UNITED STATES ICCVAM Advancing Alternatives to Animal Testing

Interagency Coordinating Committee on the Validation of Alternative Methods

Measuring U.S. Federal Agency Progress Toward Implementation of Alternative Methods in Toxicity Testing

Metrics Workgroup
Interagency Coordinating Committee on the Validation of Alternative Methods

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Summary

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) was formally established in 2000 by the ICCVAM Authorization Act (42 U.S.C. 285l-3) to increase the efficiency and effectiveness of U.S. federal agency efforts to replace, reduce, and refine the use of animals for toxicity testing (referred to as the "3Rs"). ICCVAM is composed of representatives from 17 U.S. federal regulatory and research agencies that require or use toxicological testing information.

In 2018, ICCVAM published its "Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States,"[1] which noted that "in order to assess the impact of this national strategy, effective metrics need to be created to track progress and identify objective criteria for measuring success without creating additional regulatory burden."

In 2019, the U.S. Government Accountability Office (GAO) published a report on Animal Use in Research,[2] which found that federal agencies actively promote the use of alternative methods in a variety of ways, including modifying regulations and policies, incorporating new methods into guidances, providing training on the use of alternative methods, and developing new strategic plans to minimize the use of animals. However, the GAO also noted that ICCVAM and its member agencies have not routinely developed or reported metrics that demonstrate how their efforts to encourage the use of alternative methods affect animal use recommended. Consequently, the GAO recommended that ICCVAM establish a workgroup to propose metrics to help the committee and its member agencies better monitor their progress across the range of their efforts to reduce animal use and report members' progress to the public.

The ICCVAM Metrics Workgroup found that no one set of metrics can be used by all ICCVAM member agencies. The workgroup instead recommends that each agency develop its own metrics that are relevant and practical to their unique situation. This document describes the recommendations of the ICCVAM Metrics Workgroup along with references and other materials that can be used to follow federal agency progress in promoting the use of alternative toxicological methods.

Introduction

Animals are currently used to test the potentially harmful effects of drugs, chemicals, and other products. This practice, known as *toxicity testing*, is done to protect human and environmental health. Scientists also use animals to study basic biological processes (e.g., biochemistry, physiology, behavior), or to develop therapies for diseases. This practice is often referred to simply as *research*. It is noteworthy that the definition of "animals" varies among federal agencies, but extends beyond rats, mice, and other commonly used vertebrate models.

Humane animal care in these contexts is ensured by existing animal welfare regulations and policies. These include, but are not limited to:

- The U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training ("U.S. Government Principles").[3]
- The Animal Welfare Act.[4]
- The Public Health Service Policy on Humane Care and Use of Laboratory Animals[5] as mandated by the Health Research Extension Act of 1985.[6]

Regulated industries can and do take steps to replace, reduce, or refine animal use in the early stages of product development (e.g., discovery, candidate selection, biomaterial screening). Regulatory agencies encourage such activities, but generally do not play a role in this stage of product development and thus cannot measure the impact of alternatives in this area.

Humane animal care entails minimizing pain and distress while meeting testing needs. The Animal Welfare Act, through the Animal Welfare Regulations,[7] requires principal investigators to consider alternatives to procedures that cause greater than momentary or slight pain or distress. Similarly, the Guide for the Care and Use of Laboratory Animals,[8] the Guide for the Care and Use of Agricultural Animals in Research and Teaching,[9] and the U.S. Government Principles recommend the use of alternatives to animal testing when appropriate.

Alternatives to animal testing encompass replacement, reduction, or refinement of animal use, collectively known as the 3Rs principles. Replacement substitutes the live animal (*in vivo*) with nonanimal models, such as computer simulations (*in silico*) and cell cultures (*in vitro*), or with a phylogenetically lower animal species. Reduction entails reducing the number of animals required for testing to the minimum needed to achieve testing objectives. Refinement includes modifications that alleviate pain or distress, such as enhanced methods of pain identification, appropriate anesthesia and analgesia, or earlier endpoints for euthanasia. The 3Rs principles are not mutually exclusive, but rather mutually reinforcing. For example, refinement can also be achieved by reducing the numbers of animals needed in a test. Similarly, reduction can be accomplished by replacing some or all elements of a test with nonanimal methods. Appropriate experimental design and rigor are essential to the fulfillment of the 3Rs principles as applied to animal use for toxicity testing.

