validation criteria described in Chapter 2, there may be specific and minimum mandatory technical requirements for regulatory acceptance. Depending upon the test, these include adherence to an established protocol; consistent and characterized substrates and reagents; information on test species; nature and quality of the test medium; appropriate numbers of replicates; concurrent positive and negative controls; defined assay acceptance criteria; endpoints that relate to the intended use; defined conditions of use; and a definition of what the method proposes to predict (IRAG, 1993; Balls, et al., 1995a). One cannot overemphasize that toxicological testing for regulatory purposes demands constant and strict adherence to an established protocol, SOPs, and, as far as possible, compliance with GLPs (Balls et al., 1995a). Aspects of data collection not performed according to GLPs must be fully described, along with the potential impact of such deviations. Compliance with GLPs is mandatory for data generated for regulatory submission using new or revised methods.

When a test method is presented to a regulatory agency for consideration, it should be in the form of a technical report and have the following:

- a description of the test rationale, its purpose, and a full description of the methodology, including organism/cell line, test conditions (e.g., pH and dissolved oxygen in aquatic studies), endpoints, and limitations of the test as to physical form, chemical class, and dosing pattern;
- a description of the expected range of responses, measures of central tendency and variability, and dose-response relationships;
- a description of the performance of positive and negative reference substances in comparison to control groups;
- a description of the relationship of new test measures to the range of responses in the standard test;
- all relevant raw test data, and appropriate data reduction, statistical analysis, data presentation, and interpretation;
- an independent quality assurance audit;
- demonstration of intra- and interlaboratory reproducibility (e.g., round-robin test results); and
- a statement of the extent of adherence to GLPs.

Evidence of independent peer review and evaluation of the status of validation of the method for a given purpose can facilitate the review by an agency (IRAG, 1993; Balls et al., 1995a).

3.5 Criteria and Considerations for Regulatory Acceptance

Validation is a prerequisite for regulatory acceptance of a new test method, but it is not sufficient. The validation process determines the practicality of a method in terms of its reliability and relevance for a particular application in a given regulatory program. The degrees of reliability and relevance are then considered by the regulatory agency in determining the acceptability of the method.

Acceptance criteria will depend upon the type of test being proposed (e.g., mechanistic vs. correlative; adjunct vs. definitive) or the extent of modification being proposed for an existing test. Adjunct tests conducted to provide information on a mechanism of action, for example, would be evaluated on scientific merit, and it is unlikely that extensive validation would be undertaken. A definitive test, at the other extreme, would require extensive validation, particularly if it is to replace a traditional definitive test. Modification of traditional protocols occurs to a much greater extent than acceptance of new replacement methods. Similarly, harmonization of guidelines, both nationally and internationally, will more often result in modification of traditional tests than in their replacement.

Validated methods are not automatically acceptable by regulatory agencies; they need to fit into the regulatory structure. Flexibility is essential in determining the acceptability of methods to ensure that appropriate scientific information is considered in regulatory risk assessment. A test method proposed for regulatory acceptance generally should be supported by the following attributes (see Sections 3.4 and 3.5).

- The method should have undergone independent scientific peer review by disinterested persons who are experts in the field, knowledgeable in the method, and financially unencumbered by the outcome of the evaluation.
    - There should be a detailed protocol with standard operating procedures (SOPs), a description of operating characteristics, and criteria for judging test performance and results.
    - Data generated by the method should adequately measure or predict the toxic endpoint of interest and demonstrate a linkage between either the new test and an existing test or the new test and effects in the target species.
    - There should be adequate test data for chemicals and products representative of those administered by the regulatory program or agency and for which the test is proposed.
    - The method should generate data useful for risk assessment purposes, i.e., for hazard identification, dose-response assessment, and/or exposure assessment. Methods may be useful alone or as part of a battery or tiered approach.
    - The specific strengths and limitations of the test must be clearly identified and described.
    - The test method must be robust (relatively insensitive to minor changes in protocol) and transferable among properly equipped and staffed laboratories.
    - The method should be time and cost effective.
    - The method should be one that can be harmonized with similar testing requirements of other agencies and international groups.
    - The method should be suitable for international acceptance.
    - The method must provide adequate consideration for the reduction, refinement, and replacement of animal use.

It should be noted that the acceptance process involves receipt and consideration of input from interested parties. This includes evaluation by stakeholders (e.g., test sponsors and users, groups affected by regulatory decisions) through such mechanisms as workshops and public notices in the *Federal Register*, and independent peer review. All are integral parts in determining the acceptability of a method (Balls et al., 1990b).

3.6 Process of Regulatory Acceptance

Agencies with regulatory programs should promote opportunities for interagency and international harmonization to broaden the scientific and policy base, share limited resources, reduce review time and effort for any single authority, decrease testing demands on industry, reduce reliance on animal testing, and improve the risk assessment process. Acceptance of methods by international organizations (e.g., OECD, UN Transport) will also aid in achieving acceptance by the U.S. government.

Depending on its application, there are several routes that a method may take within the Federal Government. Some methods will be applicable to several agencies, while others will be applicable to a single agency, and still others to only one program within an agency.

For methods that are designed to be used in testing paradigms within several agencies, an interagency committee should be established to facilitate and formulate a path for their validation and acceptance into the regulatory arena. The committee might be composed of representatives from each of the agencies involved. That group could either operate alone or incorporate outside consultants. Other options would be to utilize a consensus conference or public workshop to reach agreement on the applicability of a new method. The potential of combining members from science advisory groups from relevant agencies might also be explored.

For methods that will be submitted to only one agency or one program within an agency, a specific process for regulatory acceptance needs to be developed by each agency. Suggested options are to use an agencyís external science advisory group to review the method, present it to an in-house committee of scientists, or both.

3.7 Regulatory Acceptance Process Recommendations

Test method acceptance among regulatory agencies has largely been an ad hoc procedure. There is a need to streamline the process and make it more efficient. Acceptance will be aided if regulatory agencies participate in validation activities and become familiar with the strengths, weaknesses, and limitations of the methods. There is merit in having agencies involved throughout the development, optimization, validation, and acceptance phases because agencies are more likely to accept familiar methods. Industry and other external sources, including academe, may play roles in several or all steps in the process. If the test substitutes for a traditional test, regulatory agencies must be confident of their ability to evaluate toxicity using the new method compared to using the traditional method.

The effective validation of test methods can be hindered by failure to adhere to sound scientific principles, or to accurately document or report the supporting data. The requirements for test validation should be the same for all sponsors (i.e., individual scientists, regulatory agencies, and independent organizations). Test methods proposed for scientific evaluation and acceptance should be accompanied by information described in the criteria for validation (see Sec. 2.5) and acceptance (see Sec. 3.5).

Toxicology is a continually evolving science. New or revised tests for established endpoints, and tests for new endpoints, are constantly being developed. Established tests are reworked or improved, and new paradigms evolve. Often, there is insufficient coordination among programs within an agency with respect to the validation of a new or revised test method, or for deciding which test methods to recommend. There is also a lack of central focus for coordination of validation issues across the Federal Government. The evaluation of these procedures by individual agencies in isolation results in duplication of effort and may lead unnecessarily to inconsistent positions.

Basic scientific understanding of chemically induced adverse health effects is developing rapidly. Regulatory agencies with missions to protect human health and the environment need to maintain flexibility concerning new and revised methodologies that may apply to their programs. Some methods that show promise are alternative test methods that reduce, refine, or replace animal use. Mechanistic and correlative methods are being developed and both types should be considered for use.

All too often there is inadequate communication among programs within an agency, among Federal agencies, and among international bodies that provide testing guidance. Federal agencies have not always effectively communicated their testing needs to outside scientists and organizations. Scientists involved with test method development, validation, and assessment of the validation status of methods do not always solicit regulatory input, nor do regulatory agencies always solicit input from outside scientists.

Regulatory programs often unilaterally approve test methods that may also be useful to other programs and agencies. Although there are organizations dealing with the preparation of international guidelines for toxicological testing, they often apply to only some chemicals in commerce (e.g., industrial chemicals; pharmaceuticals), and there is incomplete coordination among these international bodies. An impediment to domestic interagency harmonization is a lack of coordination across international organizations. It is important to harmonize nationally and internationally, where appropriate, testing methods for regulated products, such as pharmaceuticals, pesticides, food and color additives, animal drugs, and the transport of such products. In the process of harmonizing test methods, it is important to consult with the developers and users of the methods to ensure that changes are not made that might alter the performance of the harmonized method.

Recommendations to enhance and facilitate the process culminating in regulatory acceptance and use of new methods are provided in five areas: development and validation, regulatory review of new methods, intra- and interagency coordination and harmonization, communication, and international harmonization.

3.7.1 Development and Validation

- Criteria for validation and regulatory acceptance must be taken into account in the planning and design stages of validation studies [see Executive Summary Validation Criteria (Sec. 2.5) and Regulatory Acceptance Criteria (Sec. 3.5)].
- Development of novel and innovative test methods that will provide for improved risk assessment should be encouraged and funded. Federal regulatory agencies can and should help to drive innovation.
- Testing batteries and tiered testing strategies should be accommodated in regulatory testing requirements where appropriate, and new methods should be considered for incremental acceptance.
- While both correlative and mechanistic tests can be validated and accepted, mechanistically based methods relevant to the biological or health effects of concern should be encouraged.
- Given the continuing increase in the numbers and types of test methods being developed for varying purposes, the validation process should be flexible and adaptable.
- Test methods should be evaluated by consistent validation criteria and with the same degree of rigor regardless of whether the proposal derives from academe, industry, Federal government, or other nations.
- Individuals or organizations developing or proposing new or revised test methods should be in communication with the regulatory agencies that will be asked to review and accept the methods.
- Assessment of the validation status of a new test method should involve relevant Federal agencies.

3.7.2 Regulatory Review of New Methods Methods

- An efficient and effective process leading to regulatory acceptance of alternative methods should involve regulators at all stages prior to regulatory acceptance: development, prevalidation, validation, and review.
- Current efforts to incorporate validated alternative test methods into regulatory testing strategies should be continued and expanded.
- Federal agencies should continue to hold workshops on validation and acceptance issues of concern.
- Agencies should establish internal central clearing systems for evaluation of new or revised methods submitted to the agency, and for the periodic review of methods recommended by the agency.
- Test methods should be periodically reviewed and, where appropriate, revised in light of scientific and policy developments. Considerations for such activities include the following:

  - animal and non-animal test methods that have the potential to support improved risk assessment and the potential to partially or fully replace existing toxicity tests for some or all of the products regulated should be reviewed and evaluated;
  - frequency of review of a method should be consistent with scientific activity or progress in that discipline;
  - the process should be efficient and expedient;
  - the process should include outside stakeholders;
  - the reviews and outcomes of the reviews should be made public;
  - regulations, guidelines, or recommendations should be promulgated for newly validated and accepted toxicity tests or test batteries.
- When evaluating the scientific acceptability of new or revised test methods, agencies should establish close links with the relevant scientific community to ensure continuing benefit from shared expertise.
- Concurrent submission of data from new and existing methods will help facilitate regulatory acceptance of new methods, and should be encouraged.
- Regulatory agency staff should be trained in the evaluation of data from newly accepted test methodologies.

3.7.3 Intra- and Interagency Coordination and Harmonization

- There should be interagency coordination of the evaluation of proposed test methods that are relevant to the needs of multiple agencies.
- A Federal interagency committee on test methods should be established to serve as a forum for the exchange of information, for the coordination of the review and evaluation of test methods, and for related activities. This committee should strive for interagency consistency in review and evaluation processes and interagency and international acceptance of alternative methods.
- Federal regulatory agencies should establish consistent processes and criteria for acceptance of new and revised toxicological test methods and should communicate them to interested parties.
- Federal regulatory programs should solicit input from other programs and agencies as they develop and modify test guidelines of general interest.
- Harmonization of hazard classification may be necessary before test guidelines can be harmonized.
- Proposed new or revised test methods relevant to the needs of more than one program or agency should be harmonized as appropriate.
- Interagency differences in test methods that purport to detect the same toxicological endpoints but differ unnecessarily in detail should be identified and harmonized.

