Draft Implementation Plan
for the 2008-2012 NICEATM-ICCVAM Five Year Plan

June 2012

Interagency Coordinating Committee on the
Validation of Alternative Methods

National Toxicology Program Interagency Center for the
Evaluation of Alternative Toxicological Methods

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

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Executive Summary

In 2008, the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) developed and published a five-year plan in conjunction with Federal agency program offices. The plan describes how NICEATM and ICCVAM will foster and promote research, development, translation, validation, and regulatory acceptance of alternative test methods that reduce, refine, and replace the use of animals for safety testing, while maintaining and promoting scientific quality and protecting the health of people, animals, and the environment.

This working document describes how NICEATM and ICCVAM are implementing the strategies outlined in the five-year plan. Implementation activities address the four key challenges in the five-year plan:

1. Conducting and facilitating alternative test method activities in priority areas
2. Identifying and promoting research initiatives that are expected to support the future development of innovative alternative test methods
3. Fostering the acceptance and appropriate use of alternative test methods
4. Developing partnerships and strengthening interactions with ICCVAM stakeholders in order to facilitate meaningful progress

Conducting and Facilitating Alternative Test Method Activities in Priority Areas

ICCVAM priorities emphasize alternatives for those regulatory test methods that can involve significant animal pain and distress and that can involve large numbers of animals. Currently, the four highest-priority testing areas are biologics, ocular toxicity, dermal toxicity, and acute toxicity. NICEATM and ICCVAM will identify critical knowledge and data gaps that must be addressed in order to advance alternative methods for these and other evolving priority areas (e.g., immunotoxicity, reproductive and developmental toxicity, the safety assessment of manufactured nanomaterials). ICCVAM will involve regulatory agencies, the scientific community, and other stakeholders in these activities. ICCVAM will distribute recommendations to stakeholder organizations with resources to carry out the recommended research, development and validation activities. ICCVAM and NICEATM will interact with participating stakeholders throughout the process to help develop methods that are useful for regulatory testing. Upon receiving validation study results, ICCVAM will evaluate the scientific validity of methods for regulatory testing purposes and provide recommendations to regulatory agencies on demonstrated usefulness and limitations.

Identifying and Promoting New Science and Technology

NICEATM and ICCVAM are working with Federal agencies and other stakeholders to link their research and development activities to the standardization and validation of alternative test methods. ICCVAM agencies have been asked to describe any ongoing and planned research, development, translation, and validation activities relevant to test methods that reduce, refine, and replace the use of animals. As part of this implementation plan, the role of ICCVAM working groups will be expanded to include consultation with test method developers to help

ensure that test methods are designed to meet regulatory needs. This consultation will also help optimize validation studies necessary to determine their usefulness and limitations for regulatory decision-making.

ICCVAM has established a Research and Development Working Group (RDWG) to help ICCVAM implement activities relevant to incorporating new science and technology. The RDWG is specifically charged with helping NICEATM and ICCVAM identify and promote research that incorporates new technologies expected to support the future development of new test methods and approaches that will reduce, refine, and replace animal use in toxicity testing. The RDWG will help identify test methods in the development phase that would benefit from referral and interactions with an ICCVAM test method working group.

Fostering Regulatory Acceptance and Appropriate Use of Alternative Methods

Once regulatory authorities have accepted an alternative test method, ICCVAM will promote its use by communicating the outcomes of ICCVAM review activities and/or workshops in the *Federal Register* and peer-reviewed journals and at training courses and national and international scientific meetings. Emphasis will be placed on informing the scientific community, including Institutional Animal Care and Use Committees, of new alternatives that should be considered in order to ensure compliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and Animal Welfare Act regulations, which require consideration of such methods before testing is conducted in animals.

ICCVAM will cosponsor workshops with government and nongovernment organizations, where appropriate. These workshops will 1) evaluate the state of the science related to the development and validation of alternative test methods and 2) identify high-priority research, development, translation, and validation activities necessary to advance and characterize the usefulness of such methods. The workshop results will be broadly communicated to individuals and organizations that conduct these activities.