In vitro, *in silico*, and *in chemico* (chemical reactivity methods that do not use living cells) nonanimal procedures used to replace or reduce animal use are collectively referred to as new approach methodologies (NAMs). NAMs include approaches such as bioinformatics, high-throughput screening, and systems biology technologies. Bioinformatics is an interdisciplinary field that uses biology, computer science, mathematics, and statistics to analyze biological data sets, which are often large and complex. High-throughput screening uses robotics, data processing software, liquid handling devices, and sensitive detectors to conduct chemical, genetic, or pharmacological assays. These assays can rapidly identify active compounds, antibodies, or genes that modulate specific biomolecular pathways. Systems biology is the study of complex biological systems using techniques such as 'omics' technologies, computational and mathematical analysis, and modeling technologies. 'Omics' technologies collectively characterize and quantify pools of biological molecules, such as proteins or nucleic acids, to describe the structure, function, and dynamics of an organism.

While *in vitro* and *in silico* testing approaches can characterize or predict specific aspects of toxicity, few alternatives are currently capable of completely replacing an animal test directly on a one-to-one basis. This is especially true for animal tests with repeated dosing, implantation (e.g., medical devices, where biological response may be a result of device function as well as chemistry), and multiple endpoints. All the highly complex interactions that occur *in vivo*, and many effects that only emerge after chronic dosing, cannot be evaluated by *in vitro* systems at this stage.

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

ICCVAM is a permanent committee of the National Institute of Environmental Health Sciences (NIEHS). ICCVAM receives scientific and administrative support from the U.S. National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). NICEATM is part of the Division of the National Toxicology Program within NIEHS.

Formally established in 2000 by the ICCVAM Authorization Act (42 U.S.C. 285l-3), ICCVAM is composed of representatives from 17 U.S. federal regulatory and research agencies. Each of these agencies requires, uses, generates, or disseminates toxicological and safety information. The committee promotes testing methods that protect human health and the environment while reducing animal use.

The ICCVAM Authorization Act outlines the following goals:

- Increase the efficiency and effectiveness of U.S. federal agency toxicity test method review.
- Eliminate unnecessary duplication of effort and share experience among U.S. federal regulatory agencies.
- Optimize utilization of scientific expertise outside the U.S. federal government.
- Ensure validation of new and revised test methods to meet the needs of U.S. federal agencies.
- Replace, reduce, or refine the use of animals in toxicity testing where feasible.

ICCVAM Member Agencies

- Agency for Toxic Substances and Disease Registry
- National Cancer Institute
- National Institute for Occupational Safety and Health
- National Institute of Environmental Health Sciences
- National Institute of Standards and Technology
- National Institutes of Health
- National Library of Medicine
- Occupational Safety and Health Administration
- U.S. Consumer Product Safety Commission
- U.S. Department of Agriculture
- U.S. Department of Defense
- U.S. Department of Energy
- U.S. Department of the Interior
- U.S. Department of Transportation
- U.S. Department of Veterans Affairs Office of Research and Development
- U.S. Environmental Protection Agency
- U.S. Food and Drug Administration

ICCVAM: Authority and Scope of Work

The ICCVAM Authorization Act limits the scope of ICCVAM activities to those related to alternatives for *toxicity testing* and specifically states that its activities "do 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." As such, animal use for research purposes is outside ICCVAM's scope and authority.

Validating New Alternatives

A federal agency may develop and/or validate an alternative to a regulatory toxicology testing regimen. The validated method can be submitted to ICCVAM for evaluation (https://ntp.niehs.nih.gov/whatwestudy/niceatm/resources-for-test-method-developers/submissions) and may be recommended to member agencies for use. These evaluations, as well as responses from member agencies, are posted on the NICEATM website as accepted alternative methods (https://ntp.niehs.nih.gov/whatwestudy/niceatm/accept-methods). Since an alternative may not be appropriate in all situations, each evaluation includes the regulatory acceptance/endorsement and applicable regulations for the methods used. For example, some methods may allow selection of a level of exposure to a chemical in the environment below which there is no significant risk to the general population; however, this may not be sufficient for some regulated products, such as to determine an acceptable dose of a human pharmaceutical to administer to patients or to identify the long-term effects that might occur. Regulated industries are responsible for determining which testing (*in vitro*, *in silico*, *in chemico*, or the original *in vivo* testing) is appropriate for its specific situation and will meet the relevant statutory requirements.