3.7.4 Communication

- A consistent, coordinated process of involvement and communication among all stakeholders (e.g., researchers, developers, users, regulators, and the public) at all stages (development, prevalidation, validation, review, regulatory acceptance, and implementation) will facilitate the validation and acceptance of new test methods.
- Validation and regulatory acceptance should include the opportunity for input by interested stakeholders inside and outside of government.
- The regulatory acceptance of new and revised test methods by agencies should be communicated to scientists and to various national and international organizations in journals, workshops, the *Federal Register*, and by other means.
- Agency regulations and guidelines should be readily available to the public.

3.7.5 International Harmonization

- U.S. agencies should attempt to harmonize guidelines through international organizations, such as the OECD, where appropriate.
- U.S. agencies should encourage harmonization of test guidelines across international organizations, e.g., between UN Transport and OECD, as appropriate.

TABLE 3.1
FEDERAL REGULATORY PROGRAMS INVOLVED WITH TOXICOLOGICAL TESTING

Agency	Authority	Statute	Program
CPSC	Consumer product exposures	Federal Hazardous Substances Act; Consumer Product Safety Act; Poison Prevention Packaging Act	Hazard Assessment and Reduction Program and Regulated Products Program

DOI	Drug and management chemicals for fisheries	Fish and Wildlife Coordination Act; Federal Insecticide and Fungicide and Rodenticide Act (FIFRA); Federal Food, Drug and Cosmetic Act (FFDCA)	Chemical-Drug Registration Program, National Biological Survey
	Non-Toxic Shot Program	Migratory Bird Treaty Act	Office of Migratory Bird Management, Fish and Wildlife Service
DOT	Exposure to hazardous materials in transport	Federal Hazardous Materials Transportation Law	Research and Special Programs Administration
EPA	Pesticides	FIFRA	Office of Pesticide Programs
	Industrial chemicals	Toxic Substances Control Act	Office of Pollution Prevention and Toxics
FDA	Biologicals	FFDCA; Public Health Service Act	Center for Biologics Evaluation and Research
	Medical devices; radioactive materials	FFDCA	Center for Devices and Radiological Health
	Pharmaceuticals	FFDCA	Center for Drug Evaluation and Research
	Food and color additives, cosmetics	FFDCA	Center for Food Safety and Applied Nutrition
	Veterinary drugs	FFDCA	Center for Veterinary Medicine
OSHA	Worker exposures	OSHA	Directorate of Health Standards Programs
USDA	Genetically engineered plants, microbes, and arthropods	Plant Pest Act	APHIS*
	Veterinary biologicals and diagnostics	Virus, Serum, Toxin Act	APHIS*
	Non-food compounds on foods	Federal Meat Inspection Act; Poultry Products Inspection Act	Food Safety Inspection Service

*Program has authority, but no routine toxicity testing requirements.

TABLE 3.2
RECENT EXAMPLES OF NEW OR REVISED TESTING GUIDANCE

Organization	Action	Status	Purpose	Remarks
OECD	Fixed dose procedure	Guideline	Refinement of animal use	Alternative to the acute oral LD50 test based on international validation study.
OECD	Acute toxic class method	Guideline	Reduction in animal use	Alternative to the acute oral LD50 test based on international validation study.
OECD	"Up and down" method	Draft guideline	Reduction in animal use	Alternative to the acute oral LD50 test. Guideline is being developed after a literature review found the method ready for use.
OECD	Guidelines for skin and eye irritation and corrosivity	Updated guidelines in development	Reduction and refinement in animal use	Recommends a tiered approach to testing, with full animal testing being used only as a definitive indication of the lack of corrosivity or to grade irritation. Physicochemical properties, pH, and data from validated *in vitro*assays (no examples given) should be considered when performing and scoring these tests.
OECD	Combined 28-day subchronic and developmental toxicity test	Guideline	Reduction in animal use over that used for the two tests individually	Screening test for prioritization of chemicals for further testing.

OECD	Combined 28-day subchronic developmental toxicity and reproductive effects test	Draft guideline	Reduction in animal use over that used for the three tests individually	Being reviewed. Screening test for prioritization of chemicals for further testing.
OECD	*Daphnia sp.* Reproduction Test	Draft guideline	Update of existing methods	Being considered. Method based upon international validation test.
OECD	Fish, Toxicity Test on Egg and Sac-fry Stages	Draft guideline	Revision of new guideline	Being considered. Revisions based upon member country comments.
OECD	Fish Juvenile Growth Test, 28 Days	Draft guideline	Revision of new guideline	Being considered. Revisions based upon member country comments.
OECD	Avian Acute Toxicity Test - Oral Toxicity	Being developed	New guideline	Being developed by expert working group.
OECD	Avian Reproduction TestTest	Draft guideline	Revision of existing methods.	Being developed by expert working group.
OECD	Avian Dietary Toxicity TestTest	Draft guideline	Revision of existing methods	Being developed by expert working group
OECD	Fish Acute Toxicity TestTest	Guideline	Reduction in animal use	Adopted July 1992
OECD	Bioconcentration: Flow-through Fish TestTest	Guideline	Revision of existing methods	Submitted to OECD Council, June 1995
OECD	Repeated Dose Eight-day Oral Toxicity Study in Rodents	Guideline	Revision of existing methods	Submitted to OECD Council, June 1995
OECD	Delayed Neurotoxicity of Organophosphorus Substances: Acute and 28-day Repeated Dose Studies	Guidelines	Revision of existing methods	Submitted to OECD Council, June, 1995
OECD	In vitro and in vivo genetic toxicology tests	Seven draft guidelines	Revision of six existing methods; one new guideline	Approved

OECD	Percutaneous Absorption; in vitro and in vivo methods	Two draft guidelines	New methodology	Being considered. Guidelines based upon submissions from two member countries
OECD	Neurotoxicity	Draft guideline	New methodology	Being developed by expert working group
OECD	Acute Dermal Photoirritation: Screening Test and Dose Response Test	Two draft guidelines	New methodology	Being considered. Guidelines based upon recommendations of expert working group.
ICH	Development of a systemic exposure metric as an alternative to the maximum tolerated dose for carcinogenicity studies.	Adopted	New methodology	
ICH	Elimination of the acute oral LD50	Adopted	ReductionReduction in animal use	
ICH	Elimination of the 12-month rodent toxicity study	Adopted	ReductionReduction in animal use	
ICH	International guideline for reproductive toxicity testing	Adopted	New methodology	
ICH	Evaluation of the requirement to conduct carcinogenicity studies in two rodent species.	Under study	Reduction in animal use, new methodology	
ICH	Adoption of standard genotoxicity test battery for drug products.	Proposal	Refinement in testing	Being studied by the working group

DOT	Corrositex assay Skin2*	Methods accepted for determination of corrosion or absence of corrosion potential for certain chemical classes.	In vitro assays for corrosion. Reduction and replacement in animal use.	Other U.S. agencies are being asked to consider accepting these methods
DOT	Limit test for acute inhalation toxicity	Adopted regulation	Reduction in animal use	Alternative to the LC50 test
DOT	Acute toxicity limit test	Approved regulations	Refinement	Oral, dermal, inhalation routes
EPA/OPPTS	Guidelines for Developmental and Reproductive Toxicity	Guidelines in revision	Update of existing methods	Being revised to reflect new information and technology.
EPA/OPPTS	Genetic toxicology testing strategy	Guidelines in revision	Includes both in vitro and in vivo testing in first tier	Uses animal testing to identify mutagenic potential rather than to confirm that seen in vitro activity. Testing required under TSCA Section 4 and OPP/FIFRA.
FDA/CFSAN	Guidelines for Immunotoxicity Test Testing	Draft guideline	New guideline	Being developed in response to new information about the immune system
FDA/CFSAN	Guidelines for Neurotoxicity Test Testing	Draft guideline	Update of existing methods	Being revised and updated in response to new information and advances in technology.

*No longer manufactured

4. FUTURE DIRECTIONS AND IMPLEMENTATION

FUTURE DIRECTIONS AND IMPLEMENTATION

4.1 Background

Federal agencies have historically worked independently on the development and modification of toxicological test guidelines. Regulatory agencies have often adopted similar testing requirements or guidelines in different ways in order to optimize testing for specific statutes, stakeholder needs, and costs. Guidelines for specific tests developed by many agencies and international organizations often differ in details, and guidance updating is often inconsistent or non-existent. This results in increased work on the part of government, industry, and other interested parties, at a time of significant downsizing. In addition, some agencies lack expertise in certain areas and may have to use outside consultants to help with test method review and assessment. Different test requirements among agencies and national authorities or other countries result in increased testing costs and increased use of animals because substances must be retested according to these different protocols and requirements.

4.2 Proposal

The Federal government will establish an interagency committee to coordinate the development, validation, acceptance, and harmonization nationally and internationally of toxicological test methods. This effort will help to better evaluate risks to human and animal health and the environment, reduce costs necessary to establish the safety of agents in commerce, and facilitate international trade. To accomplish this, the committee will seek to:

- utilize scientific expertise within and outside of the Federal system;
- fill gaps in scientific expertise that exist in individual agencies;
- increase the use of test methods that incorporate new scientific knowledge by deleting and revising traditional test methods and adding new ones;

- decrease redundant testing;
- reduce animal usage and improve the welfare of animals used;
- decrease total transaction costs for new and revised test methods, and;
- decrease redundancy in the validation and acceptance processes for test methods within and among agencies.

4.3 Committee Designation

The Committee is designated as the Inter-agency Coordinating Committee on the Validation of Alternative Methods (ICCVAM).

4.4 Background

The mission of the Committee is to coordinate issues throughout the Federal government that relate to the development, validation, acceptance and harmonization of toxicological test methods. It focuses on test method issues that are common to multiple agencies without impinging on considerations unique to individual programs and agencies. It recognizes that final regulatory acceptance is the purview of each Federal agency according to its regulatory mandates.

4.5 Goals

The Committee will seek to promote toxicological test methods that (1) enhance agencies ability to assess risks and make decisions; and (2) where feasible and practical, reduce animal use, refine animal procedures to make them less stressful, or replace animals in toxicological tests (the 3Rs).

4.6 Activities

The Committee may:

- evaluate the status of validation and make recommendations to agencies regarding the scientific usefulness of test methods and their potential applicability;
- coordinate technical reviews of proposed new and revised test methods of interagency interest;
- facilitate interagency communication and information sharing;
- serve as an interagency resource and communication link with parties outside of the Federal government, including academic, other government, industry, and public interest groups;
- assist agencies in assessing test method needs;
- provide guidance to agencies and other stakeholders on criteria and processes for the development, validation, and acceptance of tests;
- promote awareness of accepted U.S. test methods, and;
- advocate harmonization of test methods nationally and internationally.

4.7 Organization/Operation

The Committee will serve as a standing subcommittee of the National Toxicology Program (NTP) Executive Committee1 and will report to it for operational and policy guidance. The activities of the Committee will be summarized in the *NTP Annual Plan*. It will be composed of named representatives or their designates from Federal research and regulatory agencies that generate or use information from toxicological test methods for human health or environmental risk assessment. Members will serve as points of contact and as sources to identify technical experts from their agencies to serve on specific topical work groups. A chair will be chosen by the Director of the NTP from nominations of Committee members and will serve for a two-year period.

Operating staff will be supplied by the National Institute of Environmental Health Sciences.

1 Includes representation from the Agency for Toxic Substances and Disease Registry, Consumer Product Safety Commission, Environmental Protection Agency, Food and Drug Administration, National Cancer Institute, National Institutes of Health (NCI, NIEHS), National Institute of Occupational Safety and Health, and Occupational Safety and Health Administration.

