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ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods

Prepared by the
Interagency Coordinating Committee on the
Validation of Alternative Methods (ICCVAM)
and the
National Toxicology Program (NTP) Interagency Center for the Evaluation
of Alternative Toxicological Methods (NICEATM)

National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
Department of Health and Human Services

About ICCVAM and NICEATM

The National Institute of Environmental Health Sciences (NIEHS) established the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) in 1997 to coordinate the interagency technical review of new, modified, and alternative test methods of interagency interest and to coordinate cross-agency issues relating to the validation, acceptance, and national and international harmonization of toxicological testing methods. ICCVAM was established as a permanent interagency committee of the NIEHS under the National Toxicology program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) on December 19, 2000, by the ICCVAM Authorization Act of 2000 (Public Law 106-545; Appendix E).

The Committee is comprised of representatives from the fifteen U.S. Federal regulatory and research agencies that use or generate toxicological information. ICCVAM promotes the scientific validation and regulatory acceptance of toxicological test methods that more accurately assess the safety or hazards of chemicals and products and that refine (i.e., decrease or eliminate pain and distress), reduce, and replace animal use. NICEATM provides operational and scientific support for ICCVAM and ICCVAM-related activities. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of U.S. Federal agencies. More information about ICCVAM and NICEATM can be found at *http://iccvam.niehs.nih.gov,* by contacting NICEATM at (919) 541-2384 or by email to *iccvam@niehs.nih.gov*.

The U.S. Federal regulatory and research agencies that participate in this effort are the:

- Consumer Product Safety Commission
- Department of Agriculture
- Department of Defense
- Department of Energy
- Department of Health and Human Services
  - Agency for Toxic Substances and Disease Registry
  - Food and Drug Administration
  - National Cancer Institute
  - National Institute for Occupational Safety and Health
  - National Institute of Environmental Health Sciences
  - National Institutes of Health, Office of the Director
  - National Library of Medicine
- Department of the Interior
- Department of Labor
  - Occupational Safety and Health Administration
- Department of Transportation
- Environmental Protection Agency

On the Cover

The ICCVAM/NICEATM graphic symbolizes the important role of new and alternative toxicological methods in protecting and advancing the health of people, animals, and our environment.

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

National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

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National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
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** Alternate Principal Agency Representative

PREFACE

Before a new or modified test method is used to generate information to support regulatory decisions, it must a) undergo adequate validation to determine its reliability and accuracy for a specific proposed use, and b) be deemed acceptable by one or more regulatory agencies to fill a specific need. Criteria for validation and regulatory acceptance have been developed by the U.S. Federal government and are described in the report, *Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the Ad Hoc Interagency Coordinating Committee on the Validation of Alternative Methods* (1). Prior to the initiation of test method development or validation efforts, sponsors should consider these validation and acceptance criteria.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) developed this document, *ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods*, to assist test method sponsors and nominators in organizing the information needed by ICCVAM to assess the validation status of a new or modified test method at any stage of the validation process. This document is available online at http://iccvam.niehs.nih.gov/docs/guidelines/subguide.htm; printed copies are available on request from the National Toxicology Center (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) (NIEHS, P.O. Box 12233, MD EC-17, Research Triangle Park, NC 27709; telephone: 919-541-3398, fax: 919-541-0947, e-mail: iccvam@niehs.nih.gov). These guidelines describe:

- The ICCVAM test method nomination and submission process
- Performance standards, which communicate the basis on which a validated and accepted proprietary (i.e., copyrighted, trademarked, registered) or nonproprietary test method has been determined to have sufficient accuracy and reliability for a specific testing purpose. These performance standards should be met by proposed test methods that are based on similar scientific principles and that measure or predict the same biological or toxic effect.
- The information that should be provided in test method nominations or submissions so that ICCVAM can evaluate appropriately the extent to which the validation and acceptance criteria have been addressed, or will be addressed in proposed studies

The ICCVAM Authorization Act of 2000 (2) (**Appendix E**) directs ICCVAM to:

- Review and evaluate new, modified, or alternative test methods, including batteries of tests and test screens, that may be acceptable for specific regulatory uses
- Coordinate technical reviews of test methods of interagency interest
- Review and evaluate petitions received from the public that:
  - Identify a specific regulation, recommendation, or guideline regarding a regulatory mandate
  - Recommend new or modified test methods and provide valid scientific evidence of the potential of the recommended test method to improve prediction of adverse human or animal health or ecological effects, and to reduce, refine, or replace animal use in existing regulatory test methods.

Test method sponsors are encouraged to consult with NICEATM and ICCVAM throughout the test method development, prevalidation, and validation process, as well as during preparation of submissions. The objective of these interactions is to maximize the likelihood that validation studies and submissions will adequately characterize the usefulness and limitations of the proposed test method. Complete submissions are essential and serve as a basis for assessing the validation status of a proposed test method through an independent ICCVAM peer review process. This interactive process enhances the likelihood that agencies will have sufficient data and information to determine the extent that a test method can generate information that will meet their regulatory needs.

These guidelines now include guidance on the process for submitting nominations to ICCVAM for test methods that are proposed for further consideration, but which may require further compilation of data or even additional validation studies. Test method nominators are encouraged to consult with NICEATM and ICCVAM prior to submitting nominations. The objective of this interaction is to ensure that the nominations contain as much information as possible and to ensure that the proposed test methods have regulatory applicability.

The initial ICCVAM submission guidelines, first released in May 1998, incorporated much of the guidance developed for data submissions for the Second Workshop of the Interagency Regulatory Alternatives Group (3). Revised submission guidelines were published in 1999, based on experience gained with the first two test methods reviewed by ICCVAM – the Local Lymph Node Assay and Corrositex®. This second revision reflects further experience gained with the evaluation of other alternative test methods (Frog Embryo Teratogenesis Assay – *Xenopus*, the Up-and-Down Procedure for Acute Oral Toxicity, EPISKIN™, EpiDerm™, the Rat Skin Transcutaneous Electrical Resistance assay, and *in vitro* estrogen–receptor/androgen–receptor binding and transcriptional activation assays) and incorporates procedures revised in response to the ICCVAM Authorization Act of 2000. ICCVAM continues to welcome suggestions for improving the usefulness of these guidelines.

We gratefully acknowledge the ICCVAM agency representatives, working group members, and peer review panel members who contributed to the preparation of the original document and to subsequent revisions. We also appreciate the constructive suggestions received from scientists who used earlier versions of the guidelines to prepare submissions to ICCVAM.

Leonard M. Schechtman, Ph.D.
Chair, ICCVAM

William S. Stokes, D.V.M., Diplomate A.C.L.A.M.
Director, NICEATM
Executive Director, ICCVAM

1.0 INTRODUCTION

ICCVAM is responsible for coordinating the interagency technical review of new or modified alternative test methods of interagency interest, and coordinating cross-agency issues relating to the validation, acceptance, and national and international harmonization of toxicological test methods throughout the U.S. Federal government. ICCVAM was established as a permanent interagency committee of the National Institute of Environmental Health Sciences (NIEHS) under NICEATM by the ICCVAM Authorization Act of 2000 (Public Law 106-545) (2; **Appendix E**). Priority is given to test methods that may provide for improved prediction of adverse human, animal, or ecological effects, and those that might reduce[1], refine[2], or replace[3] animal use.

In the *ad hoc* ICCVAM report on the validation and regulatory acceptance of toxicological test methods (1), various stages were identified to move a proposed test method from concept to regulatory acceptance (**Figure 1**). A critical stage is the communication of a proposed test method by the sponsor or nominator to ICCVAM for consideration and review. NICEATM, on behalf of ICCVAM, receives proposed test method nominations or submissions and communicates with the submitting organization or individual. Typically, the ICCVAM evaluation process involves an initial assessment by NICEATM of the adequacy and completeness of the proposed test method nomination or submission, and a determination by ICCVAM of the priority that the proposed test method will have for technical evaluation (see **Section 2**). Once a proposed test method has been accepted for evaluation, ICCVAM assembles an interagency working group of government scientists with scientific and regulatory expertise in the appropriate scientific disciplines to collaborate with NICEATM on the evaluation process. Depending on the validation status of the proposed test method, ICCVAM, in conjunction with NICEATM, develops recommendations and priorities for further efforts. Such efforts might include an expert workshop, an expert panel meeting, a peer review meeting, an expedited peer review process, or a validation study (**Figure 2**).

Following this review process, ICCVAM develops and forwards recommendations on the usefulness and limitations of the proposed test method for regulatory purposes to Federal agencies, in accordance with Public Law 106-545 (2). Based on their specific statutory mandates, each agency then makes a determination regarding the acceptability of the test method. Agencies are required to respond to ICCVAM within 180 days of receipt of an ICCVAM test method recommendation. If the test method is accepted, appropriate actions (e.g., revision of existing regulations, publication of guidelines and/or guidance documents) are taken to inform the regulated community.

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[1]Reduction alternative: A new or modified test method that reduces the number of animals required.
[2]Refinement alternative: A new or modified test method that refines procedures to lessen or eliminate pain or distress in animals or enhances animal well-being.
[3]Replacement alternative: A new or modified test method that replaces animals with nonanimal systems or one animal species with a phylogenetically lower one.