In vitro and *in silico* testing approaches can characterize or predict specific aspects of toxicity, and have been successfully applied to predicting toxicity for acute endpoints such as eye irritation or skin sensitization. However, few alternatives are currently capable of completely replacing an animal test directly on a one-to-one basis. This is especially true for animal tests that include repeated dosing, implantation (e.g., medical devices, where biological response may be a result of device function as well as chemistry), and multiple endpoints.

GAO Report on Animal Use in Research

In response to a request by Congress, the U.S. Government Accountability Office (GAO) investigated and reported (https://www.gao.gov/assets/710/701635.pdf) on how federal agencies ensure that nonanimal toxicity testing methods are being considered and used. The GAO found that federal agencies actively promote and encourage the use of alternative methods in a variety of ways, including modifying regulations and policies, incorporating new methods into guidance documents, training on the use of alternative methods, and developing new strategic plans to minimize the use of animals. However, the GAO also found that federal agencies have not routinely developed or reported metrics that demonstrate progress and success of their efforts to encourage the use of alternative methods to reduce overall animal use. As a result, the GAO recommended to Congress that federal agencies establish a workgroup through ICCVAM to propose metrics for assessing the progress on the development and promotion of alternative methods. The GAO further recommended that agencies include those metrics in the ICCVAM Biennial Report. Importantly, the GAO report did not differentiate between *research* and *toxicity testing*, instead using the term *research* to describe both activities.

U.S. federal agencies encourage the use of alternatives to animal testing, but do not have the authority to ban the use of animal test methods. In some cases, such a mandate would only result in additional testing, such as when the drug or chemical being tested will be marketed in other countries that require animal testing or when the animal testing was already done for previous products. In these instances, the company would have to perform the animal testing in the countries that still require it, in addition to the nonanimal testing required in the U.S. Because U.S. agencies ask for all available data, the companies would still send the animal data to the U.S. agency.

ICCVAM Metrics Workgroup

ICCVAM formed a Metrics Workgroup (MWG) to provide guidance that will assist federal agencies in assessing and monitoring progress toward the implementation of alternatives to live animal use in toxicity testing. The workgroup members are from federal agencies that conduct toxicity testing or use information from animal tests for making decisions about the toxicity of drugs, chemicals, and other products. As the ICCVAM Authorization Act limits the scope of ICCVAM activities to those related to alternatives to toxicity testing, the MWG has not attempted to address development of metrics for use of animals or alternatives in research activities. Thus, the charge to the MWG, based on the GAO recommendation, is to "develop metrics that the agencies could use to assess the progress they have individually or collectively made toward reducing, refining, or replacing animal use in [toxicity] testing." These metrics are provided as recommendations from ICCVAM to federal agencies, as ICCVAM does not have legal authority to direct agencies to develop or report metrics.

The MWG determined that it would not be practical or appropriate to define a single metric or set of metrics to encompass the breadth of activities of all agencies represented in ICCVAM. The myriad of regulatory authorities each federal agency operates under creates different mandates and provides for varying access to information on animal use. As a result, various agencies are not able to use common metrics to track progress. The MWG recommends that each agency should develop its own metrics that are relevant and practical to the specific activities of the agency. The MWG is also aware that sponsoring organizations may take steps to replace, reduce, or refine animal use in the early stages of product development (e.g., discovery phase, candidate selection, and biomaterial screening), but regulatory agencies generally do not play a role in this stage of industry product development and so cannot measure the impact of alternatives in this area. The MWG recommends the use of both quantitative and/or qualitative metrics (described below in Agency Metrics) to assess progress when relevant and practical.

The MWG found that no one set of metrics can be used by all ICCVAM member agencies. The MWG instead recommends that each agency develop its own metrics that are relevant and practical to their unique situation.