The Committee will carry out work of interest to Federal agencies on toxicological test methods. It will interact with parties outside the Federal government, including other government bodies, industry, and public interest groups, through meetings, workshops, *Federal Register* solicitations, and other means.

Agencies will share resources to maximize Committee output without over-taxing individual programs and agencies. Opportunities and mechanisms to work with experts and with stakeholders outside of government will be sought to develop scientific consensus on issues related to development and validation of new test methods. This effort will include scientific peer review of proposed new test methods to evaluate their validation status with regard to demonstrated reliability and relevance.

4.8 ICCVAM Process

The various stages involved in the process of moving a new test method from concept to regulatory acceptance and use is illustrated in Figure 4. l. A flow diagram illustrating the role of the ICCVAM in this process is provided in Figure 4.2. General concepts related to the process are as follows:

4.8.1 Test Method Sponsors

- Test method sponsors may communicate with the ICCVAM prior to or any time during the development, validation, and submission process.
- Proposals may be submitted to either the ICCVAM Office or the designated coordinating office in an individual agency. The ICCVAM Office or agency coordinating office will determine if the method is of potential applicability to more than one agency or program (e.g., carcinogenicity testing) and if so, will forward it to the ICCVAM for consideration. If a method is likely to have applicability to only one agency or program, then the method will be forwarded to the respective agency coordinating office (e.g., a new method for neurovirulence testing of polio vaccines). The ICCVAM will not normally address methods applicable to only one program or agency.

4.8.2 ICCVAM Review

- The ICCVAM will establish expert interagency workgroups to evaluate test method submissions. These workgroups will be composed of experts from the member agencies and at least one liaison member from the ICCVAM. In some instances, the workgroup may need the services of ad hoc consultants.
- Workgroups will review methods for their relevance to regulatory risk assessment, and determine if:

  - additional information should be requested from the test sponsor;
  - sufficient information is available to warrant an independent, scientific peer review;
  - the method has been sufficiently validated and peer reviewed and should be submitted to appropriate agencies for consideration, or;
  - a workshop should be convened to further discuss the science and available data on a method or group of methods, or to discuss a proposed validation study design.
- Recommendations from the workgroup will be forwarded to the ICCVAM, which will review and carry out those actions deemed appropriate (i.e., arrange for scientific peer review, etc.).
- Workshops and peer reviews will be public and announced in the *Federal Register*, and an opportunity provided for public comment.

4.8.3 Independent Peer Review

The ICCVAM will coordinate independent, interagency peer reviews. Nomina-tions for peer review panel members will be solicited from member agencies and stakeholder groups, including academe, industry, government, public interest groups, and the international community. Each concerned ICCVAM member agency will provide a liaison to the peer review panel to provide information regarding respective regulatory requirements and scope of regulatory responsibility.

- Peer review meetings will be public, and announced in the *Federal Register*.
- Results of the peer review will be published and made readily available in the public domain.
- The ICCVAM will consider the peer review results and forward their recommendations with the peer review report to each agency.
- Test method sponsors may elect to arrange for independent peer review by third parties prior to submission of a method to an agency or ICCVAM.

4.8.4 Regulatory Acceptance

- Each regulatory agency will review the recommendations forwarded by ICCVAM and consider new test methods for approval as appropriate. The rationale for non-approval of methods will be provided by agencies to the ICCVAM and the test sponsor.

4.8.5 International Organizations

- Communication and coordination with international organizations will be accomplished via the respective coordinating agency, e.g. FDA for ICH, EPA for OECD, DOT for UN Transport, etc. The ICCVAM will coordinate activities and information exchange with the European Centre for the Validation of Alternative Methods (ECVAM).
- Agency coordinators for these international organizations may utilize the ICCVAM to communicate applicable proposed new methods to other agencies and programs.

Figure 4.1

Stages in the Development of New Toxicological Testing Methods

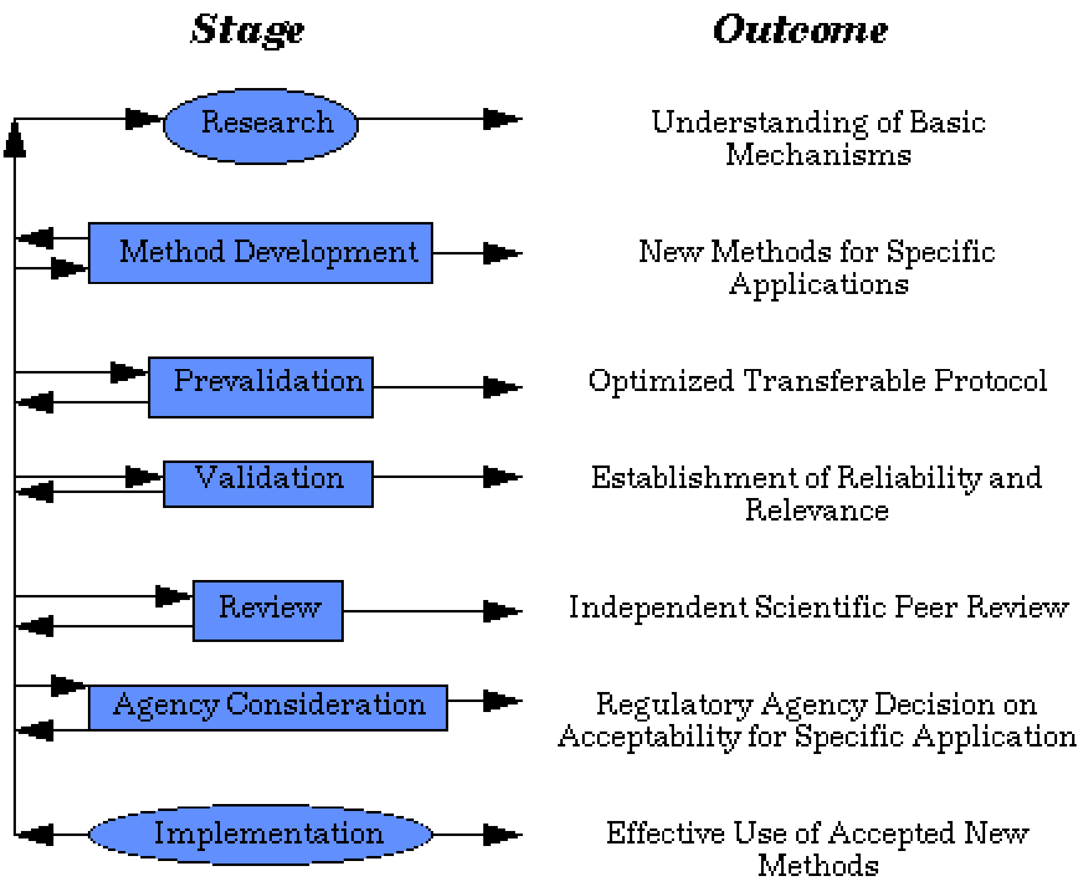
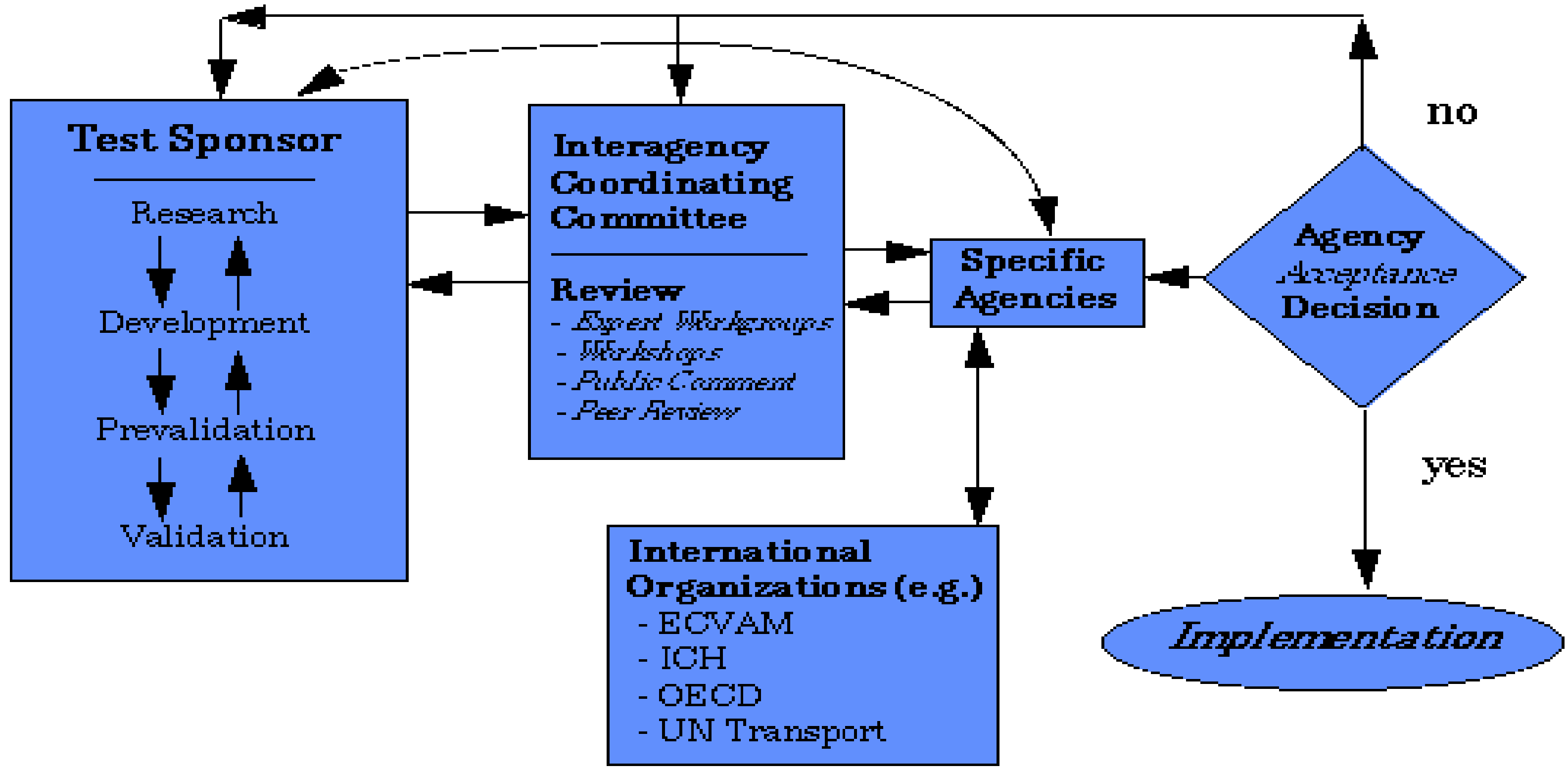


Figure 4.

New Toxicological Methods: ICCVAM/Agency Process Flow



APPENDIX A - GLOSSARY

Accuracy: (a) The closeness of agreement between a test result and an accepted reference value. (b) The proportion of correct outcome of a method. Often used interchangeably with **concordance** (see two-by-two table).

Adjunct test: A test that provides information that adds to or helps interpret the results of other tests, and provides information useful for the risk assessment process.

Algorithm: A step-by-step procedure for solving a problem; a formula.

Alternative test method: A test method that: a) reduces the number of animals required, b) refines procedures to lessen or eliminate pain or distress to animals, or enhances animal well-being, or c) replaces animals with non-animal systems or one animal species with a phylogenetically lower one (e.g., a mammal with an invertebrate). [Note: Alternative test methods are sometimes broadly defined as any new test method not currently being used, e.g. a new or revised method proposed as an alternative to a traditional method.]

Assay: The experimental system used; used interchangeably with **test**.

Coded chemicals: Chemicals labeled by code rather than name so that they can be tested and evaluated without knowledge of their identity or anticipation of the test results. Coded chemicals are used to avoid intentional or unintentional bias when evaluating laboratory performance or performance of test methods.