Figure 1. Test Method Validation Process

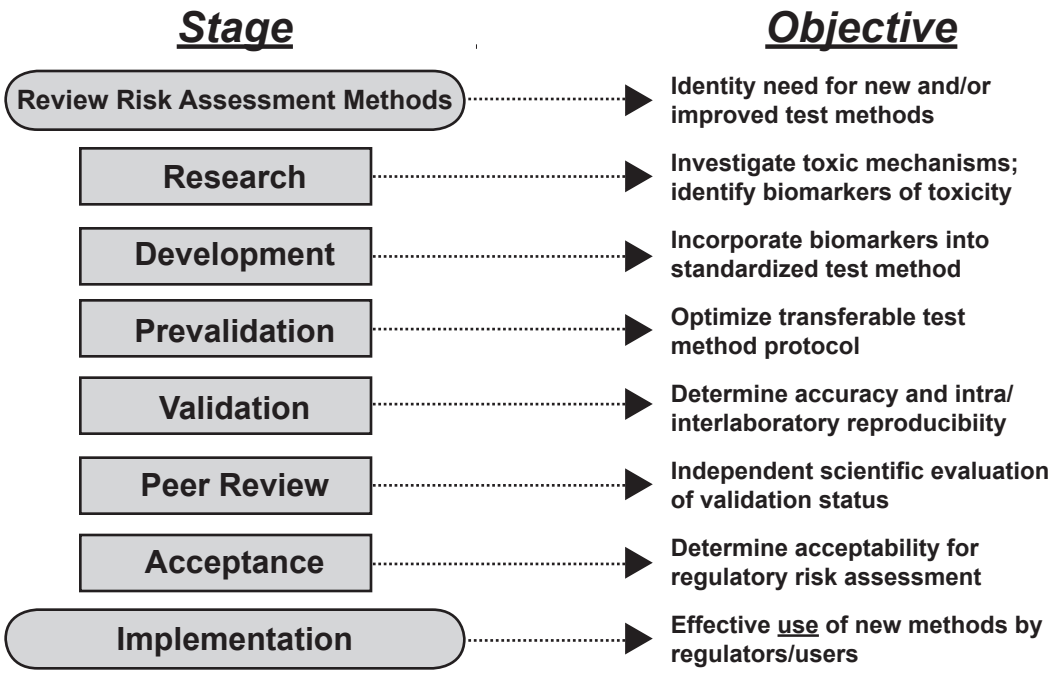
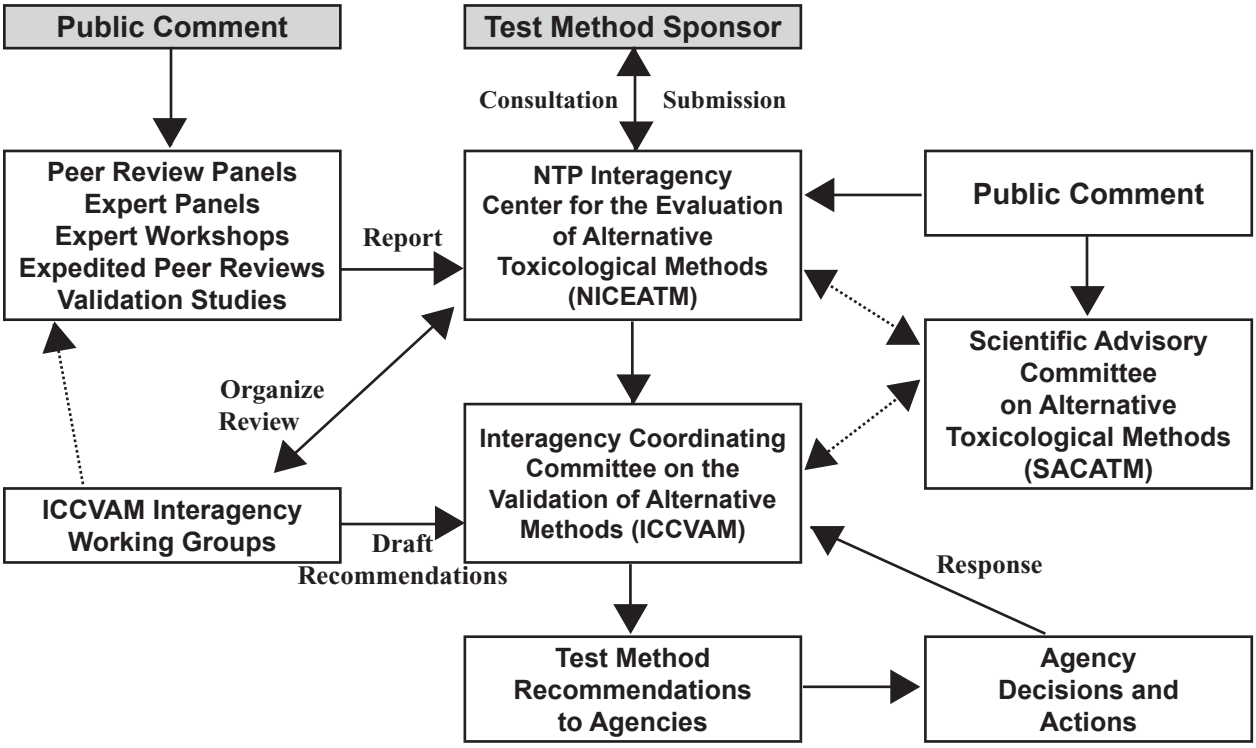


Figure 2. ICCVAM Test Method Evaluation Process



The purpose of this document is to provide guidance to sponsors and nominators on the information needed by ICCVAM to evaluate the validation status of a proposed test method (1, 4, 5). In preparing a nomination or submission, the outline in **Appendix A** should be used to discuss the extent to which each of the validation and acceptance criteria (**Appendix D**) have been addressed or will be addressed in proposed validation studies. Sponsors and nominators may be asked to provide additional information to augment or complement the information described in these guidelines.

Validation is defined as the process by which the reliability[4] and relevance[5] of a procedure for a specific purpose are established. The test method submission must contain sufficient information for an independent scientific peer review panel to assess the validation status of the proposed test method and for agencies to assess the acceptability of the proposed test method for providing useful information for hazard or risk assessment. Nominations should be accompanied by as much of the requested information outlined in this document as possible. Although there is no mandatory minimum requirement for information to provide with nominations, complete information will expedite ICCVAM consideration of the proposed test method.

The test method nomination or submission to ICCVAM should include:

- An introduction, including the scientific and regulatory rationale for the proposed test method
- Information on the development of the proposed test method protocol and its key components
- Characterization of the substances used for validation studies on the proposed test method
- The reference data used to assess the accuracy and reliability of the proposed test method
- Test method data and results
- An assessment of the accuracy of the proposed test method
- An assessment of the reliability (repeatability/reproducibility) of the proposed test method
- An assessment of test method data quality
- Other scientific reports and reviews pertinent to the proposed test method
- An assessment of animal welfare considerations (refinement, reduction, and replacement)
- Practical considerations (e.g., test method study costs, time needed to perform a study, ease of transferability of the test method among laboratories)
- A comprehensive and complete list of references
- Supporting materials (e.g., the proposed test method protocol) in appendices

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[4]*Reliability:* A measure of the degree to which a test method can be performed reproducibly within and among laboratories over time. It is assessed by calculating intra- and inter-laboratory reproducibility and intralaboratory repeatability.

[5]*Relevance:* The extent to which a test method correctly predicts or measures the biological effect of interest in humans or another species of interest. Relevance incorporates consideration of the "accuracy" or "concordance" of a test method.

Nominations and submissions should be submitted to NICEATM in both printed and electronic formats. The preferred software for electronic submission of text is Microsoft® Word and the preferred format for databases is Microsoft® Excel. However, other software programs may be used.

Although ICCVAM recognizes that there may be a need to maintain confidentiality of proprietary information, the designation of materials as confidential is discouraged because this limits an open and transparent evaluation. Submission of adequate and complete information will facilitate the ICCVAM review process. The amount and type of information needed to substantiate the usefulness of a proposed test method for a specified regulatory purpose will vary depending on its nature and its proposed use. An organizational outline to be followed when preparing the nomination or submission is provided in **Appendix A**.

Sponsors and nominators should communicate with NICEATM/ICCVAM throughout the development, prevalidation, and validation process, and during the nomination or submission process. If requested and appropriate, ICCVAM may solicit interagency comments on proposed test method protocols and prevalidation or validation studies. Requests for comment on proposed prevalidation or validation study designs should include descriptions of the scientific basis and regulatory applicability of the proposed test method, the scientific rationale for the proposed prevalidation or validation studies, and responses to each section of the submission guidelines.

The NICEATM office is located at NIEHS, which is headquartered in Research Triangle Park, NC (NIEHS, P.O. Box 12233, MD EC-17, Research Triangle Park, NC 27709; telephone: 919-541-2384; fax: 919-541-0947; e-mail: iccvam@niehs.nih.gov). NICEATM serves as a communication link between test sponsors and Federal agencies during the development and validation process. In collaboration with ICCVAM, NICEATM convenes expert workshops, expert panel meetings, peer review meetings, and expedited peer reviews, and conducts validation studies when appropriate and recommended by ICCVAM.

2.0 ICCVAM TEST METHOD NOMINATION AND SUBMISSION PROCESS

This section describes the process by which "test method nominations[6]" and "test method submissions[7]" to ICCVAM are considered and prioritized for review and evaluation (**Figure 3**). Submissions should be accompanied by all requested information. Although there is no mandatory minimum requirement for information to provide with nominations, ICCVAM consideration of the proposed test method will be expedited by providing as much information as possible. The minimum information required for submissions and recommended to accompany nominations is summarized in **Section 4**. Areas where the requested information is not available or is incomplete should be indicated, along with the scientific approach(es) planned to generate those data.