Agency Metrics

The MWG recommends that agencies use quantitative and qualitative metrics separately or together to assess progress in implementing alternatives in toxicity testing.

Quantitative Metrics

Quantitative metrics include counting entities or actions, such as the number of animals used in toxicity testing or number of educational opportunities (e.g., training, webinars, and publications) provided. The U.S. Environmental Protection Agency webpage *Adopting 21st-Century Science Methodologies—Metrics* for pesticides (listed below under Resources and Information) provides examples of how reductions of animal use can be quantitatively characterized. Animal numbers can also be obtained from ordering records and annual reports that are submitted to the U.S. Department of Agriculture (USDA) as required for Animal Welfare Act-regulated activities and species. However, counting animals may not be the best approach to assessing progress in implementation of alternatives, and in some cases could be misleading. For example, raw numbers may not provide a complete picture of the extent to which an alternative is being implemented. Furthermore, activity of a given laboratory may vary (e.g., number of compounds and types of tests) from year to year. Annual reports provided to the USDA only include the number of animals used, and do not describe the reason for their use (e.g., research vs. toxicity testing, the compounds being studied, U.S. vs. foreign regulatory requirements). For these reasons, comparing the total number of animals used from year to year may not correlate to the progress in the implementation of alternatives.

Qualitative Metrics

Qualitative metrics include the development or implementation of alternatives, or the provision of educational opportunities that raise awareness regarding alternatives. Educational opportunities include training, publications, and presentations given by agency scientists. These activities raise awareness of the availability and appropriate use of alternatives. While not a direct measure of how often an alternative test method is used, they document the efforts toward increasing implementation of an alternative. Other examples of qualitative metrics could include published guidance and agency strategic plans.

Conclusion

The MWG encourages ICCVAM member agencies to develop their own tailored metrics that demonstrate progress in replacing, reducing, and refining animal use in toxicity testing. Federal agencies are uniformly proud of their progress in this area. However, agency representatives agree that more could be done to inform stakeholders of progress being made.

The MWG found that no one set of metrics can be used by all ICCVAM member agencies. The MWG instead recommends that each agency develop its own metrics that are relevant and practical to their unique situation. Further, the MWG encourages that the metrics used by each federal agency be communicated to the public through both the NICEATM website and, if available, the websites of individual federal agencies.

Resources and Information on Federal Agency Efforts to Reduce Animal Use

The resources below provide examples of how ICCVAM and its member agencies report progress toward reducing animal use in testing.

ICCVAM Agency Activities to Promote Alternatives and Measure Success

In coordination with the MWG, ICCVAM agencies are developing webpages to inform their stakeholders about progress on adoption of alternatives and reduction of animal use. A list of those efforts and links to agency-specific information on metrics will be made available and maintained at https://ntp.niehs.nih.gov/whatwestudy/niceatm/iccvam/metric.

Examples include:

- EPA New Approach Methods: Efforts to Reduce Use of Animals in Chemical Testing (U.S. Environmental Protection Agency)
  Provides news about EPA activities to develop and use NAMs, as well as materials from EPA's annual conference on the State of the Science on Development and Use of NAMs for Chemical Safety Testing.
- Adopting 21st Century Science Methodologies – Metrics (Office of Pesticide Programs, U.S. Environmental Protection Agency)
  Describes metrics to assess the progress made toward reducing, refining, and replacing animal use in testing required by EPA for pesticide registration.
- Advancing Alternative Methods at FDA (U.S. Food and Drug Administration)
  Describes activities of the FDA's Alternative Methods Working Group.
- Assessing Contaminant Hazards Without a Critter – Advancements in Alternatives to Animal Toxicity Testing (U.S. Geological Survey, U.S. Department of the Interior)
  Describes approaches taken by the U.S. Geological Survey (USGS) to replace, reduce, and refine animal use for ecotoxicity testing.
- USGS Ecosystems Mission Area: Animal Welfare Assurance (U.S. Geological Survey, U.S. Department of the Interior)
  Provides resources for Institutional Animal Care and Use Committees supporting the USGS Ecosystems Mission Area.
- Animal Welfare Information Center (National Agricultural Library, U.S. Department of Agriculture)
  Provides information for improved animal care and use in research, testing, and teaching.
- Recommended Procedures Regarding the CPSC's Policy on Animal Testing (U.S. Consumer Product Safety Commission)
  Describes testing required under the Federal Hazardous Substances Act and acceptable alternatives to traditional animal tests.