Concordance: The proportion of all chemicals tested that are correctly classified as positive or negative; often used interchangeably with **accuracy** (see two-by-two table). A measure of test performance. The **concordance** is highly dependent on the **prevalence** of positives in the population being examined.

Definitive test: A test which generates adequate data to determine the particular hazard of a substance without additional testing. A test upon which decisions regarding safety can be made.

Correlative methods: Test methods whose usefulness depends on a correlation or association between the endpoint measured and the biological effect of concern rather than on known or demonstrated mechanistic relationships. Used interchangeably with **empirical methods**.

Dose-response assessment: That part of risk assessment associated with evaluating the relationship between the dose of an agent administered or received and the incidence and/or severity of an adverse health or ecological effect.

Empirical methods: Test methods whose usefulness depends upon a correlation or association between the endpoint measured and the biological effect of concern rather than on known or demonstrated mechanistic relationships. Used interchangeably with **correlative methods**.

Endpoint: The biological or chemical process, response, or effect assessed by a test method.

Exposure assessment: That part of risk assessment associated with the determination of how much exposure to a substance or agent there is to humans or other target species.

False positive: A nonactive substance incorrectly identified as positive by a test.

False positive rate: The proportion of all negative (inactive) substances that are falsely identified as positive. An indication of test performance.

False negative: An active substance incorrectly identified as negative by a test.

False negative rate: The proportion of all positive (active) substances falsely identified as negative. An indication of test performance.

Good Laboratory Practices (GLPs): Regulations promulgated by the FDA and EPA that describe recordkeeping and quality assurance procedures for laboratory records that will be the basis for data submissions to the agencies. Also described in an OECD Guidance Document.

Hazard: An adverse health or ecological effect. A hazard potential produces only if an exposure occurs that leads to the possibility of an adverse effect being manifested.

Hazard classification: Assignment of a chemical or product hazard into a category of severity based on the results of a standard test method for a specific toxic endpoint; most commonly used for labeling purposes.

Hazard identification: That part of **risk assessment** associated with the determination of whether exposure to a particular substance is or might be associated with adverse health or ecological effects.

Hierarchical test approach: An approach where series of tests to measure or elucidate a particular toxic effect are used simultaneously or in an ordered sequence. In a typical hierarchical testing approach, one or a few tests are initially used; the results from these tests determine which (if any) subsequent tests are to be used. Decisions regarding hazard may be made at each stage in the testing procedure.

Interlaboratory reproducibility: A measure of whether different qualified laboratories using the same protocol and test chemicals can produce qualitatively and quantitatively similar results. Interlaboratory reproducibility is determined during the **prevalidation** and **validation** processes and indicates the extent to which a test can be successfully transferred among laboratories.

Intralaboratory reproducibility: The first stage of **validation**; a determination of whether qualified people within the same laboratory can successfully replicate results using a specific test protocol at different times.

Mechanistically-based methods: Methods that provide a direct relationship between the biological effects observed with the biological effects of interest.

Mechanistic studies/tests: Studies or tests designed to obtain an understanding of the biologic or chemical events responsible for, or associated with, the effect observed, and that provide information concerning the molecular, cellular, or physiological mechanisms by which substances exert their effects on living cells and organisms.

Nonparametric methods: A statistical approach that treats the data as a set of discrete entities rather than as a sample taken from a continuous distribution. The distribution of the underlying population is estimated by selecting discrete, individual samples for analysis.

Operational characteristics: Operational characteristics of a test refers to its performance under typical conditions, as measured by its reproducibility, its sensitivity, specificity, positive and negative predictivity, and **concordance** (where appropriate), and the types of substances that the test is effective or ineffective at identifying.

Parametric methods: A statistical approach that assumes that the distribution of values in the population from which the data were sampled can be described by a continuous function. The constants in that algebraic expression are evaluated using mathematical techniques.

Potency: A measure of the relative biological or chemical activity of a substance. The potency of a substance can differ for different biological or biochemical effects.

Prediction model: A procedure used to convert the results from a test method into a prediction of the toxic effect of interest. A prediction model contains four elements: a definition of the specific purpose(s) for which the test is to be used, a definition of all possible results that may be obtained, an **algorithm** that converts each test result into a prediction of the toxic effect of interest, and an indication of the **accuracy** of the prediction.

Predictivity (negative): The proportion of correct negative responses among materials testing negative (see two-by-two table). A measure of test performance. The negative predictivity is a function of the sensitivity of the test and the prevalence of negatives among the chemicals tested.

Predictivity (positive): The proportion of correct positive responses among materials testing positive (see two-by-two table). A measure of test performance. The positive predictivity is a function of the sensitivity of the test and the prevalence of positives among the chemicals tested.

Prevalence: The proportion of positives in the population of agents tested (see two-by-two table).

Prevalidation: The process during which standardized test protocols are constructed for use in validation studies, and laboratories are selected and shown to be competent to perform validation studies.

Protocol: The precise step-by-step description of a test, including the listing of all necessary reagents and all criteria and procedures for the evaluation of the test data.

Quality assurance: A management process by which adherence to laboratory testing standards, requirements, and recordkeeping procedures is assessed independently by individuals other than those performing the testing.

Reduction alternative: A new or revised test method that reduces the number of animals required.

Reference chemicals: Chemicals selected for use in the validation process. These chemicals should be representative of the classes of chemicals for which the test is expected to be used and should represent different levels of expected responses. Different sets of reference chemicals may be required for the different stages of the validation process, and for different types of tests.

Reference species: The species used in the traditional test method to which a new or revised test is being compared. This may be the target species when it is also the species of interest, or it may be a surrogate species when it is not possible to perform testing on the target species.

Reference value: An agreed upon value for comparison of results among test procedures and/or among laboratories.

Refinement alternative: A new or revised test method that refines procedures to lessen or eliminate pain or distress to animals, or enhances animal well-being.

Relevance: Describes the relationship of a test to the effect of interest and whether a test is meaningful and useful for a particular purpose. The extent to which a test method will correctly predict or measure the biological effect of interest.

Reliability: A measure of the degree to which a test can be performed reproducibly within and among laboratories over time.

Repeatability: The closeness of agreement between test results obtained within a single laboratory when the procedure is performed on the same substance under identical conditions within a given time period.

Replacement alternative: A new or revised test method that replaces animals with non-animal systems or one animal species with a phylogenetically lower one (e.g., a mammal with an invertebrate).

Reproducibility: The variability between single test results obtained in a single laboratory (intralaboratory reproducibility) or in different laboratories (interlaboratory reproducibility) using the same protocol and test samples (see Intra- and interlaboratory reproducibility).

Risk: The probability or degree of concern that an agent will cause an adverse effect given some exposure.

Risk assessment: Evaluation of the potential adverse health and environmental effects to a target species from exposures to environmental agents (see **hazard identification**, dose response assessment, and risk characterization).

Risk characterization: That part of risk assessment associated with the description of the nature and magnitude of the potential adverse effects from exposure to an agent, including strengths, weaknesses, and uncertainties in the assessment.

Robustness: The insensitivity of a test method to departures from the specified test conditions when conducted in different laboratories or over a range of conditions under which the test method might normally be used.

Round-robin testing: A multi-laboratory collaborative validation study in which all laboratories test the same substances using identical test protocols. The purpose of the study is to determine interlaboratory reproducibility of a test method [sometimes referred to as 'ring testing'].

Screen/screening test: A rapid, simple test conducted for the purposes of a general classification of substances according to general categories of hazard. The results of a screen are generally used for preliminary decision making and to set priorities for more **definitive tests**. A screening test may have a truncated response range, e.g., be able to reliably identify active chemicals but not inactive chemicals.

Sensitivity: The proportion of all positive chemicals that are correctly classified as positive in a test. A measure of test performance (see two-by-two table).

Specificity: The proportion of all negative chemicals that are correctly classified as negative in a test. A measure of test performance (see two-by-two table).

Standard operating procedures (SOPs): Formal, written procedures that describe how specific laboratory operations are to be performed. Required by GLPs.

Substitute method: A new or revised test method proposed for use in lieu of a currently used method, regardless of whether that method is for a definitive, screening or **adjunct test**.

Surrogate: A test or species used in the place of another test or target species.

Target species: The species for which information on the potential toxicity of a chemical is sought.

Test: The experimental system used; used interchangeably with **assay**.

Test battery: A series of tests, usually performed at the same time or in close sequence. Each test in the battery generally measures a different component of a multifactorial toxic effect.

Test method: A process or procedure used to obtain information on the characteristics of a chemical or agent. Toxicological test methods generate information regarding the ability of a chemical or agent to produce a specified biological effect under specified conditions. Used interchangeably with **test** and **assay**.

Transferability: The ability of a test method or procedure to be accurately and reliably performed in different, competent laboratories.

True Negative: A negative test result, that accurately reflects the tested-for activity of the chemical.

True Positive: A positive test result, that accurately reflects the tested-for activity of the chemical.

Two-by-two (2x2) table:
The 2x2 table can be used for calculating

		New Test Outcome		
		Positive	Negative	Total
Reference Test	Positive	a	c	a+c
Classification	Negative	b	d	b+d
	Total	a+b	c+d	a+b+c+d

---

accuracy (**concordance**) (a+d/a+b+c+d), negative predictivity (d/c+d), positive predictivity (a/a+b), prevalence (a+c/a+b+c+d), sensitivity (a/a+c), specificity (d/b+d), **false positive rate** (b/b+d) and **false negative rate** (c/a+c).

Valid Method: A method determined to be acceptable for a specific use and application.

Validated Method: A test method for which the reliability and relevance for a specific purpose have been established in validation studies.

Validation: The process by which the reliability and relevance of a procedure are established for a specific purpose.

APPENDIX B

COMPARISON OF SELECTED AGENCY PROCESSES FOR TEST METHOD ACCEPTANCE[1]

Question	EPA-OPPT/OPP	ATSDR	FDA-CFSAN
1. Does your agency have a working definition of test validation	No	No	No
2. How is it determined that a new or revised test method is valid?	OPPT. Review of data by expert work groups, work shops and general acceptance by the scientific community are used to determine validity. OPP. New test methods are reviewed by the Science Advisory Board and/or the Scientific Advisory Panel in a public peer-review setting.	A lead/division/office or specific cross-agency work group is designated to evaluate the method. The group's findings are used to propose a preliminary position to be presented to the ATSDR Science Forum. If the consensus is to pursue the method, its viability and utility are explored further.	DFS examines the test for validity and evaluates the results statistically and empirically. The method is then examined by a collaborative study with 8-12 laboratories to demonstrate precision and accuracy using standard methods and unknowns. The new method is compared with existing methods when they are available. DMS. AOAC International Procedures, especially interlaboratory laboratory collaborative studies are used. When a complete collaborative study is impractical or impossible to perform, intralaboratory validation is accepted. DTR. Scientific consensus, usually derived from symposia or other means of interacting with the scientific community to obtain comments about

			the appropriateness of new and revised test procedures is used.
3. Does your agency use specific principles or guidelines for validation of test methods or reference published guidelines such as the ASTM guidelines or GLPs?	OPPTS does not have specific principles or guidelines but recommends adherence to ASTM methods, OECD Guidelines, and GLPs.	No specific principles or guidelines are in effect. For toxicology testing, adherence to GLPs to the extent possible is expected.	DFS. Guidelines are available through Congressional and Consumer and International Affairs Staff. USP, AOAC, GLP and peer review articles are also used. DMS and DTR. The DMS and DTR use GLPs in conducting studies of new and revised test procedures.
4. What information must be provided before a new or revised test will be considered for evaluation?	OPPT. Varies on a case-by-case-basis.	The following information is sought through appropriate questions: 1) development, verification, and acceptance (reproducibility, sensitivity, specificity, predictive value, accuracy, publication, peer review, current use); 2) validation and comparison with methods currently in use (advantages and disadvantages); 3) cost benefit analysis (without a compromise in science); 4) uniform and consistent applicability of the method agency-wide (interpretation, easily explainable to risk assessors and public); 5) urgency and need in terms of conducting	DFS. Looks at the intended use and value of the test method, the detailed methodology, data, limits, ruggedness testing, critical conditions and components, and comparison with existing alternative methods. DTR. There is no specific requirement for any information before a new test or test modification is considered for evaluation. The decision to modify or establish a new method is usually determined by knowledge of scientific literature (advancement in the scientific field), which demonstrates the need for modification or establishment of new