The Director of NICEATM solicits and tracks the status of proposed test method submissions and nominations, provides updates to ICCVAM, and arranges for a preliminary evaluation of submissions and nominations by NICEATM, as resources permit. Preliminary evaluations summarize the extent to which proposed test method submissions or nominations address the following ICCVAM prioritization criteria:

- The extent to which the proposed test method is:
  - Applicable to regulatory testing needs
  - Applicable to multiple agencies/programs
- Warranted, based on the extent of expected use or application and impact on human, animal, or ecological health
- The potential for the proposed test method, compared to current test methods accepted by regulatory agencies, to:
  - Refine animal use (decreases or eliminates pain and distress)
  - Reduce animal use
  - Replace animal use
- The potential for the proposed test method to provide improved prediction of adverse health or environmental effects, compared to current test methods accepted by regulatory agencies
- The extent to which the test method provides other advantages (e.g., reduced cost and time to perform) compared to current methods

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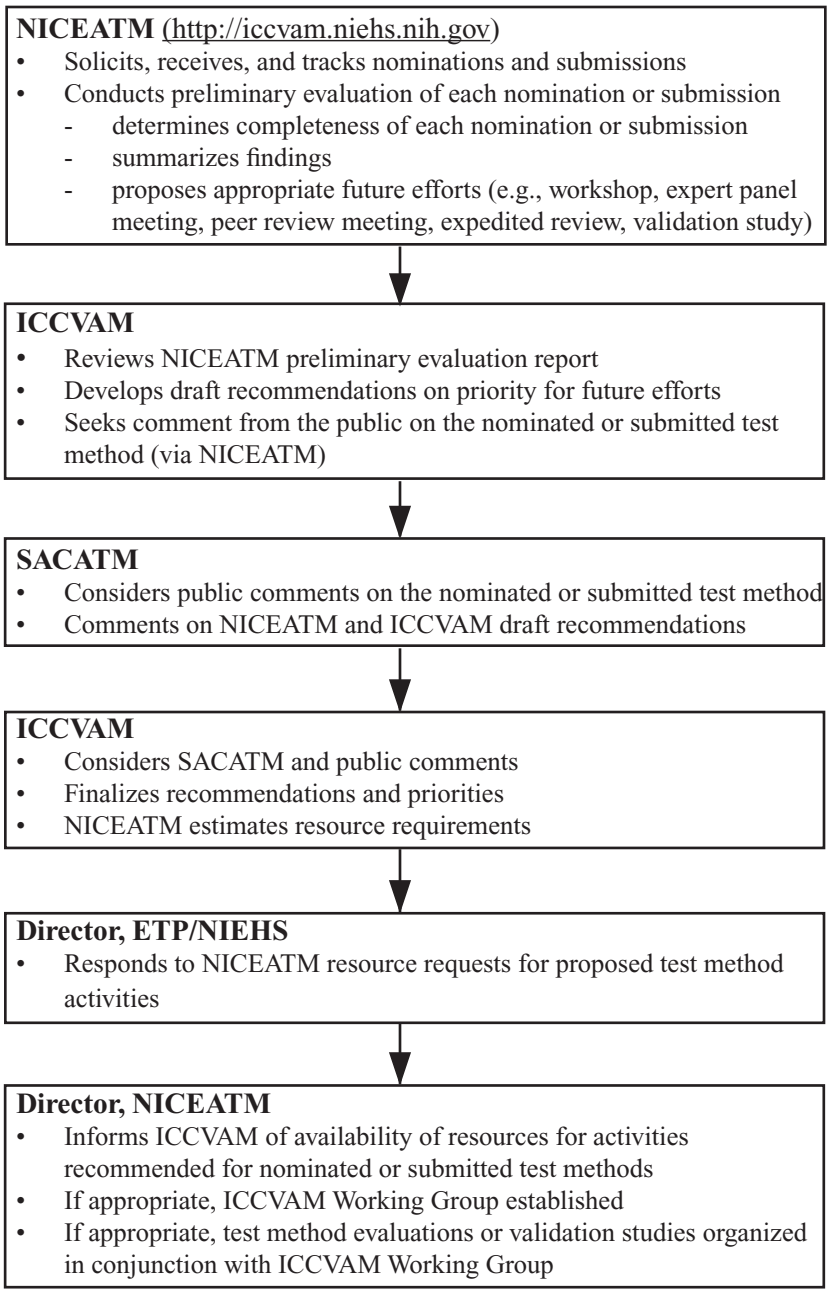
[6]*Test method nomination:* A test method proposed to ICCVAM for review and evaluation for which a complete test method submission is not available. Examples include: (1) test methods for which adequate validation studies presumably have been completed but lack a complete submission package; (2) test methods that appear promising based on limited prevalidation or validation data and are proposed for additional validation studies; (3) test methods that have been developed and are proposed for prevalidation or validation studies; and (4) test methods that are recommended for a workshop or other activity.

[7]*Test method submission:* A test method proposed to ICCVAM for review and evaluation for which adequate validation studies have been completed to characterize the usefulness and limitations of the test method for a specific proposed regulatory testing requirement or application, and adequate documentation of the scientific validity has been prepared in accordance with ICCVAM test method submission guidelines.

Figure 3. ICCVAM Test Method Submission and Nomination Process



- The completeness of the nomination or submission with regard to ICCVAM test method submission guidelines

The Director of NICEATM provides the results of NICEATM preliminary evaluations to ICCVAM, including recommendations and relative priority for further evaluations (e.g., workshop, expert panel meeting, peer review meeting, expedited review process) or validation studies. ICCVAM then:

- Reviews the NICEATM preliminary evaluation report
- Determines whether the test method is of sufficient interest and applicability to one or more agencies to warrant further evaluation
- Develops draft recommendations regarding priority for evaluation, the conduct of validation studies, or other activities

The Director of NICEATM provides the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) with a status report on test method submissions and nominations, the results of NICEATM and ICCVAM preliminary evaluations, and any draft recommendations. The SACATM comments on the draft test method evaluations and recommendations in terms of future ICCVAM efforts. ICCVAM also seeks comment from the public, using electronic methods (ICCVAM listserve groups, the ICCVAM/NICEATM web site) and printed materials and publications (*Federal Register*). ICCVAM considers comments from the SACATM and the public, develops final recommendations, and prioritizes future evaluation and validation efforts.

The Director of NICEATM estimates resource requirements for proposed evaluations and/or validation studies and forwards these, along with ICCVAM, NICEATM, and SACATM recommendations, to the Director of the Environmental Toxicology Program (ETP)/NIEHS with a request for funding, when necessary. The ETP Director responds with information on the availability of the requested resources for the recommended activity.

The Director of NICEATM informs ICCVAM of the availability of funding to conduct the recommended activities. When resources are available to support a recommended activity (workshop, expert panel meeting, independent peer review, expedited review, validation study), ICCVAM establishes an interagency working group of knowledgeable scientists to work with NICEATM in organizing the appropriate evaluation or validation study. In collaboration with ICCVAM and the appropriate working group, NICEATM organizes workshops, expert panel meetings, independent peer reviews, validation studies, or expedited reviews, as appropriate, to evaluate the validation status of the proposed test method.

3.0 PERFORMANCE STANDARDS FOR TEST METHODS

Prior to the acceptance of a proposed test method for regulatory testing applications, validation studies are conducted to assess reliability and accuracy. The purpose of performance standards is to communicate the basis on which a new proprietary (i.e., copyrighted, trademarked, registered) or nonproprietary test method was determined to have sufficient accuracy and reliability for a specific testing purpose. Performance standards may be developed and recommended by ICCVAM as part of its evaluation of the validation status of a proposed test method. Performance standards, based on test methods accepted by regulatory agencies, can be used to evaluate the reliability and accuracy of other proposed test methods that are based on similar scientific principles and measure or predict the same biological or toxic effect.

The three elements of performance standards are:

- **Essential test method components** (previously referred to as "minimum procedural standards" [6]): These consist of essential structural, functional, and procedural elements of a validated test method that should be included in the protocol of a mechanistically and functionally similar proposed test method. These components include unique characteristics of the test method, critical procedural details, and quality control measures. Adherence to essential test method components will help to assure that a proposed test method is based on the same concepts as the corresponding validated test method.

- **Minimum list of reference chemicals:** These are used to assess the accuracy and reliability of a mechanistically and functionally similar proposed test method. These chemicals are a representative subset of those used to demonstrate the reliability and the accuracy of the validated test method. To the extent possible, these reference chemicals should:
  - Be representative of the range of responses that the validated test method is capable of measuring or predicting
  - Have produced consistent results in the validated test method and in the *in vivo* reference test method and/or the species of interest
  - Reflect the accuracy of the validated test method
  - Have well-defined chemical structures
  - Be readily available
  - Not be associated with excessive hazard or prohibitive disposal costs

  These reference chemicals are the minimum number that should be used to evaluate the performance of a proposed, mechanistically and functionally similar test method. The chemicals should not be used to develop the prediction model for the proposed test method. If any of the recommended chemicals are unavailable, other chemicals for which adequate reference data are available could be substituted. To the extent possible, the substituted chemical(s) should be of the same chemical class as the original chemical(s). If desired, additional chemicals representing other chemical or product classes and for which adequate reference data are available can be used to more comprehensively evaluate the accuracy of the proposed test method. However, these additional chemicals should not include any that had been used to develop the proposed test method.