Resources Provided by NICEATM and ICCVAM

ICCVAM Annual and Biennial Reports

These reports highlight ICCVAM member agency activities supporting toxicology innovation, as well as regulatory agency initiatives to promote the 3Rs and to provide information about the use of new approach methodologies. The most recent report describes ICCVAM, ICCVAM agency, and NICEATM accomplishments from 2018-2019, including:

- Publication of A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States.
- Development of the Collaborative Acute Toxicity Modeling Suite, *in silico* models of acute oral systemic toxicity that predict five specific endpoints needed by regulatory agencies.
- Expansion of the Integrated Chemical Environment, an online resource providing curated data and tools to facilitate the safety assessment of chemicals.
- Development of plans to replace, reduce, or refine animal use for testing by the U.S. Department of Defense, EPA, and FDA.

ICCVAM Agency Activities: News Published Since Last Biennial Report

Contains announcements of ICCVAM agency activities occurring since the last ICCVAM Biennial Report. Announcements are distributed via NICEATM News, an email list that distributes announcements of interest to those developing alternatives to animal use for chemical safety testing. Subscribe to NICEATM News.

Strategic Roadmap: Implementation

Provides details of progress and planned activities for implementation of the Strategic Roadmap in the following areas:

- Acute Systemic Toxicity
- Eye and Skin Irritation
- Skin Sensitization

ICCVAM-Recommended Test Method Protocols

Contains links to ICCVAM-recommended protocols for specific test methods and supporting information.

Alternative Methods Accepted by U.S. Agencies

Lists methods for chemical safety testing that are accepted by regulatory authorities as alternatives to required animal tests, as well as guidances to support alternatives to animal use. This page links to the European Union's Tracking System for Alternative Methods (TSAR) resource, which tracks progress of an alternative method from submission for validation through to its final adoption by inclusion into the regulatory framework. TSAR includes methods evaluated by ICCVAM, and ICCVAM interacts with the European Union and other international test method evaluation organizations through the International Cooperation on Alternative Test Methods.

Testing Regulations and Guidelines

Lists regulations and guidelines relevant to alternative methods implementation.

Regulatory Applications of 3Rs

Provides specific examples of how U.S. federal agencies have applied 3Rs approaches to testing requirements, including acceptance of non-animal methods or guidance on non-testing approaches, such as waivers, that can be used to reduce animal testing.

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Notes

[1] ICCVAM. 2018. A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States. Available: https://ntp.niehs.nih.gov/go/iccvam-rdmp. https://dx.doi.org/10.22427/NTP-ICCVAM-ROADMAP2018.

[2] Animal Use in Research: Federal Agencies Should Assess and Report on Their Efforts to Develop and Promote Alternatives GAO-19-629: Published: Sep 24, 2019. Publicly Released: Sep 24, 2019.

[3] IRAC [Interagency Research Animal Committee]. 1985. U.S. Government Principles for Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training. Federal Register, May 20, 1985. Washington: Office of Science and Technology Policy. Available at https://olaw.nih.gov/policies-laws/gov-principles.htm; accessed October 18, 2020.

[4] The Animal Welfare Act: Title 7, Chapter 54, Sections 2131-2159.

[5] Public Health Service Policy on Humane Care and Use of Laboratory Animals. 2015. Published by the Department of Health and Human Services, and the National Institutes of Health-Office of Laboratory Animal Welfare.

[6] Health Research Extension Act of 1985. Public Law 99-158. November 20, 1985.

[7] Title 9, Chapter 1, Subchapter A, Parts 1-4: Animal Welfare.

[8] Guide for the Care and Use of Laboratory Animals. 8th Edition. Institute of Laboratory Animal Research, The National Academies Press, Washington, D.C. 2011.

[9] Guide for the Care and Use of Agricultural Animals in Research and Teaching. 3rd Edition. American Dairy Science Association, American Society of Animal Science, and Poultry Science Association. 2010.

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