		agency business; 6) feasibility and logistics for field application in human populations; and, 7) public acceptance.	procedures.
5. What criteria or principles does your agency use to determine when a new or revised test method is acceptable (e.g., development of standard protocol, intra/inter-laboratory testing; certain degree of specificity, sensitivity, etc.?	OPPT. Varies on a case-by-case basis; OECD acceptance is an important consideration.	No written criteria or principles to determine acceptability of new or revised test methods . Given information on the method as described in question #4, ATSDR will apply biomedical judgment to determine acceptability.	DFS. Uses all of the criteria listed. DTR. No established principles or criteria. Modifications of methods and new methods are reviewed by scientists having expertise in a particular field and based on that review, the method is considered acceptable or non-acceptable. DMS: In the course of interlaboratory collaborative studies, standard protocols, intra/interlaboratory testing, sensitivity and specificity are determined.
6. What is the process used to determine that a proposed new or revised test method is acceptable? Do acceptance procedures vary among programs within your agency?	OPPT. OECD acceptance; procedure may vary in some instances.	ATSDR generally follows some variation of the following steps: 1) designates a lead division/office to define rationale/need along with supporting material. A specific cross-agency work group may be tasked with the responsibility; 2) the findings of the work group are used to propose a preliminary position to be presented to the	DFS. Committee review and comparison testing followed by oversight from the responsible individual. Examination of modification by interested parties. Information may be published in the Federal Register. This could be incorporated in the Code of Federal Regulations. The process could stop at some of the points mentioned depending

Science Forum. If the consensus is to pursue this method, the viability and utility of the method are explored further; 3) in some cases, a tentative position could be presented to the Board of Scientific Counselors; 4) the proposal may be brought before the Office of the General Counsel for legal implications and the Tri-Agency Superfund Applied Research Committee for technical evaluation. May also announce intent in the Federal Register to consider the proposal and seek public comments; 5) in many cases external peer-review of the proposed method, protocol, or position the agency intends to take is initiated; 6) evidence that tests can be performed in human field studies that have public health relevance in addition to clinical utility; and 7) approval by the Assistant Administrator may be sought.

on the impact of the method or method change.

DTR. Modifications of methods and new methods are reviewed by scientists having expertise in a particular field and based on that review, the method is considered acceptable or non-acceptable for DTR.

DMS. AOAC International procedures, especially interlaboratory collaborative studies, are used.

7. What type of management concurrence is needed for acceptance of a new test or test modification, e.g., reviewing individual, division director, program/office director, agency head?	OPPT. Division Director and above.	Although no formal process is in place, in most cases, approval by the Division/Office Director is required; concurrence of the Assistant Administrator may be sought as appropriate.	DFS. Committee review is presented to the responsible individual. The level of the individual depends on the impact on public health and welfare, and/or how important and/or sensitive are the related issues. DTR. There is no formal concurrence by management. The scientist having the greatest expertise is usually relied on for the determination that a method is sufficient for its intended purposes.
8. Do your procedures include an opportunity for outside comment on proposed new test methods or requirements (e.g., peer review process, Federal Register, workshops)? At what stage of the approval process does this occur?	OPPT. Federal Register publication, OECD review process, OECD workshops, etc. OPP. Guidelines are published by NTIS and may be part of work shops. There is a peer review process for new guidelines and significant modifications of existing guidelines. New guidelines or those which undergo significant modification are sent to the SAP/SAB for review.	ATSDR may choose to announce its intent to consider the proposal in the Federal Register and seek public comments. In many cases, external peer-review of the proposed method, protocol or position the agency intends to take on a given issue is initiated. Generally, this is done later in the process, after the agency has thoroughly researched the proposal, and prior to final agency approval.	DFS. The test method during development may be examined in collaborative studies with industry and/or academia. When applicable, Federal Register publication would follow the interpretation of the collaborative study. OSRS. DMS publishes its preferred methods in the Bacteriological Analytical Manual. Methods in toxicology are widely disseminated and comments received. Symposia and/or workshops may be held to further obtain opinions.

9. Do you coordinate the review of proposed new test methods with other agencies that may use or have a requirement for the same or similar testing? If so, how is this achieved.	OPPTS. Through the OECD review process.	For testing relevant to hazardous substance data needs, the proposal may be brought before the OGC for legal implications and the TASARC for technical evaluation. TASARC is composed of representatives of ATSDR, EPA and NTP and is charged to coordinate and assure the initiation of a research program to fill priority data needs of ATSDR's priority hazardous substances relevant to the objectives of CERCLA 104(1)(5) as amended.	DFS. Achieved through personnel communication, collaboration, scientific meetings, peer review publications, and other publications. Depending on the impact the information may pass through the General counsel, the Federal Register, Legislative Affairs, External Affairs, and/or Senior Science review. DTR. Other government agencies are usually provided information on the new or refined test method(s). This is usually accomplished by having knowledge of who is the appropriate individual(s) in sister agencies and conveying the information describing the new or refined test method(s) to that individual.
10. Once a new or revised test is considered acceptable by your agency, how is that information communicated to the public, e.g., printed or electronically available guidelines, Federal Register notice, published in the Code of Federal Regulations?	OPPT. Publication in the CFR (this may be subject to change); printed or electronically available.	ATSDR has not derived a general distribution plan for making available such information. Depending upon the information to be distributed, the Division/Office generally can apply some discretion. For example, alternative methods accepted for filling data needs via	DFS. Publication in the AOAC, USP, and other official publications as the publication of guidelines through the Federal Register process. DTR. The Federal Register has been used as well as the development of specific compendia containing the new

		ATSDR's voluntary research program were announced in the Federal Register while standardized test batteries for use in environmental field studies were made available through the development and distribution of agency publications.	methods (Redbook). The latter is widely disseminated as well as being available through the Federal Records Center.
11. Are there recent examples of new or revised test methods that have been developed by or submitted to your agency for approval that were either accepted or rejected? What was the rationale for acceptance or rejection?	OPPTS. An alternative test for corrosion is undergoing review; the OECD tests for the fixed-dose method, the acute toxic class method, dermal irritation and the combined 28-day subchronic/developmental study as well as other new and revised OECD guidelines were accepted.	Recently, ATSDR Division of Toxicology announced 117 final priority data needs for 38 hazardous substances. Simultaneously, the agency requested volunteers to fill these data needs. In response, a member of the regulated industry proposed using physiologically based pharmacokinetic models in lieu of conventional testing to fill data needs for one of these 117 chemicals. The proposal was brought to the OGC for legal implications and the TASARC committee for technical evaluation. It was the opinion of OGC that ATSDR could pursue the initiative with the industry provided the proposal was consistent with credible science.	DMS. The 8th edition of the Bacteriological Analytical Manual is in press. The revised Redbook containing toxicological methods is due to be completed in 1996. Both documents are internally generated and the issue of acceptance/rejection does not apply. The latest revision of the Redbook has introduced methods for screening food additives for neurotoxicologic and immunotoxicologic potential. DTR has received and is evaluating suggestions for modifications to these two toxicology test areas that have been suggested by industry and other interested parties.

		ATSDR sent the proposal for external peer review; the process is ongoing. Upon completion of the study, it will be sent for external peer review and placed in the public record.	
12. Does your agency currently participate in the review of proposed new international test methods? If so, does this process allow for adequate input from your agency?	OPPTS participates in the review of proposed new and revised OECD guidelines; the process allows for adequate input.	ATSDR participates in the review and comment process for new and revised OECD testing guidelines. This effort is coordinated through the Division of Toxicology and involves review and comment by appropriate disciplinary experts and concurrence from the director of the division.	DFS. DFS participates in the review process and most of the time the process allows adequate input. DMS. CFSAN currently receives copies of drafts of toxicological tests under study and development of scientific consensus by international organizations like OECD. These drafts are sent to the most relevant technical expert in the center. These individuals make comments that are sent back to OECD for their consideration.
13. Does your agency accept data conducted in accordance with current OECD or other applicable national or international testing guidelines? If certain data using such test guidelines would not be applicable, please explain.	OPPTS. Data conducted in accordance with current OECD guidelines are potentially acceptable.	Generally, such data would be viewed in an overall weight of evidence evaluation for a particular substance and endpoint.	DFS. Yes. Some of the OECD guidelines have been accepted. The reviewer would want to be assured that the data were produced in accordance with the acceptable guideline(s). DTR. CFSAN accepts data from tests conducted in accordance with OECD guidelines. At times the

			results from these tests may not resolve the scientific question at issue and then additional (and perhaps different) testing may be required.
14. Are there mandates/policies within your agency to minimize or refine animal use or to seek substitutes (replacements) for animals in testing?	OPPTS. Yes.	No such written mandates or policies exist at ATSDR although verbal discussions to this effect have frequently occurred.	DFS. Yes. DTR. The Agency has a policy regarding minimization and refinement of animal use as well as seeking substitutes for animals..
15. Does an animal care and use committee in your agency: a) review proposed new or revised test methods that use animals prior to acceptance of the method by your agency; or b) review the proposed use of animals for testing conducted within your agency with regard to numbers, handling and manipulation of test animals?	OPPTS. Neither part of the question is applicable to OPPT.	ATSDR does not have animal toxicology testing capabilities; thus, there does not exist an animal use and care committee at ATSDR.	DFS. Yes to both parts of the question. DTR. The CFSAN has an Animal Care and Use Committee which reviews proposed new or revised test methods that use animals for studies conducted by CFSAN, not by other institutions.
16. Does your agency fund the development and validation of new or revised test methods? Is this done within your own laboratories or via contracts, grants or other mechanisms? Briefly describe any efforts in this area.	OPPTS. Not applicable. ORD. The Office of Research and Development uses all of these means to develop and validate new and revised test methods.	To date, the agency's efforts in this area have come under the auspices of its voluntary research program; thus, no funds have been expended in the development of new of revised methods.	DFS. Yes. This is done with the agency laboratories. We do have some contracts, grants, CRADA, and collaborations with industry and academia. DTR. The CFSAN by virtue of providing resources for research conducted with animals, funds the development and validation of new or

revised test methods. The DTR has a number of studies employing non-whole animal methods in the areas of neurotoxicology, developmental toxicology, mechanistic toxicology, and toxicity screening.

[1]Compiled from responses to agency survey conducted by ICCVAM. Only three programs/agencies are included for purposes of comparison of processes.

APPENDIX C

INTERNATIONAL ORGANIZATIONS CONCERNED WITH TOXICOLOGICAL TESTING

Organization for Economic Cooperation and Development (OECD)

OECD plays a pivotal role in the acceptance of assays by the international regulatory community primarily for industrial chemicals but also for pesticides and consumer and occupational exposures. OECD comprises 28 member countries including most of the countries of the European Union, Australia, New Zealand, Japan, Canada, Mexico, and the U.S. Guidelines developed and accepted by OECD member countries are generally accepted by other non-OECD countries for regulatory purposes.

Built into the OECD process is the Mutual Acceptance of Data (MAD) principle, wherein it is agreed that data generated under an approved test guideline will be acceptable to regulatory agencies within the OECD member countries. The U.S. has not always followed this principle because some chemical products are not directly addressed by OECD. However, when an agency requires more or a different type of data than is available using OECD guidelines, it is free to request additional information.

Although the process for developing OECD test guidelines can be long, it ensures that all interested parties have the opportunity to comment. Proposals may go to a meeting of experts for discussion before gaining comment internationally and final approval. OECD operates on the basis of consensus, and a test is not accepted until all member countries agree on its applicability and ability to satisfy various regulatory mandates. The U.S., through the EPA, solicits input into the development of each test guideline and presents to the OECD Secretariat a national position that takes into account the comments of regulatory agencies, public interest groups, and the regulated industry (Koëter, 1994).