- **Accuracy and reliability values:** These are the comparable performance that should be achieved by the proposed test method when evaluated using the minimum list of reference chemicals.

The ICCVAM process for developing performance standards for new test methods is as follows:

- NICEATM and the appropriate ICCVAM working group develop proposed performance standards for consideration during the ICCVAM evaluation process. If performance standards are proposed by a test method sponsor, these will be considered by ICCVAM during the evaluation process. Generally, the performance standards will be based on the information and data provided in the test method submission or on other available applicable data.

- The ICCVAM/NICEATM Peer Review Panel evaluates the proposed performance standards for completeness and appropriateness during its evaluation of the validation status of the proposed test method. The proposed performance standards will be made available with the test method submission to the public for comment prior to and during the Peer Review Panel meeting.

- The appropriate ICCVAM working group, with the assistance of NICEATM, prepares the final performance standards for ICCVAM approval, taking into consideration the recommendations of the Peer Review Panel and public comments.

Performance standards recommended by ICCVAM would be incorporated into ICCVAM test method evaluation reports, which are provided to Federal agencies and made available to the public. Regulatory authorities may then reference the performance standards in the ICCVAM report when they communicate their acceptance of a new test method. In addition, performance standards adopted by regulatory authorities could be provided in guidelines issued for new test methods. Availability of ICCVAM test method evaluation reports are announced routinely in the *Federal Register*, NTP Newsletters, and ICCVAM/NICEATM e-mail listserve groups.

4.0 SUBMISSION GUIDELINES FOR PROPOSED TEST METHODS

4.1 Introduction and Rationale for the Proposed Test Method

The sponsor should use this section to introduce the proposed test method and describe its regulatory and scientific rationale. A description must be provided of how the proposed test method can be used in the context of current or anticipated regulatory applications (e.g., as a screen in a tiered testing strategy, as an adjunct test to provide mechanistic information, as a substitute or replacement for an existing test method). The mechanistic basis of the proposed test method and the context in which it will be used to measure or predict the toxicological activity of a test material or substance should be discussed, as well as what is known and not known about the similarities and differences of modes and mechanisms of action in the test system compared to the species of interest (e.g., humans for human health-related toxicity testing). If applicable, the extent to which the proposed test method meets the performance standards of a mechanistically and functionally similar validated and accepted test method should be addressed. The sponsor should indicate the relevant classes of chemicals and products that can and cannot be evaluated using the proposed test method and any known limitations. Finally, the sponsor should indicate where and how the proposed test method might be included in the overall safety or hazard assessment process. In particular, if the proposed test method is part of tiered or battery approaches, the weight given to the new method relative to other tests in the tier or battery should be addressed.

4.2 Test Method Protocol Components

The sponsor should explain and describe the basis for decisions on critical functional, structural, and procedural elements of the test method protocol (a complete, detailed protocol for the proposed test method should be provided in an appendix to the submission). This would include the extent to which the protocol for the proposed test method is similar to the protocol of a validated mechanistically and functionally similar test method for which performance standards exist. The basis for any protocol modifications made during the validation of the proposed test method should be discussed. The technical parameters of the proposed test method (e.g., vehicles, exposure time), the nature of the response evaluated, and the basis for proposed concurrent controls should be described. Concurrent controls (negative, solvent, and positive), as appropriate, provide a basis for experiment-to-experiment comparisons and are usually part of the acceptance criteria for a given experiment. The acceptable ranges for the control responses and historical data used to establish the acceptable range should be included.

The nature of the data to be collected, the methods used for data collection, the type of media in which data are stored, measures of variability, the statistical or nonstatistical methods used to analyze and evaluate the data (including methods used to analyze for a dose-response relationship), and the decision criteria (and their rationale) used to classify the response as positive or negative, if applicable, must be described. The procedure for dose selection and the number of animals required, if any, for dose selection and the actual test should be stated. Both the statistical and nonstatistical methods used for data evaluation should be justified. Any confidential information associated with the proposed test method should be indicated clearly; however, the inclusion of confidential information is discouraged.

The number of replicate and/or repeat experiments needed to ensure an adequate study must be provided, and the basis for the design should be described. If replicate or repeat experiments are not part of the proposed test method protocol, a rationale for their exclusion must be provided.

The basis for selection of the proposed test method system must be provided. If an animal model is used, the rationale for selecting the species, strain or stock, sex, acceptable age range, diet, frequency of dosing, the number of doses, and other applicable protocol components should be included.

If the test method employs proprietary components, the procedures used to ensure their integrity (in terms of reliability and accuracy) from "lot-to-lot" and over time should be described. Also, procedures that the user may employ to verify the integrity of the proprietary components should be described.

4.3 Substances Used for Validation of the Proposed Test Method

The rationale for the numbers and types of substances tested during the validation process should be described. The specific chemical or formulation names and relevant chemical and product classes for the substances tested must be specified. A test method may be more effective for the evaluation of certain classes of chemicals. In addition, not all data sets will be homogeneous for a given chemical characteristic (e.g., water solubility). In such cases, it may be useful to separate the data set into smaller, more uniform subsets for data analysis. To the extent possible, the following information should be provided for each test substance:

- Chemical Abstracts Service Registry Number (CASRN)
- Physical and chemical characteristics
- Concentrations tested
- Purity
- Source
- Stability of the test substance in the test medium

Any characteristics thought to have direct impact on test method accuracy and/or reliability should be described. Information concerning coding of substances during validation studies should be included. In the case of mixtures, the constituents and their relative concentrations should be stated, whenever possible. A suggested spreadsheet format for listing this information is provided in **Appendix B**. Information regarding the use of coded substances and blind testing during the validation process should be included. For a proposed test method mechanistically and functionally similar to a validated test method with established performance standards, the extent to which the reference chemicals recommended in the performance standards were tested in the proposed test method should be discussed, and any deviations from this list should be justified. In situations where a listed reference chemical is unavailable, the criteria used to select a replacement chemical should be described. To the extent possible, when compared to the original reference chemical, the replacement chemical should be from the same chemical/product class and produce similar effects in the *in vivo* reference test method. In addition, if applicable, the replacement chemical should have been tested in the comparable validated test method. Also where applicable, the rationale for

adding additional chemicals and the adequacy of data from the *in vivo* reference test method or the species of interest should be provided.

4.4 *In Vivo* Reference Data Used to Assess the Accuracy of the Proposed Test Method

If the proposed test method is intended to replace or substitute for an existing *in vivo* reference test method, then a comparison of data between the proposed test method and the *in vivo* reference test method is necessary. The submission should include:

- Comparative data for the same substances tested using the *in vivo* reference test method and, where available, from human studies. If possible, individual animal and human data should be provided.
- The criteria used to select the *in vivo* reference test method (or human) data
- The source of the *in vivo* reference test method data (e.g., the literature citation for published information, the laboratory study director, the year generated for unpublished data)
- A description of the protocol(s) employed to generate the *in vivo* reference test method or human data. Any modifications to the *in vivo* reference test method protocol(s) should be stated clearly for each data set, along with a discussion of the potential impact of these modifications on the assessment of the accuracy of the proposed test method.
- A description of the quality of the *in vivo* reference test method data, including the extent of Good Laboratory Practices (GLP) compliance (7-12) and the use of coded test chemicals
- The availability of original study data for the *in vivo* reference test method studies
- A summary of the availability and use of other, relevant toxicity information from the species of interest (e.g., data from human studies, accidental exposures for human health-related toxicity test methods, results of postmarketing surveillance)

4.5 Test Method Data and Results

The data generated by testing chemicals and substances using the proposed test method protocol are provided here. Any protocol modifications made during the development process and their impact should be stated clearly for each data set. All data, both original and derived, should be submitted, along with each laboratory's summary judgment regarding the outcome of each study. The submission should include data (and explanations) from all studies, whether successful or not. The statistical approach used to evaluate the data should be described and justified.

It is also important to describe the "lot-to-lot" consistency of the test chemicals, the time frame of the various studies, and the laboratory(ies) in which the studies were conducted. A coded designation for each laboratory involved in an interlaboratory evaluation of test method reliability and accuracy is acceptable. Any original data not submitted should be available for review, if requested. Presenting all available data, including data from published sources, is essential for an adequate scientific assessment of the proposed test method.

Results should be presented in graph or tabular form for easy comparison of results from the reference test methods with those from the proposed test method. A suggested tabular format for presenting the results is provided in **Appendix B**.

4.6 Test Method Accuracy

The sponsor should describe the accuracy of the proposed test method with respect to its ability to measure or predict the effect of interest. The accuracy (i.e., sensitivity, specificity, positive and negative predictivity, false positive and negative rates) of the proposed test method should be compared to that obtained for the in vivo reference test method currently accepted by regulatory agencies and to data or recognized toxicity information from the species of interest (e.g., humans for human-health-related toxicity testing). In instances where the proposed test method is measuring or predicting an endpoint for which there is no pre-existing test method, the frequency of correct predictions should be compared to relevant information from the species of interest. In cases where the proposed test method is mechanistically and functionally similar to a validated test method with established performance standards, the accuracy of both test methods should be compared. When the results obtained using the proposed test method is discordant from that obtained using the comparable validated test method, the frequency of correct predictions of each test method compared to recognized toxicity information from the species of interest should be presented. The basis for any discordance in results for the following comparisons should be discussed.