OECD had been concerned primarily with traditional toxicological test methods for human health and ecotoxicology, as well as other endpoints, but is now becoming more involved in the use of alternatives in testing. OECD has recently accepted two alternatives to the acute oral LD50 test, the fixed dose procedure and the acute toxic class method, and is at work on the development of a third, the up-and-down method, all of which are aimed at reducing animal use and/or suffering. In addition, it has reduced animal use for the skin and eye irritation/corrosion tests (*Table 3.2*).

International Conference on Harmonization (ICH)

The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) is an organization dedicated to the adoption of standardized methods for the development of human drugs and biologicals. As part of this effort, the ICH is evaluating current toxicology testing standards to identify and eliminate duplicated, unnecessary, or obsolete standards and to reduce the use of animals in drug safety evaluation.

The ICH is composed of pharmaceutical regulatory agencies and manufacturing trade associations from the European Union, Japan, and the United States. There are three expert working groups: safety, quality, and efficacy. Safety is concerned with animal toxicology and related areas. Quality works on chemistry and manufacturing standards, and efficacy is concerned with clinical issues.

There are five steps in the ICH approval process. Step 1 consists of preliminary discussions of the topic at hand by the relevant working group as mandated by the ICH Steering Committee, composed of representatives from the six member organizations. In step 2, a draft document is signed by the organizations and sent to the regulatory agencies for a 6-month period in accordance with their normal internal and/or external procedures for comment and review. In the U.S., the step 2 document is published in the *Federal Register* allowing for public review and comment. In step 3, comments are collected and exchanged among regulatory bodies in the organization. At this stage, revisions are incorporated into new drafts and signed by designated representatives of the ICH working groups. During step 4, a final draft is discussed within the Steering Committee and signed by representatives of the three regulatory bodies, which then recommend adoption of the draft document. During step 5, the recommendations are incorporated into the domestic regulations of the three regulatory bodies within ICH.

The ICH has completed or has pending a number of testing issues including (1) elimination of the LD50 test, (2) elimination of the requirement for a 12-month rodent toxicity study,(3) adoption of an improved standard for male reproductive toxicity testing; (4) development of a systemic exposure metric as an alternative to the maximum tolerated dose for carcinogenicity studies, (5) development of a toxicokinetics guideline,(6) evaluation of the requirement to conduct carcinogenicity studies in two rodent species, and (7) adoption of a standard genotoxicity test battery (*Table 3. 2*).

United Nations Committee on Transport

The United Nations Committee of Experts on the Transport of Dangerous Goods (UN Transport) is the focal point for international activity regarding hazardous materials in transport. It is also the only international body dealing with regulatory testing that affects most countries of the world. Most efforts are focused on physical hazards from chemical exposure (e.g., flammability), but some deal with acute health effects, such as dermal corrosion and acute toxicity, and with environmental hazards. The group agrees on testing protocols, criteria for evaluation of test data, and a system of communicating hazards including labeling and marking of packages, placarding of tanks, and documentation of emergency response information. Work is completed in two-year cycles, and agreements are brought into national regulations.

Appendix D

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Appendix E-NRC Assessment Paradigms

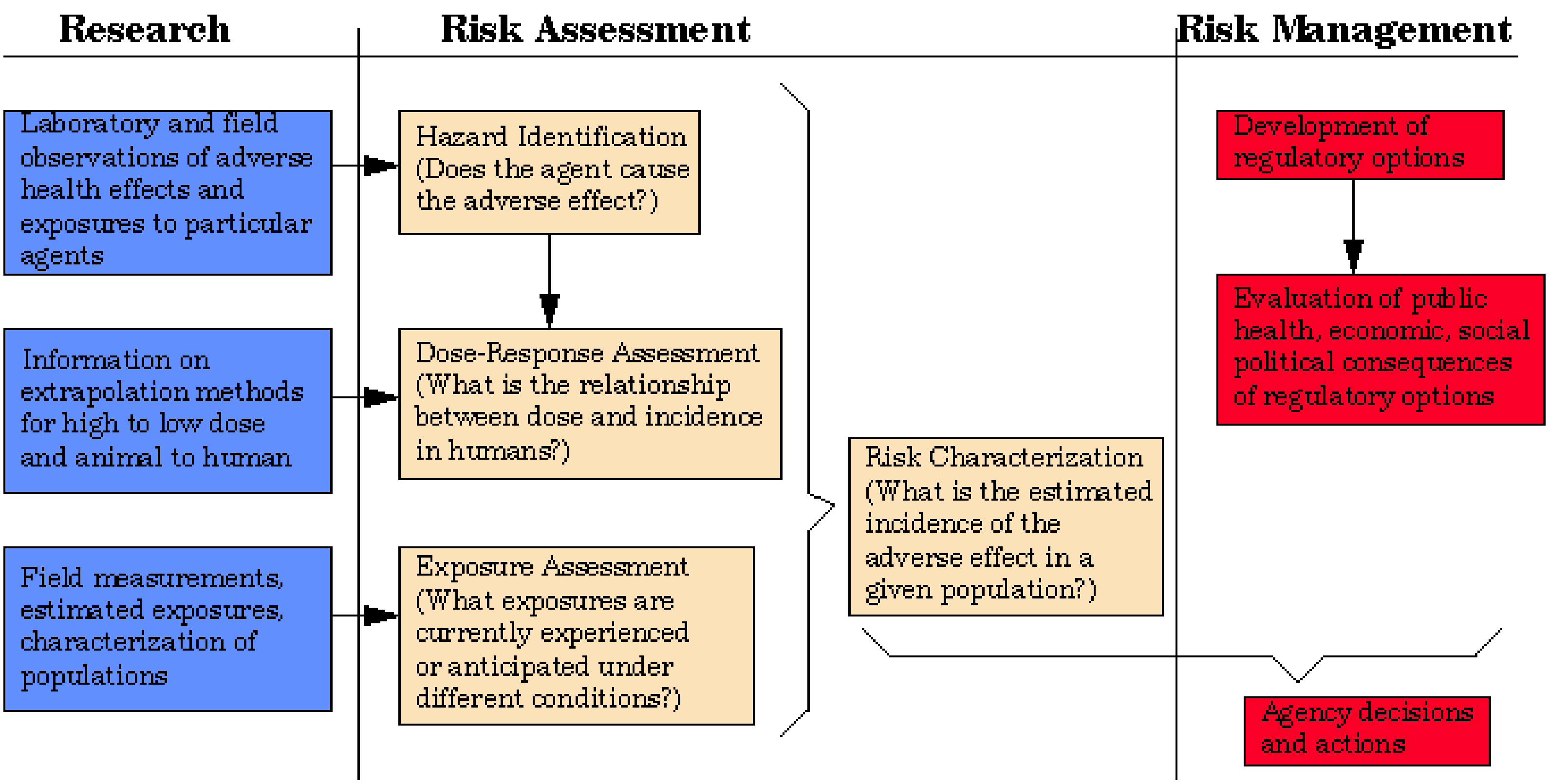

Figure 3-1 Elements of Risk Assessment and Risk Management
NRC (National Research Council) 1983
Risk Assessment in the Federal Government: Managing the Process.
Washington, DC: National Academy Press

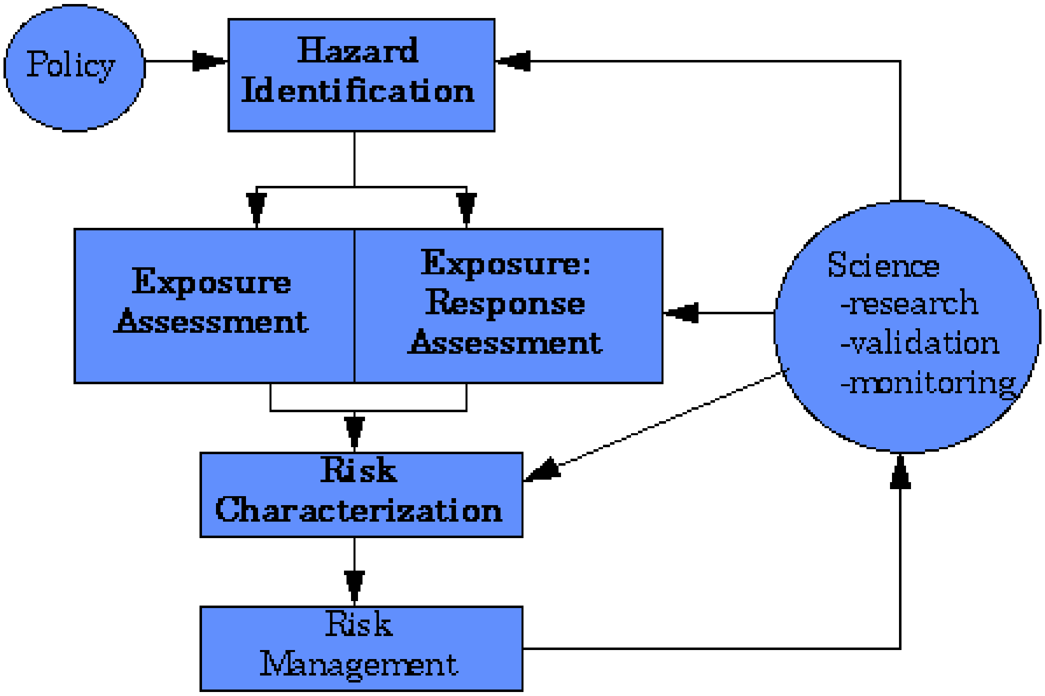

Figure 3-2 Extension of the 1983 NAS Risk Assessment Paradigm to include Ecological as well as Human Health Risks.
NRC (National Research Council) 1993. Issues in Risk Assessement, Washington, DC: National Academy Press

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APPENDIX F

1993 NIH REVITALIZATION ACT, SECTIONS 1301 AND 205

LEGI-SLATE Report for the 103rd Congress Mon., June 14, 1993 1:41pm (EDT)

Bill, Sponsor and Short Title:
S.1 by KENNEDY, EDWARD (D-MA) ñ National Institutes of Health Revitalization Act of 1993

Official Title (caption):
A bill to amend the Public Health Service Act to revise and extend the programs of the National Institutes of Health, and for other purposes.

Item 81: (34) TITLE XIII--NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

Item 82: (32) SEC. 1301. APPLIED TOXICOLOGICAL RESEARCH AND TESTING PROGRAM

(a) In General.--Subpart 12 of part C of title IV of the Public Health Service Act (42 U.S.C. 2851) is amended by adding at the end the following section:

ìAPPLIED TOXICOLOGICAL RESEARCH AND TESTING PROGRAM

'Sec. 463A. (a) There is established within the Institute a program for conducting applied research and testing regarding toxicology, which program shall be known as the Applied Toxicological Research and Testing Program.

'(b) In carrying out the program established under subsection(a), the Director of the Institute shall, with respect to toxicology, carry out activities--

'(1) to expand knowledge of the health effects of environmental agents;

'(2) to broaden the spectrum of toxicology information that is obtained on selected chemicals;

'(3) to develop and validate assays and protocols, including alternative methods that can reduce or eliminate the use of animals in acute or chronic safety testing;

'(4) to establish criteria for the validation and regulatory acceptance of alternative testing and to recommend a process through which scientifically validated alternative methods can be accepted for regulatory use;

'(5) to communicate the results of research to government agencies, to medical, scientific, and regulatory communities, and to the public; and

'(6) to integrate related activities of the Department of Health and Human Services.'

(b) Technical Amendment.--Section 463 of Public Health Service Act (42 U.S.C. 2851) is amended by inserting after 'Sciences' the following: '(in this subpart referred to as the ëInstituteí)'.

S.1 As finally approved by the House and Senate (Enrolled)

Item 35: (55) SEC. 205. PLAN FOR USE OF ANIMALS IN RESEARCH.

SEC. 205. PLAN FOR USE OF ANIMALS IN RESEARCH.

(a) In General - Part A of Title IV of the Pubic Health Service Act, as amended by section 204 of this Act, is amended by adding at the end the following new section:

'PLAN FOR THE USE OF ANIMALS IN RESEARCH

'SEC. 404C. (a) The Director of NIH, after consultation with the committee established under subsection (e), shall prepare a plan-

'(1) for the National Institutes of Health to conduct or support research into-

'(A) methods of medical research and experimentation that do not require the use of animals;

'(B) methods of such research and experimentation that reduce the number of animals used in such research;

'(C) methods of such research and experimentation that produce less pain and distress in such animals; and

'(D) methods of such research and experimentation that involve the use of marine life (other than marine mammals);

'(2) for establishing the validity and reliability of the methods described in paragraph (1);

'(3) for encouraging the acceptance by the scientific community of such methods that have been found to be valid and reliable; and

'(4) for training scientists in the use of such methods that have been found to be valid and reliable.

'b) Not later than October 1, 1993, the Director of NIH shall submit to the Committee on Energy and Commerce of the House of Representatives, and to the Committee on Labor and Human Resources of the Senate, the plan required in subsection (a) and shall begin implementation of the plan.

'(c) The Director of NIH shall periodically review, and as appropriate, make revisions in the plan required under subsection (a). A description of any revision made in the plan shall be included in the first biennial report under section 403 that is submitted after the revision is made.

'd) The Director of NIH shall take such actions as may be appropriate to convey to scientists and others who use animals in biomedical or behavioral research or experimentation information respecting the methods found to be valid and reliable under section (a)(2).

'(e)(1) The Director of NIH shall establish within theNational Institutes of Health a committee to be known as the Interagency Coordinating Committee on the Use of Animals in Research (in this subsection referred to as the 'Committee').

'(2) The Committee shall provide advice to the Director of NIH on the preparation of the plan required in subsection (a).

'(3) The Committee shall be composed of--

> '(A) the Directors of each of the national research institutes and the Director of the Center for Research Resources (or the designees of such Directors); and
>
> '(B) representatives of the Environmental Protection Agency, the Food and Drug Administration, the Consumer Product Safety Commission, the National Science Foundation, and such additional agencies as the Director of NIH determines to be appropriate, which representatives shall include not less than one veterinarian with expertise in laboratory-animal medicine.'

(b) Conforming Amendment.óSection 4 of the Health Research Extension Act of 1985 (Public Law 99-158; 99 Stat. 880 is repealed.

APPENDIX G

FEDERAL REGISTER NOTICE, DECEMBER 7, 1994

[Appeared in *Federal Register*, Vol. 59, No. 234, Wed., December 7, 1994, pp. 63100-63101]

Public Health Service

National Institute of Environmental Health Sciences: Validation and Acceptance of Alternative Testing Methods: Request for Comments

Introduction

Section 1301 of the National Institutes of Health Revitalization Act of 1993 (Public Law No. 103-43) directed the National Institute of Environmental Health Sciences (NIEHS) to establish an Applied Toxicologi-cal Research and Testing Program to conduct applied research and testing regarding toxicology. The Act specified that the toxicology-related activities to be carried out by the program would include: (i) establishing criteria for the validation and regulatory acceptance of alternative testing methods; and (ii) recommending a process through which scientifically validated alternative methods can be accepted for regulatory use. The purpose of this announcement is to invite interested parties to provide information for consideration in the formulation of these criteria and processes.

Background

In response to the directives in Public Law No. 103-43, the NIEHS has established the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to develop recommendations relating to the validation and acceptance of new and revised testing methods that would be useful to Federal agencies. Many new and revised test methods represent alternative methods, models, and approaches in that they: a) result in the reduction of the total number of animals required in a test; b) incorporate refinements of procedures to lessen or eliminate pain or distress to animals; or c) provide for the partial or total replacement of animals with non-animal systems, or the replacement of one animal species with another (e.g., a mammalian species replaced by a nonmammalian or invertebrate species).

The Committeeís goals include recommending criteria and processes that will:
1) encourage the development of new methods and improvement of existing test methods to generate data useful for risk assessment; 2) lead to the scientific validation of new and improved test methods; 3) increase the likelihood of regulatory acceptance of scientifically valid new test methods; and
4) encourage the refinement and reduction of animal use in testing, and the replacement of animals with non-animal methods and/or phylogenetically lower species, when scientifically feasible.

Action

Comments and information are invited from interested parties regarding criteria for the validation and acceptance of alternative testing methods, and processes for the regulatory acceptance of scientifically validated alternative methods. Information is sought regarding the following broad topics:

- types of information necessary to evaluate the practical utility of a test method;
- essential components and processes applicable to the validation of test methods;

- principles and criteria for assessing the validity of a test method; i.e., do considerations vary depending upon whether the test is: a) in vivo vs. in vitro; b) a screen or a replacement; or c) mechanistically-based or not;
- factors relevant to the acceptance of validated test methods by regulatory and scientific agencies.

The Committee will consider such comments and information prior to the preparation of a draft document. Opportunity for comment on the Committeeís draft document will be announced at a later date, and a public meeting will also be announced.

Comments and information should be sent within 60 days of the publication of this announcement to Dr. William Stokes, NIEHS, MD-A2-05, P.O. Box 12233, Research Triangle Park, North Carolina 27709. For further information regarding this request, please contact Dr. Stokes by mail at the above address, by FAX at 919/541-0719, by telephone at 919/541-7997, or by Internet e-mail at Stokes@NIEHS.NIH.GOV.

Signed by:
Richard A. Griesemer, D.V.M., Ph.D
Deputy Director, NIEHS

APPENDIX H

FEDERAL REGISTER NOTICE, NOVEMBER 3, 1995

[Appeared in Federal Register, 60 FR 55849, Wed., November 3, 1995, pp. 55849-50]

Public Health Service

Request for Comments on the Draft Report on Validation and Regulatory Acceptance of Toxicological Test Methods; Announcement of the National Toxicology Program (NTP) Workshop on Validation and Regulatory Acceptance of Alternative Toxicological Test Methods

The draft report on *Validation and Regulatory Acceptance of Toxicological Test Method* is available and public review and comment are encouraged. Registration is open for an NTP Workshop scheduled for December 11-12, 1995, that will provide the opportunity to participate in the review of this Report and to comment on the recommendations generated at the Workshop.

BACKGROUND ON THE REPORT

One of the over-arching goals of the NTP is developing and validating improved alternative toxicological test methods. Consistent with the goal, the NIH Revitalization Act of 1993 (P.L. 103-43, sec. 1301) stated that the National Institute of Environmental Health Sciences (NIEHS), the primary component of the NTP, would: (*a*) *establish criteria for the validation and regulatory acceptance of alternative testing methods; and* (*b*) *recommend a process through which scientifically validated alternative methods can be accepted for regulatory use.*

An ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) was established by NIEHS to develop a report recommending criteria and processes for validation and regulatory acceptance of toxicological testing methods. Fifteen Federal regulatory and research agencies have participated in this effort, including:

- Agency for Toxic Substances and Disease Registry (ATSDR)
- Consumer Product Safety Commission (CPSC)
- Department of Agriculture (USDA)
- Department of Defense (DOD)
- Department of Energy (DOE)
- Department of the Interior (DOI)
- Department of Transportation (DOT)
- Environmental Protection Agency (EPA)
- Food and Drug Administration (FDA)
- National Institute for Occupational Safety and Health (NIOSH)
- National Institutes of Health (NIH)
- National Cancer Institute (NCI)
- National Institute of Environmental Health Sciences (NIEHS)
- National Library of Medicine (NLM)
- Occupational Safety and Health Administration (OSHA)

The draft Report is applicable to all proposed toxicological testing methods for health and ecological endpoints, including those termed 'alternatives.' Alternative test methods are those that incorporate some aspect of reduction, refinement, and replacement of animal use. Such methods: result in the reduction of the total number of animals required; incorporate refinements of procedures to lessen or eliminate pain or distress to animals and enhance animal well-being; or provide for the partial or total replacement of animals with non-animal systems, or the replacement of an animal species with a phylogenetically lower species (e.g., a mammalian species replaced by an invertebrate species).

The ICCVAM determined that the goals of the Report are to:

- Communicate the criteria and processes that Federal agencies should employ in considering new and revised test methods;
- Encourage the development of new methods and improvement of existing test methods;
- Provide more effective guidance for scientists for the validation and evaluation of new and revised test methods;
- Contribute to the increased likelihood of regulatory acceptance of scientifically valid new and revised test methods;
- Encourage, when scientifically feasible, the reduction and refinement of animal use in testing, and the replacement of animals with non-animal methods and phylogenetically lower species;
- Encourage the use of validated and accepted new and revised test methods.

COMMENTS ON THE REPORT

Public review of the draft Report is critical to its completion and is encouraged. To receive a copy of the Report, please contact the NTP Liaison Office at NIEHS, P.O. Box 12233, MD A3-01, Research Triangle Park, NC 27709, or by FAX to: (919) 541-0295. Written comments received by November 20, 1995, will be distributed for consideration during the workshop. Written comments submitted after November 20 but before January 2, 1996, will be considered by the Committee in preparing a final Report. Submit comments to Dr. William Stokes, NIEHS, P.O. Box 12233, MD B2-04, Research Triangle Park, NC 27709, or by FAX to (919) 541-0719. For further information about the Report, please contact one of the ICCVAM co-chairs -- Dr. William Stokes, NIEHS, or Dr. Richard Hill, EPA, Mail Code 7101, 401 M Street, S.W., Washington, DC 20460, or FAX (202) 260-1847.

BACKGROUND ON THE WORKSHOP

A workshop on Validation and Regulatory Acceptance of Alternative Toxicological Test Methods will be held on December 11-12, 1995, in Arlington, Virginia, to receive comments from the public and invited review panels on the draft Report. The Workshop meeting structure will include opening and closing Plenary Sessions and three Breakout Groups that will address: (1) Validation Criteria; (2) Regulatory Acceptance Criteria and Processes; and (3) Proposals for Future Directions.

Specific goals of the Workshop:

- To obtain comments and recommendations and strengthen the usefulness of the Report for the scientific community.
- To discuss comments received in response to this notice and other announcements.
- To obtain comments and recommendations relevant to the effective implementation of the processes

described in the Report.

Comments and recommendations from the Workshop will be considered by the ICCVAM in preparing a final Report.

REGISTRATION FOR THE WORKSHOP

Registration materials for the workshop can be obtained by contacting the NTP Liaison Office at NIEHS, P.O. Box 12233, MD A3-01, Research Triangle Park, NC 27709, or by FAX to: (919) 541-0295. Please indicate on the registration form if you wish to speak. Oral presentations from participants requesting time during the closing plenary session will be limited to five minutes in length to allow for a maximum number of presentations. Written comments accompanying the oral statements are encouraged and should be received by close of business on November 20, 1995, to ensure consideration by the workshop breakout groups.

Signed by:
Kenneth Olden, Ph.D.
Director, National Toxicology Program
National Toxicology Program (NTP)

Workshop on

Validation and Regulatory Acceptance of Alternative Toxicological Test Methods

December 11-12, 1995

Crystal Gateway Marriott Hotel
1700 Jefferson Davis Highway
Arlington, Virginia

Sponsored by:
the National Institute of Environmental Health Sciences

Organized by the
***ad hoc* Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)**

Background

One of the over-arching goals of the National Toxicology Program (NTP) is developing and validating alternative test systems. Consistent with the goal, the NIH Revitalization Act of 1993 stated that the National Institute of Environmental Health Sciences (NIEHS), the primary component of the NTP, would: (a) establish criteria for the validation and regulatory acceptance of alternative testing methods; and (b) recommend a process through which scientifically validated alternative methods can be accepted for regulatory use.

An ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) was established by NIEHS to develop a report recommending criteria and processes for validation and regulatory acceptance of toxicological testing methods. Fifteen Federal regulatory and research agencies have participated in this effort, including:

- Agency for Toxic Substances and Disease Registry (ATSDR)
- Consumer Product Safety Commission (CPSC)
- Department of Agriculture (USDA)
- Department of Defense (DOD)
- Department of Energy (DOE)
- Department of Interior (DOI)
- Department of Transportation (DOT)
- Environmental Protection Agency (EPA)
- Food and Drug Administration (FDA)
- National Institute for Occupational Safety and Health (NIOSH)
- National Institutes of Health (NIH)
- National Cancer Institute (NCI)
- National Institute of Environmental Health Sciences (NIEHS)
- National Library of Medicine (NLM)
- Occupational Safety and Health Administration (OSHA)

The ICCVAM determined that this report should be applicable to all proposed toxicological testing methods, including those termed 'alternatives.' This decision was based on the premise that the criteria and processes for validation and regulatory acceptance of test methods considered 'alternatives' should be no different than for other test methods. Alternative test methods are those that incorporate some aspect of reduction, refinement, and replacement of animal use. Such methods:

- Result in the reduction of the total number of animals required;
- Incorporate refinements of procedures to lessen or eliminate pain or distress to animals and enhance animal well-being; or
- Provide for the partial or total replacement of animals with non-animal systems, or the replacement of an animal species with a phylogenetically lower species (e.g., a mammalian species replaced by an invertebrate species).

Specific Goals of the Report

The ICCVAM determined that the goals of the Report are to:

- Communicate the criteria and processes that Federal agencies should employ in considering new and revised test methods;
- Encourage the development of new methods and improvement of existing test methods;
- Provide more effective guidance for scientists for the validation and evaluation of new and revised test methods;
- Contribute to the increased likelihood of regulatory acceptance of scientifically valid new and revised test methods;

- Encourage, when scientifically feasible, the reduction and refinement of animal use in testing, and the replacement of animals with non-animal methods and phylogenetically lower species;
- Encourage the use of validated and accepted new and revised test methods.

Public Review of Report

Broad public review of the report is critical to its completion. The report will be available for review and comment beginning in November and this workshop will provide an additional forum for this input.

Specific Goals of the Workshop

1. To obtain comments and recommendations on the draft report described above that will strengthen the usefulness of the report for the scientific community. Comments will be sought from the following:
   - Invited panelists, including representatives from industry, academe, public interest groups, and the international community.
   - Workshop registrants at specific times during plenary and breakout sessions.

2. To discuss comments received in response to the *Federal Register* notice and other announcements inviting comments on the draft report.

3. To obtain comments and recommendations relevant to the effective implementation of the processes described in the Report.

Breakout Sessions	**Salons 1, 2 and 3** **Arlington Ballroom**

	Validation Criteria	**Regulatory Acceptance & Criteria Processes**	**Proposals for Future Directions**
Chair/ Co-chair	David Brusick Warren Schaeffer	Steve Niemi	Patricia Williams
Executive Secretary	Oliver Flint	Lorraine Twerdok	Penelope Fenner-Crisp
	Daniel Bagley Paul Bailey John Bantle Leon Bruner Rodger Curren John Frazier	Mark Chamberlain James Emerson Susan Hurt Myra Karstadt Herman Koëter Karen Kohrman	Michael Balls Betsy Carlton Mary Ann Danello Alan Goldberg Sarah Goodman John Harbell

	Thomas Goldsworthy Gilly Griffin A. Wallace Hayes Kevin Renskers Robert Scala Horst Spielmann Janet Springer John Stegeman	Michael McClain Hiroshi Ono Gary Patterson Verne Ray Daniel Sauder Gregory Smith Martin Stephens	Yuji Kurokawa George Lucier James O'Steen Richard Phillips Andrew Rowan Loretta Schuman Katherine Stitzel Jan Willem van der Laan Neil Wilcox
ICCVAM Liaison	Errol Zeiger	Angela Auletta	Joy Cavagnaro David Hattan
ICCVAM Committee and Agency Reps.	William Allaben Robert Finch Sidney Green Kailash Gupta Helene Guttman* Vera Hudson David Longfellow Barnett Rattner Harry Salem Hugh Tilson	Joseph Contrera George Cushmac Victor Fung Bryan Hardin Anita O'Connor Marilyn Wind	John Bucher Richard Hill Louis Sibal William Stokes

*member to 10/95

Sunday, December 10, 1995

7:30 - 9:30 p.m.	**Registration**	**Arlington Ballroom (Lobby)**
7:30 - 9:30 p.m.	**Meeting of Co-Chairs, Liaisons, and Executive Secretaries**	**Arlington Ballroom (*Salon 1*)**

Monday, December 11, 1995

7:30 - 8:30 am	**Registration and Continental Breakfast**	**Arlington Ballroom (Lobby)**

Opening Plenary Session

Arlington Ballroom (Salon 3)

Chair: Kenneth Olden

8:30 a.m. **'Opening Remarks'**
Kenneth Olden, Director
National Institute of Environmental Health Sciences (NIEHS)
and the National Toxicology Program (NTP)

8:45 a.m.	**'The Role of the National Toxicology Program in Test Method Development and Validation'** **George Lucier, Director, Environmental Toxicology Program, NIEHS**	
9:15 a.m.	**'Review of Federal Toxicological Testing Activities'** **Richard Hill, Science Advisor, Office of Prevention, Pesticides and Toxic Substances, Environmental Protection Agency**	
9:45 a.m.	**'Review of Workshop and ICCVAM Objectives; Charges to the Breakout Groups'** **William Stokes, Associate Director for Animal and Alternative Resources, NIEHS**	
10:00 a.m.	**Break** **Breakout Sessions**	**(see chart, previous page)** **Arlington Ballroom**
10:30 - 12:00 p.m.	**Breakout Groups (see chart, previous page)**	**(Salons 1, 2 and 3)**
12:00 - 1:30 p.m.	**Lunch Break (on your own)**	
1:30 - 5:00 p.m.	**Breakout Group Sessions (Continued)**	**(Salons 1, 2 and 3)**
5:10 - 5:45 p.m.	**Meeting of Co-Chairs, Liaisons and Executive Secretaries**	**(Arlington Coatroom)**

Evening Session — **Salon 4**

6:15 - 7:00 p.m.	**Cash Bar with Complimentary Dry Snacks**	**(Salon 4 Foyer)**
7:00 p.m.	**Dinner** **'An Overview and Current Activities for the European Centre for the Validation of Alternative Methods'** **Professor Michael Balls, Director, European Centre for the Validation of Alternative Methods, Joint Research Centre, Environment Institute, Commission of the European Union, Ispra, Italy**	

Tuesday, December 12, 1995

Breakout Sessions Continued — **(see chart, previous page)**

7:45 - 8:30 am	**Registration and Continental Breakfast**	**Arlington Ballroom Lobby**
8:30 - 12:00 am	**Breakout Session Continued (see chart, previous page)**	**(Salons 1, 2 and 3)**
12:00 - 1:30 p.m.	**Lunch (on your own)**	

Closing Plenary Session — **Arlington Ballroom (Salon 3)**

Chair: Richard Hill

1:30 - 3:00 p.m. **Breakout Groups: Presentations and Discussions**

1:30 - 1:50 p.m.	**Validation Criteria**	**Co-Chairs: David Brusick, Warren Schaeffer**
1:50 - 2:00 p.m.	**Discussion**	
2:00 - 2:20 p.m.	**Regulatory Acceptance Criteria and Processes**	**Chair: Steve Niemi**
2:20 - 2:30 p.m.	**Discussion**	
2:30 - 2:50 p.m.	**Proposals for Future Directions**	**Chair: Patricia Williams**
2:50 - 3:00 p.m.	**Discussion**	
3:00 - 3:30 p.m.	**Break**	
Chair: John Bucher		
3:30 - 5:15 p.m.	**Public Comments and Open Discussion**	
3:30 - 3:35 p.m.	**Mr. Kurt Enslein, Health Design, Inc.**	
3:35 - 3:40 p.m.	**Dr. George Becking, World Health Organization**	
3:45 - 3:50 p.m.	**Mr. Mark Benjamin, Xenometrix, Inc.**	
3:50 - 3:55 p.m.	**Dr. Yasuo Ohno, National Institute of Health Sciences, Japan**	
3:55 - 4:00 p.m.	**Ms. Martha Armstrong, MSPCA/AHES**	
4:00 - 4:05 p.m.	**David Neumann, ILSI Risk Science Institute**	
4:05 - 4:10 p.m.	**Dr. Spencer Farr, Xenometrix, Inc.**	
4:10 - 4: 15 p.m.	**Mr. Shayne C. Gad, Gad Consulting Services**	
4:15 - 4:20 p.m.	**Dr. Alan M. Goldberg, Johns Hopkins University**	
4:20 - 4:25 p.m.	**Dr. Horst Spielmann, ZEBET, Germany**	
4:30 - 5:00 p.m.	**Open Discussion**	
5:00 - 5:30 p.m.	**'Closing Remarks' William Stokes**	
5:30 p.m.	**Adjourn**	

Breakout Group Descriptions

Breakout Groups will develop recommendations in the areas described below. There will be time in each group to allow public comment by observers. The number of observers will be limited by space available and to ensure a size that will enable the recommendations to be completed by the end of the workshop.

1. Validation Criteria

The focus of this group will be the review of the draft ICCVAM chapter on validation criteria. The chapter discusses scientific assessment of the reliability and relevance of new and revised toxicological testing methods. The group will address the adequacy and completeness of the concepts and criteria for the evaluation of screening tests, adjunct tests, substitute methods for existing tests, and tests for new toxicological endpoints. The criteria for validation of mechanistically-based tests compared with empirical tests will be discussed, as well as considerations for in vitro and in vivo methods. The chapter recommendations will be evaluated for their adequacy.

2. Regulatory Acceptance Criteria and Processes

This group will discuss the chapter of the draft report that addresses determining the acceptability of validated test methods for regulatory use. The concepts, criteria, and processes for regulatory acceptance will be assessed for their adequacy and completeness. Minimum information that should be required in the submission of a proposed new test method will be reviewed. The group will address the criteria for acceptance of methods to generate data for use in hazard identification and classification for risk assessment purposes. The process for reviewing methods proposed at the agency, Federal, and international levels will be discussed.

3. Proposal for Future Directions

Practical strategies for effective implementation of the report and its recommendations will be the focus of this discussion group. The role of groups inside and outside of government in evaluating the status of validated methods will be discussed. The group will discuss proposals relating to the processes for validation and regulatory acceptance of new and revised toxicological test methods. The group will address how industry, government, academe, and public interest groups can work together both nationally and internationally as stakeholders to more efficiently develop, validate, and adopt new improved testing methods for regulator use.

Public Comment Session

Public comment and open discussion during the closing plenary session will provide the opportunity for additional views and comments. Oral presentations from participants requesting time will be limited to 5 minutes in length to allow for a maximum number of presentations. Please indicate your interest in speaking on the registration form.

Written comments on the draft report can be forwarded to:

NTP Liaison Office
P.O. Box 12233
MD: A3-01
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Written comments should be received by November 20, 1995, for distribution to the breakout group chairs and consideration during the workshop.

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Workshop Organizing Committee

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William Stokes,NIEHS

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Angela Auletta, EPA	Sidney Green, FDA	Harry Salem, DOD
James Beall, DOE	Helene Guttman, USDA	Loretta Schuman, OSHA
Christina Blakeslee, NIH	Bryan Hardin, NIOSH	Doug Sharpnack, NIOSH
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Conference Management Committee

Sandy Lange   Pam Wigington   Anna Lee Sabella   Alma Britton   Tonia James

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APPENDIX H.3 - LIST OF WORKSHOP ATTENDEES

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