- The proposed test method and currently accepted *in vivo* reference test methods
- The proposed test method and, if applicable, the comparable validated test method with established performance standards
- The proposed test method and the accepted *in vivo* reference test method in predicting responses in the species of interest, where data from the species of interest is available

The submission should include a discussion of the strengths and limitations of the proposed test method and should describe salient issues of data interpretation.

4.7 Test Method Reliability (Repeatability/Reproducibility)

An assessment of test method reliability (repeatability and reproducibility) must be provided. This assessment should include discussion of the rationale for the selection of the substances used to evaluate intra- and interlaboratory reproducibility, and the extent to which they represent the range of possible test outcomes. Outlying values should be identified and discussed. A quantitative statistical analysis of the extent of intra- and inter-laboratory variability, such as that described in ASTM Publication Number E691-92 (13) or coefficient-of-variation analysis, should be included. Measures of central tendency and variation, should be summarized for historical control data (negative, vehicle, and positive, where applicable). In cases where the proposed test method is mechanistically and functionally similar to a validated test method with established performance standards, the reliability of the two test methods should be compared and the potential impact of any differences discussed.

4.8 Test Method Data Quality

The extent of adherence to national and international GLP guidelines (7-12) for the data presented in the submission, as well as the results of any data quality audits, should be included here. Deviations from GLP guidelines and the impact of any noncompliance detected in audits should be described. Information on the availability of laboratory notebooks and other data retained

by the sponsor(s) for external audits by ICCVAM should be stated. Unpublished data should be supported by laboratory notebooks.

4.9 Other Scientific Reports and Reviews

The submission should discuss all data from other published or unpublished studies conducted using the proposed test method. Comment should be provided on any conclusions presented in independent peer-reviewed reports or other scientific reviews of the proposed test method. The conclusions of such scientific reports or reviews should be compared to the conclusions reached in the submission. Any other ongoing or planned evaluations of the proposed test method should be described. In cases where the proposed test method is mechanistically and functionally similar to a validated test method with established performance standards, the results of studies conducted subsequent to the ICCVAM evaluation should be included, and any impact on the reliability and accuracy of the proposed test method discussed.

4.10 Animal Welfare Considerations (Refinement, Reduction, and Replacement)

A description should be included of how the proposed test method will refine, reduce, or replace animal use as compared to current methods used for the endpoint of interest. If the proposed test method requires the use of animals, the rationale for such use should be provided. A description of the sources used to determine the possible availability of alternative test methods that would refine, reduce, or replace animal use for the endpoint of interest should be provided (14, 15). The description should include, at a minimum, the databases searched, the search strategy, the search date(s), the database search results, and the rationale for not utilizing available alternative methods. The basis for determining the appropriate number of animals for the proposed test method should be described. If the test involves potential animal pain and distress, the procedures and approaches that have been incorporated to minimize and, whenever possible, to eliminate the occurrence of such pain and distress should be discussed.

4.11 Practical Considerations

The cost and time involved in conducting a study using the proposed test method should be specified and compared to the reference test method(s) and, if applicable, to the mechanistically and functionally validated test method with established performance standards. Also include the following:

- A discussion of the facilities and major fixed equipment needed to conduct the test method
- The general availability of other necessary equipment and supplies
- The required level of training, expertise, and demonstrated proficiency needed by the study personnel

4.12 References

A listing of all publications referenced in the submission should be provided.

4.13 Supporting Materials

The appendices should contain:

- A detailed protocol for the proposed test method
- Copies of all relevant publications, including those containing data from the proposed test method, the *in vivo* reference test method, and if applicable, a comparable validated test method
- All available nontransformed original data used to evaluate the validity of the proposed test method
- Suggested performance standards for consideration by NICEATM and ICCVAM, if performance standards for the proposed test method do not exist. Examples of performance standards can be located on the ICCVAM/NICEATM web site at http://iccvam.niehs.nih.gov.

5.0 REFERENCES

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APPENDIX A: OUTLINE FOR NOMINATIONS AND SUBMISSIONS TO ICCVAM[1]

1.0 Introduction and Rationale for the Proposed Test Method

1.1 Introduction

1.1.1 Describe the historical background for the proposed test method, from original concept to present. This should include the rationale for its development, an overview of prior development and validation activities, and, if applicable, the extent to which the proposed test method is mechanistically and functionally similar to a validated test method with established performance standards.

1.1.2 Summarize and provide the results of any peer review conducted to date and summarize any ongoing or planned reviews.

1.1.3 Clearly indicate any confidential information associated with the test method; however, the inclusion of confidential information is discouraged.

1.2 Regulatory rationale and applicability

1.2.1 Describe the current regulatory testing requirement(s) for which the proposed test method is applicable.

1.2.2 Describe the intended regulatory use(s) (e.g., screen, substitute, replacement, or adjunct) of the proposed test method and how it will be used to substitute, replace, or complement any existing regulatory testing requirement(s).

1.2.3 Where applicable, discuss the similarities and differences in the endpoint measured in the proposed test method and the currently used *in vivo* reference test method and, if appropriate, between the proposed test method and a comparable validated test method with established performance standards.

1.2.4 Describe how the proposed test method fits into the overall strategy of hazard or safety assessment. If a component of a tiered assessment process, indicate the weight that should be applied relative to other measures.

1.3 Scientific basis for the proposed test method

1.3.1 Describe the purpose and mechanistic basis of the proposed test method.

1.3.2 Describe what is known and not known about the similarities and differences of modes and mechanisms of action in the proposed test method as compared to the species of interest (e.g., humans for human health-related toxicity testing).

[1]Where the requested information is not yet available, this should be indicated. Plans for generating information that is not available should be described.

1.3.3 Describe the intended range of substances amenable to the proposed test method and/or the limits of the proposed test method according to chemical class or physicochemical factors.

2.0 Test Method Protocol Components

Note: A complete, detailed protocol should be included as an appendix to the nomination or submission.

2.1 Provide an overview of how the proposed test method is conducted. If appropriate, this would include the extent to which the protocol for the proposed test method adheres to established performance standards.

2.2 Provide a detailed description and rationale, if appropriate, for the following aspects of the proposed test method:

2.2.1 Materials, equipment, and supplies needed

2.2.2 Dose-selection procedures, including the need for any dose range-finding studies or acute toxicity data prior to conducting a study, if applicable

2.2.3 Endpoint(s) measured

2.2.4 Duration of exposure

2.2.5 Known limits of use

2.2.6 Nature of the response assessed

2.2.7 Appropriate vehicle, positive, and negative controls and the basis for their selection

2.2.8 Acceptable range of vehicle, positive and negative control responses and the basis for the acceptable ranges

2.2.9 Nature of the data to be collected and the methods used for data collection

2.2.10 Type of media in which data are stored

2.2.11 Measures of variability

2.2.12 Statistical or nonstatistical methods used to analyze the resulting data, including methods to analyze for a dose-response relationship. Justify and describe the method(s) employed.

2.2.13 Decision criteria and the basis for the prediction model used to classify a test chemical (e.g., positive, negative, or equivocal), as appropriate

2.2.14 Information and data that will be included in the study report and availability of standard forms for data collection and submission

2.3 Explain the basis for selection of the test method system. If an animal model is being used, this should include the rationale for selecting the species, strain or stock, sex, acceptable age range, diet, and other applicable parameters.

2.4 If the test method employs proprietary components, describe what procedures are used to ensure their integrity (in terms of reliability and accuracy) from "lot-to-lot" and over time. Also describe procedures that the user may employ to verify the integrity of the proprietary components.

2.5 Describe the basis for the number of replicate and repeat experiments; provide the rationale if experiments are not replicated or repeated.

2.6 Discuss the basis for any modifications to the proposed test method protocol that were made based on results from validation studies.

2.7 If applicable, discuss any differences between the protocol for the proposed test method and that for a comparable validated test method with established performance standards.

3.0 Substances Used for Validation of the Proposed Test Method (See Appendix B)

3.1 Describe the rationale for the chemicals or products selected for use in the validation process. Include information on the suitability of the substances selected for testing, indicating any chemicals that were found to be unsuitable.

3.2 Discuss the rationale for the number of substances that were tested.

3.3 Describe the chemicals or products evaluated. For each chemical or product, include the following information:

3.3.1 Chemical or product name, or if a mixture, provide information on all components

3.3.2 CASRN

3.3.3 Chemical and product class

3.3.4 Physical/chemical characteristics (e.g., water and lipid solubility, pH, pKa, etc.). Any characteristics thought or known to impact test method accuracy and/or reliability should be clearly described

3.3.5 Stability of the test substance in test medium

3.3.6 Concentrations tested

3.3.7 Purity, including the presence and identity of contaminants and stabilizing additives

3.3.8 Supplier or source

3.4 Describe the coding procedures used in the validation studies.

3.5 For proposed test methods that are mechanistically and functionally similar to a validated test method with established performance standards, discuss the extent to which the recommended reference chemicals were tested in the proposed test method. In situations where a listed reference chemical was unavailable, the criteria used to select a replacement chemical should be described. To the extent possible,

when compared to the original reference chemical, the replacement chemical should be from the same chemical/product class and produce similar effects in the *in vivo* reference test method. In addition, if applicable, the replacement chemical should have been tested in the mechanistically and functionally similar validated test method. If applicable, the rationale for adding additional chemicals and the adequacy of data from the *in vivo* reference test method or the species of interest should be provided.

4.0 *In Vivo* Reference Data Used for an Assessment of the Accuracy of the Proposed Test Method

4.1 Provide a clear description of the protocol(s) used to generate data from the *in vivo* reference test method. If a specific guideline has been followed, it should be provided. Any deviations should be indicated, including the rationale for the deviation.

4.2 Provide the *in vivo* reference test method data used to assess the accuracy of the proposed test method. Individual human and/or animal reference test data, if available, should be provided. Provide the source of the reference data, including the literature citation for published data, or the laboratory study director and year generated for unpublished data.

4.3 If not included in the submission, indicate if original records are available for the *in vivo* reference test method data.

4.4 Indicate the quality of the *in vivo* reference test method data, including the extent of GLP compliance and any use of coded chemicals.

4.5 Discuss the availability and use of relevant toxicity information from the species of interest (e.g., human studies and reported toxicity from accidental or occupational exposure for human health-related toxicity testing).

4.6 Discuss what is known or not known about the accuracy and reliability of the *in vivo* reference test method.

5.0 Test Method Data and Results

5.1 Describe the proposed test method protocol used to generate each submitted set of data. Any differences from the proposed test method protocol should be described, and a rationale or explanation for the difference provided. Any protocol modifications made during the development process and their impact should be clearly stated for each data set.

5.2 Provide all data obtained to evaluate the accuracy and reliability of the proposed test method. This should include copies of original data from individual animals and/or individual samples, as well as derived data. The laboratory's summary judgment regarding the outcome of each test should be provided. The submission should include data (and explanations) from all studies, whether successful or not.

5.3 Describe the statistical approach used to evaluate the data resulting from studies conducted with the proposed test method.

5.4 Provide a summary, in graphic or tabular form, of the results. The suggested tabular format for providing data for use in an assessment of accuracy is provided in **Appendix B**.

5.5 For each set of data, indicate whether coded chemicals were tested, whether experiments were conducted without knowledge of the chemicals being tested, and the extent to which experiments followed GLP guidelines.

5.6 Indicate the "lot-to-lot" consistency of the test substances, the time frame of the various studies, and the laboratory in which the study or studies were conducted. A coded designation for each laboratory is acceptable.

5.7 Indicate the availability of any data not submitted for external audit, if requested.

6.0 Test Method Accuracy

6.1 Describe the accuracy (e.g., concordance, sensitivity, specificity, positive and negative predictivity, false positive and negative rates) of the proposed test method compared with the reference test method. Explain how discordant results in the same or multiple laboratories from the proposed test were considered when calculating accuracy.

6.2 Discuss results that are discordant with results from the *in vivo* reference method.

6.3 Discuss the accuracy of the proposed test method compared to data or recognized toxicity from the species of interest (e.g., humans for human health-related toxicity testing), where such data or toxicity classification are available. This is essential when the method is measuring or predicting an endpoint for which there is no preexisting method. In instances where the proposed test method was discordant from the *in vivo* reference test method, describe the frequency of correct predictions of each test method compared to recognized toxicity information from the species of interest.

6.4 State the strengths and limitations of the proposed test method, including those applicable to specific chemical classes or physical-chemical properties.

6.5 Describe the salient issues of data interpretation, including why specific parameters were selected for inclusion.

6.6 In cases where the proposed test method is mechanistically and functionally similar to a validated test method with established performance standards, the results obtained with both test methods should be compared with each other and with the *in vivo* reference test method and/or toxicity information from the species of interest.

7.0 Test Method Reliability (Repeatability/Reproducibility)

7.1 Discuss the selection rationale for the substances used to evaluate the reliability (intralaboratory repeatability and intra- and interlaboratory reproducibility) of the proposed test method as well as the extent to which the chosen set of substances represents the range of possible test outcomes.

7.2 Provide analyses and conclusions reached regarding the repeatability and reproducibility of the proposed test method. Acceptable methods of analyses might include those described in ASTM E691-92 (13) or by coefficient of variation analysis.

7.3 Summarize historical positive and negative control data, including number of experiments, measures of central tendency, and variability.

7.4 In cases where the proposed test method is mechanistically and functionally similar to a validated test method with established performance standards, the reliability of the two test methods should be compared and any differences discussed.

8.0 Test Method Data Quality

8.1 State the extent of adherence to national and international GLP guidelines (7-12) for all submitted data, including that for the proposed test method, the *in vivo* reference test method, and if applicable, a comparable validated test method. Information regarding the use of coded chemicals and coded testing should be included.

8.2 Summarize the results of any data quality audits, if conducted.

8.3 Discuss the impact of deviations from GLP guidelines or any noncompliance detected in the data quality audits.

8.4 Address the availability of laboratory notebooks or other records for an independent audit. Unpublished data should be supported by laboratory notebooks.

9.0 Other Scientific Reports and Reviews

9.1 Summarize all available and relevant data from other published or unpublished studies conducted using the proposed test method.

9.2 Comment on and compare the conclusions published in independent peer-reviewed reports or other independent scientific reviews of the proposed test method. The conclusions of such scientific reports and reviews should be compared to the conclusions reached in this submission. Any ongoing evaluations of the proposed test method should be described.

9.3 In cases where the proposed test method is mechanistically and functionally similar to a validated test method with established performance standards, the results of studies conducted with the validated test method subsequent to the ICCVAM evaluation should be included and any impact on the reliability and accuracy of the proposed test method should be discussed.

10.0 Animal Welfare Considerations (Refinement, Reduction, and Replacement)

10.1 Describe how the proposed test method will refine (reduce or eliminate pain or distress), reduce, or replace animal use compared to the reference test method.

10.2 If the proposed test method requires the use of animals, the following items should be addressed:

10.2.1 Describe the rationale for the need to use animals and describe why the information provided by the proposed test method requires the use of animals (i.e., cannot be obtained using nonanimal methods).

10.2.2 Include a description of the sources used to determine the availability of alternative test methods that might further refine, reduce, or replace animal use for this testing. This should, at a minimum, include the databases searched, the search strategy used, the search date(s), a discussion of the results of the search, and the rationale for not incorporating available alternative methods.

10.2.3 Describe the basis for determining that the number of animals used is appropriate.

10.2.4 If the proposed test method involves potential animal pain and distress, discuss the methods and approaches that have been incorporated to minimize and, whenever possible, eliminate the occurrence of such pain and distress.

11.0 Practical Considerations

11.1 Discuss the following aspects of proposed test method transferability. Include an explanation of how this compares to the transferability of the *in vivo* reference test method and, if applicable, to a comparable validated test method with established performance standards.

11.1.1 Discuss the facilities and major fixed equipment needed to conduct a study using the proposed test method.

11.1.2 Discuss the general availability of other necessary equipment and supplies.

11.2 Discuss the following aspects of proposed test method training. Include an explanation of how this compares to the level of training required to conduct the *in vivo* reference test method and, if applicable, a comparable validated test method with established performance standards.

11.2.1 Discuss the required level of training and expertise needed for personnel to conduct the proposed test method.

11.2.2 Indicate any training requirements needed for personnel to demonstrate proficiency and describe any laboratory proficiency criteria that should be met.

11.3 Cost Considerations

Discuss the cost involved in conducting a study with the proposed test method. Discuss how this compares to the cost of the *in vivo* reference test method and, if applicable, with that of a comparable validated test method with established performance standards.

11.4 Time Considerations

Indicate the amount of time needed to conduct a study using the proposed test method and discuss how this compares with the *in vivo* reference test method and, if applicable, with that of a comparable validated test method with established performance standards.

12.0 References

12.1 List all publications referenced in the submission.

13.0 Supporting Materials (Appendices)

13.1 Provide the complete, detailed protocol for the proposed test method.

13.2 Provide the detailed protocol(s) used to generate reference data for this submission and any protocols used to generate validation data that differ from the proposed protocol.

13.3 Provide copies of all relevant publications, including those containing data from the proposed test method, the *in vivo* reference test method, and if applicable, a comparable validated test method with established performance standards.

13.4 Include all available nontransformed original data for both the proposed test method, the *in vivo* reference test method, and if applicable, a comparable validated test method with established performance standards.

13.5 If appropriate performance standards for the proposed test method do not exist, performance standards for consideration by NICEATM and ICCVAM may be proposed. Examples of established performance standards can be located on the ICCVAM/NICEATM web site at http://iccvam.niehs.nih.gov.

APPENDIX B: SUGGESTED FORMATS FOR PRESENTING DATA

Characterization of Substances Tested

In addition to a written description of the substances tested, presentation in the following table format is recommended. This information should be provided in printed and electronic formats (Microsoft® Word and Excel are preferred, but other software programs are acceptable).

Chemical or Product Name	CASRN	Chemical Class	Product Class	Concentrations Tested	**Purity**	Supplier or Source of Substance	Physical and Chemical

Test Method Accuracy Assessment

The following format is suggested for presenting the information used in the accuracy assessment. Additional detailed information also should be provided in tabular or printed format as described in **Section 4** of these guidelines. This information should be provided in printed and electronic formats (Microsoft® Word and Excel are preferred, but other software programs are acceptable).

Chemical or Product Name	CASRN	Chemical Class	Product Class	Result Using Proposed Test Method (quantitative)	Result Using Proposed Test Method (+/-)	Result Using Reference Test Method (quantitative)*	Result Using Reference Test Method (+/-)	References or Data Sources	Comments

*Where possible, data from the *in vivo* reference test method should be separated into single columns for each species with available information. Human data should be always presented independently of nonhuman data. If applicable, corresponding data obtained using the mechanistically and functionally similar validated test method with established performance standards should be provided.

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APPENDIX C: GLOSSARY[1]

Accuracy: (a) The closeness of agreement between a test method result and an accepted reference value. (b) The proportion of correct outcomes of a test method. It is a measure of test method performance and one aspect of "relevance". The term is often used interchangeably with "concordance" (see also "two-by-two" table). Accuracy is highly dependent on the prevalence of positives in the population being examined.

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.

Assay: The experimental system used. Often used interchangeably with "test" and "test method".

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 test results. Coded chemicals are used to avoid intentional or unintentional bias when evaluating laboratory or test method performance.

Concordance: The proportion of all chemicals tested that are correctly classified as positive or negative. It is a measure of test method performance and one aspect of "relevance". The term is often used interchangeably with "accuracy" (see also "two-by-two" table). Concordance is highly dependent on the prevalence of positives in the population being examined.

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 or severity of an adverse health or ecological effect.

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

Essential test method components: Structural, functional, and procedural elements of a validated test method that should be included in the protocol of a mechanistically and functionally similar proposed test method. These components include unique characteristics of the test method, critical procedural details, and quality control measures. Adherence to essential test method components is necessary when the acceptability of a proposed test method is being evaluated based on performance standards derived from a mechanistically and functionally similar validated test method. [Note: Previously referred to as "minimum procedural standards" (6).]

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

False positive rate: The proportion of all negative substances that are falsely identified by a test method as positive (see "two-by-two" table). It is one indicator of test method accuracy.

False negative: A substance incorrectly identified as negative by a test method.

False negative rate: The proportion of all positive substances falsely identified by a test method as negative (see "two-by-two" table). It is one indicator of test method accuracy.

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[1]NIEHS. 1997. Validation and regulatory acceptance of toxicological methods: A report of the *ad hoc* Interagency Coordinating Committee on the Validation of Alternative Methods. NIH Publication No. 97-3981. NIEHS, Research Triangle Park, NC.

Good Laboratory Practices (GLPs): Regulations promulgated by the U.S. Food and Drug Administration (FDA), and the U.S. Environmental Protection Agency (EPA), and principles and procedures adopted by the Organisation for Economic Co-operation and Development (OECD) and Japanese authorities that describe record keeping and quality assurance procedures for laboratory records that will be the basis for data submissions to national regulatory agencies.

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

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 method can be transferred successfully among laboratories.

Intralaboratory 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.

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 and the biological effects of interest.

Performance: The accuracy and reliability characteristics of a test method (see "accuracy", "reliability").

Performance standards: Standards, based on a validated test method, that provide a basis for evaluating the comparability of a proposed test method that is mechanistically and functionally similar. Included are (1) essential test method components; (2) a minimum list of reference chemicals selected from among the chemicals used to demonstrate the acceptable performance of the validated test method; and (3) the comparable levels of accuracy and reliability, based on what was obtained for the validated test method, that the proposed test method should demonstrate when evaluated using the minimum list of reference chemicals.

Prediction model: A formula or algorithm used to convert the results obtained using a test method into a prediction of the toxic effect of interest. A prediction model contains four elements: (1) a definition of the specific purpose(s) for which the test method is to be used, (2) specifications of all possible results that may be obtained, (3) an algorithm that converts each study result into a prediction of the toxic effect of interest, and (4) specifications as to the accuracy of the prediction.

Predictivity (negative): The proportion of correct negative responses among substances testing negative by a test method (see "two-by-two" table). It is one indicator of test method accuracy. Negative predictivity is a function of the sensitivity of the test method and the prevalence of negatives among the substances tested.

Predictivity (positive): The proportion of correct positive responses among materials testing positive by a test method (see "two-by-two" table). It is one indicator of test method accuracy.

Positive predictivity is a function of the sensitivity of the test method and the prevalence of positives among the substances tested.

Prevalence: The proportion of positive or negative substances in the population of substances tested (see "two-by-two" table).

Prevalidation: The process during which a standardized test method protocol is developed and evaluated for use in validation studies. Based on the outcome of those studies, the test method protocol may be modified or optimized to increase intra- and/or inter-laboratory reproducibility for use in further validation studies.

Proprietary test method: A test method for which manufacture and distribution is restricted by patents, copyrights, trademarks, etc.

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

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

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

Reference chemicals: Chemicals selected for use during the research, development, prevalidation, and validation of a proposed test method because their response in the *in vivo* reference test method or the species of interest is known (see "reference test method"). Reference chemicals should represent the classes of chemicals for which the proposed test method is expected to be used and cover the range of expected responses (negative, weak to strong positive). Different sets of reference chemicals are likely to be required for the various stages of validation.

After a proposed test method has been recommended or accepted as valid for its intended purpose (i.e., has been recommended as a validated test method to Federal agencies), a representative subset of chemicals used during the validation process may be selected to validate a mechanistically and functionally similar test method. To the extent possible, this subset of reference chemicals should:

- Be representative of the range of responses that the validated test method is capable of measuring or predicting
- Have produced consistent results in the validated test method and in the reference test method and/or the species of interest
- Reflect the accuracy of the validated test method
- Have well-defined chemical structures
- Be readily available
- Not be associated with excessive hazard or prohibitive disposal costs

This list of reference chemicals would represent the minimum number of chemicals that should be used to evaluate the performance of a proposed, mechanistically and functionally similar test method with established performance standards. If any of the recommended chemicals are unavailable, other chemicals for which adequate reference data are available could be substituted. If desired, additional chemicals representing other chemical or product classes and for which adequate reference data are available can be used to more comprehensively evaluate the accuracy of the proposed test method.

Reference species: The species used in the reference (or traditional) test method to which a new or modified 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 test method: The accepted *in vivo* test method used for regulatory purposes to evaluate the potential of a test substance to be hazardous to the species of interest.

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

Relevance: The extent to which a test method correctly predicts or measures the biological effect of interest in humans or another species of interest. Relevance incorporates consideration of the "accuracy" or "concordance" of a test method.

Reliability: A measure of the degree to which a test method can be performed reproducibly within and among laboratories over time. It is assessed by calculating intra- and inter-laboratory reproducibility and intralaboratory repeatability.

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

Reproducibility: The consistency of individual 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 exposure to an agent will cause an adverse effect in the species of interest.

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 generally are 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 classified correctly as positive in a test method. It is a measure of test method accuracy (see "two-by-two" table).

Specificity: The proportion of all negative chemicals that are classified correctly as negative in a test method. It is a measure of test method accuracy (see "two-by-two" table).

Standard operating procedures (SOPs): Formal, written procedures that describe how specific laboratory operations are to be performed. These are required by GLP guidelines.

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

Test: The experimental system used; used interchangeably with "test method" and "assay".

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

Test method nomination: Test methods proposed to ICCVAM for review and evaluation for which a complete test method submission is not available. Four examples are (1) test methods for which adequate validation studies presumably have been completed but lack a complete submission package; (2) test methods that appear promising based on limited prevalidation or validation data and are proposed for additional validation studies; (3) test methods that have been developed and are proposed for prevalidation or validation studies; and (4) test methods that are recommended for a workshop or other activity.

Test method nominator: The organization or individual that submits a test method nomination to ICCVAM for consideration.

Test method sponsor: The organization or individual that puts forward a test method submission to ICCVAM for consideration.

Test method submission: A test method proposed to ICCVAM for consideration for which adequate validation studies have been completed to characterize the usefulness and limitations of the test method for a specific proposed regulatory testing requirement or application, and adequate documentation of the scientific validity has been prepared in accordance with ICCVAM test method submission guidelines.

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

Two-by-two table: The two-by-two table can be used for calculating 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]).

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

Validated test method: An accepted test method for which validation studies have been completed to determine the accuracy and reliability of this method for a specific proposed use.

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

APPENDIX D: ICCVAM VALIDATION AND REGULATORY ACCEPTANCE CRITERIA[1]

Validation Criteria

For a new or modified test method to be considered validated for regulatory risk assessment purposes, it generally should 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 test method given its purpose and the supporting database. Because test methods 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.

- 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 method's 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 method 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 method within and among laboratories must have been demonstrated. Data must be provided describing the level of intra- and inter-laboratory reproducibility and how it varies over time. The degree to which biological variability affects this test reproducibility should be addressed.

- The test method's 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, including 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

[1]NIEHS. 1997. Validation and regulatory acceptance of toxicological methods: A report of the *ad hoc* Interagency Coordinating Committee on the Validation of Alternative Methods. NIH Publication No. 97-3981. NIEHS, Research Triangle Park, NC.

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 nonanimal 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 impacts.

- 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.

Regulatory Acceptance Criteria

Validated test 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 attributes listed below:

- The test method should have undergone independent scientific peer review by disinterested persons who are experts in the field, knowledgeable of the test method, and financially (and otherwise) unencumbered by the outcome of the evaluation.

- There should be a detailed test method protocol with standard operating procedures (SOPs), a list of operating characteristics, and criteria for judging test performance and results.

- Data generated by the test method should adequately measure or predict the endpoint of interest and demonstrate a linkage between the new test method and an existing test method or between the new test method and effects in the target species.

- There should be adequate test method data for chemicals and products representative of those administered by the regulatory program or agency and for which the test is proposed.

- The test method should generate data useful for risk assessment purposes (i.e., for hazard identification, dose-response assessment, or exposure assessment). Such test methods may be useful alone or as part of a battery or tiered approach.

- The specific strengths and limitations of the test method 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 test method should be time and cost effective.

- The test method should be one that can be harmonized with similar testing requirements of other agencies and international groups.

- The test method should be suitable for international acceptance.

- The test method must provide adequate consideration for the reduction, refinement, and replacement of animal use.

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APPENDIX E: ICCVAM Authorization Act (Public Law 106-545)

Public Law 106–545
106th Congress

An Act

To establish, wherever feasible, guidelines, recommendations, and regulations that promote the regulatory acceptance of new or revised scientifically valid toxicological tests that protect human and animal health and the environment while reducing, refining, or replacing animal tests and ensuring human safety and product effectiveness.

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled,

SECTION 1. SHORT TITLE.

This Act may be cited as the "ICCVAM Authorization Act of 2000".

SEC. 2. DEFINITIONS.

In this Act:

(1) ALTERNATIVE TEST METHOD.—The term "alternative test method" means a test method that—

(A) includes any new or revised test method; and

(B)(i) reduces the number of animals required;

(ii) refines procedures to lessen or eliminate pain or distress to animals, or enhances animal well-being; or

(iii) replaces animals with non-animal systems or one animal species with a phylogenetically lower animal species, such as replacing a mammal with an invertebrate.

(2) ICCVAM TEST RECOMMENDATION.—The term "ICCVAM test recommendation" means a summary report prepared by the ICCVAM characterizing the results of a scientific expert peer review of a test method.

SEC. 3. INTERAGENCY COORDINATING COMMITTEE ON THE VALIDATION OF ALTERNATIVE METHODS.

(a) IN GENERAL.—With respect to the interagency coordinating committee that is known as the Interagency Coordinating Committee on the Validation of Alternative Methods (referred to in this Act as "ICCVAM") and that was established by the Director of the National Institute of Environmental Health Sciences for purposes of section 463A(b) of the Public Health Service Act, the Director of the Institute shall designate such committee as a permanent interagency coordinating committee of the Institute under the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods. This Act may not be construed as affecting the authorities of such Director regarding ICCVAM that were in effect on the day before the date of the enactment of this Act, except to the extent inconsistent with this Act.

(b) PURPOSES.—The purposes of the ICCVAM shall be to—

(1) increase the efficiency and effectiveness of Federal agency test method review;

(2) eliminate unnecessary duplicative efforts and share experiences between Federal regulatory agencies;

(3) optimize utilization of scientific expertise outside the Federal Government;

(4) ensure that new and revised test methods are validated to meet the needs of Federal agencies; and

(5) reduce, refine, or replace the use of animals in testing, where feasible.

(c) COMPOSITION.—The ICCVAM shall be composed of the heads of the following Federal agencies (or their designees):

(1) Agency for Toxic Substances and Disease Registry.
(2) Consumer Product Safety Commission.
(3) Department of Agriculture.
(4) Department of Defense.
(5) Department of Energy.
(6) Department of the Interior.
(7) Department of Transportation.
(8) Environmental Protection Agency.
(9) Food and Drug Administration.
(10) National Institute for Occupational Safety and Health.
(11) National Institutes of Health.
(12) National Cancer Institute.
(13) National Institute of Environmental Health Sciences.
(14) National Library of Medicine.
(15) Occupational Safety and Health Administration.
(16) Any other agency that develops, or employs tests or test data using animals, or regulates on the basis of the use of animals in toxicity testing.

(d) SCIENTIFIC ADVISORY COMMITTEE.—

(1) ESTABLISHMENT.—The Director of the National Institute of Environmental Health Sciences shall establish a Scientific Advisory Committee (referred to in this Act as the "SAC") to advise ICCVAM and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods regarding ICCVAM activities. The activities of the SAC shall be subject to provisions of the Federal Advisory Committee Act.

(2) MEMBERSHIP.—

(A) IN GENERAL.—The SAC shall be composed of the following voting members:

(i) At least one knowledgeable representative having a history of expertise, development, or evaluation of new or revised or alternative test methods from each of—

(I) the personal care, pharmaceutical, industrial chemicals, or agriculture industry;

(II) any other industry that is regulated by the Federal agencies specified in subsection (c); and

(III) a national animal protection organization established under section 501(c)(3) of the Internal Revenue Code of 1986.

(ii) Representatives (selected by the Director of the National Institute of Environmental Health Sciences) from an academic institution, a State government agency, an international regulatory body, or any corporation developing or marketing new or revised or alternative test methodologies, including contract laboratories.

(B) NONVOTING EX OFFICIO MEMBERS.—The membership of the SAC shall, in addition to voting members under subparagraph (A), include as nonvoting ex officio members the agency heads specified in subsection (c) (or their designees).

(e) DUTIES.—The ICCVAM shall, consistent with the purposes described in subsection (b), carry out the following functions:

(1) Review and evaluate new or revised or alternative test methods, including batteries of tests and test screens, that may be acceptable for specific regulatory uses, including the coordination of technical reviews of proposed new or revised or alternative test methods of interagency interest.

(2) Facilitate appropriate interagency and international harmonization of acute or chronic toxicological test protocols that encourage the reduction, refinement, or replacement of animal test methods.

(3) Facilitate and provide guidance on the development of validation criteria, validation studies and processes for new or revised or alternative test methods and help facilitate the acceptance of such scientifically valid test methods and awareness
of accepted test methods by Federal agencies and other stakeholders.

(4) Submit ICCVAM test recommendations for the test method reviewed by the ICCVAM, through expeditious transmittal by the Secretary of Health and Human Services (or the designee of the Secretary), to each appropriate Federal agency, along with the identification of specific agency guidelines, recommendations, or regulations for a test method, including batteries of tests and test screens, for chemicals or class of chemicals within a regulatory framework that may be appropriate for scientific improvement, while seeking to reduce, refine, or replace animal test methods.

(5) Consider for review and evaluation, petitions received from the public that—

(A) identify a specific regulation, recommendation, or guideline regarding a regulatory mandate; and

(B) recommend new or revised or alternative test methods and provide valid scientific evidence of the potential of the test method.

(6) Make available to the public final ICCVAM test recommendations to appropriate Federal agencies and the responses from the agencies regarding such recommendations.

(7) Prepare reports to be made available to the public on its progress under this Act. The first report shall be completed not later than 12 months after the date of the enactment of this Act, and subsequent reports shall be completed biennially thereafter.

SEC. 4. FEDERAL AGENCY ACTION.

(a) IDENTIFICATION OF TESTS.—With respect to each Federal agency carrying out a program that requires or recommends acute or chronic toxicological testing, such agency shall, not later than 180 days after receiving an ICCVAM test recommendation, identify and forward to the ICCVAM any relevant test method specified in a regulation or industry-wide guideline which specifically, or in practice requires, recommends, or encourages the use of an animal acute or chronic toxicological test method for which the ICCVAM test recommendation may be added or substituted.

(b) ALTERNATIVES.—Each Federal agency carrying out a program described in subsection (a) shall promote and encourage the development and use of alternatives to animal test methods (including batteries of tests and test screens), where appropriate, for the purpose of complying with Federal statutes, regulations, guidelines, or recommendations (in each instance, and for each chemical class) if such test methods are found to be effective for generating data, in an amount and of a scientific value that is at least equivalent to the data generated from existing tests, for hazard identification, dose-response assessment, or risk assessment purposes.

(c) TEST METHOD VALIDATION.—Each Federal agency carrying out a program described in subsection (a) shall ensure that any new or revised acute or chronic toxicity test method, including animal test methods and alternatives, is determined to be valid for its proposed use prior to requiring, recommending, or encouraging the application of such test method.

(d) REVIEW.—Not later than 180 days after receipt of an ICCVAM test recommendation, a Federal agency carrying out a program described in subsection (a) shall review such recommendation and notify the ICCVAM in writing of its findings.

(e) RECOMMENDATION ADOPTION.—Each Federal agency carrying out a program described in subsection (a), or its specific regulatory unit or units, shall adopt the ICCVAM test recommendation unless such Federal agency determines that—

(1) the ICCVAM test recommendation is not adequate in terms of biological relevance for the regulatory goal authorized by that agency, or mandated by Congress;

(2) the ICCVAM test recommendation does not generate data, in an amount and of a scientific value that is at least equivalent to the data generated prior to such recommendation, for the appropriate hazard identification, dose-response assessment, or risk assessment purposes as the current test method recommended or required by that agency;

(3) the agency does not employ, recommend, or require testing for that class of chemical or for the recommended test endpoint; or

(4) the ICCVAM test recommendation is unacceptable for satisfactorily fulfilling the test needs for that particular agency and its respective congressional mandate.

SEC. 5. APPLICATION.

(a) APPLICATION.—This Act shall not apply to research, including research performed using biotechnology techniques, or research related to the causes, diagnosis, treatment, control, or prevention of physical or mental diseases or impairments of humans or animals.

(b) USE OF TEST METHODS.—Nothing in this Act shall prevent a Federal agency from retaining final authority for incorporating the test methods recommended by the ICCVAM in the manner determined to be appropriate by such Federal agency or regulatory body.

(c) LIMITATION.—Nothing in this Act shall be construed to require a manufacturer that is currently not required to perform animal testing to perform such tests. Nothing in this Act shall be construed to require a manufacturer to perform redundant endpoint specific testing.

(d) SUBMISSION OF TESTS AND DATA.—Nothing in this Act precludes a party from submitting a test method or scientific data directly to a Federal agency for use in a regulatory program.

Approved December 19, 2000.