Developing Partnerships and Strengthening Interactions with ICCVAM Stakeholders

ICCVAM will foster international collaboration by including experts from the international scientific community in workshops that review the state of the science for particular test method areas. Where appropriate, NICEATM and ICCVAM will also invite representatives from international organizations such as the Organisation for Economic Co-operation and Development (OECD) and from OECD member countries to attend and participate in relevant NICEATM and ICCVAM-sponsored workshops, peer reviews, and other scientific activities. Similarly, to further ensure the development of scientifically valid international test guidelines, NICEATM and ICCVAM will encourage participation of their scientists in U.S. delegations to OECD test guideline meetings, expert consultations, and workshops.

Introduction

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is an interagency committee created by the National Institute of Environmental Health Sciences (NIEHS) in 1997 and established as a permanent committee by the ICCVAM Authorization Act of 2000. Administered by NIEHS under the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), ICCVAM is composed of members from 15 Federal agencies. The committee's mission is to facilitate development, validation, and regulatory acceptance of new, revised, and alternative test methods that reduce, refine, and replace the use of animals in testing while maintaining and promoting scientific quality and the protection of human health, animal health, and the environment.

An overall goal is for ICCVAM to assume a greater leadership role in promoting research, development, translation, validation, and regulatory acceptance of alternative test methods. NICEATM and ICCVAM developed a Five-Year Plan that builds on the ICCVAM mission, vision, and strategic priorities to achieve progress and to inform the public of their plans and approaches.[1] To implement this plan, NICEATM and ICCVAM will work with a broad range of stakeholders, including Federal agencies, national and international validation and test guideline organizations, industry, academia, and the animal welfare community. Success will depend on these interactions both within and outside of ICCVAM agencies. ICCVAM will take a proactive leadership role and identify and develop collaborations that will include experienced scientists that can bring state-of-the-art science to the forefront.

ICCVAM, as an interagency committee, does not have resources to conduct research, development, and validation studies. Rather, it depends on its many stakeholders to conduct and achieve successful test method research, development, translation, and validation efforts. Many Federal agencies and other organizations conduct research that could ultimately result in the development and validation of an alternative test method for regulatory use. These test methods can then be evaluated by ICCVAM for potential regulatory use.

ICCVAM's priorities are based on agency priorities[2] as well as other criteria[3] that include:

- The potential impact that alternative test methods may have on reducing, refining, or replacing the use of animals for testing, taking into consideration the severity of pain and distress and numbers of animals involved
- The potential for the proposed test method(s) to better predict adverse health or environmental effects
- The applicability of testing alternatives across agencies

ICCVAM uses these criteria to prioritize test method nominations and submissions for evaluation.

Several Federal agencies are responsible for safeguarding human and animal health and the environment. To assess health and environmental risks, Federal agencies have developed and

[1] http://iccvam.niehs.nih.gov/docs/5yearplan.htm
[2] Testing priorities of individual Federal agencies may differ because of the different statutory mandates under which they operate.
[3] http://iccvam.niehs.nih.gov/SuppDocs/submission.htm

adopted testing methods to evaluate the potential hazards or safety of chemicals, and other products. However, new and revised toxicological test methods are being developed with increasing frequency as scientists seek to incorporate new science and technology. ICCVAM and NICEATM serve a unique role in helping to evaluate the usefulness and limitations of these methods and achieving the acceptance of those found to be scientifically valid for regulatory purposes. This interagency cooperation provides an efficient and effective mechanism for Federal test method review and helps to ensure that new and revised test methods meet the needs of Federal agencies while reducing, refining and replacing the use of animals in testing where scientifically feasible.

In implementing the strategies outlined in the Five-Year Plan, NICEATM and ICCVAM will address four key challenges: 1) conducting and facilitating activities in priority areas, 2) identifying and promoting research initiatives that are expected to support the future development of innovative alternative test methods, 3) fostering the acceptance and appropriate use of alternative test methods through outreach and communication, and 4) developing partnerships and strengthening interactions with ICCVAM stakeholders in order to facilitate meaningful progress. While ICCVAM has accomplished much during its first 10 years, this document focuses on the plans to implement the goals and objectives set forth for the next 5 years.

Challenge #1: Conduct and Facilitate Alternative Test Method Activities In Priority Areas

ICCVAM priorities emphasize alternatives for those regulatory test methods that can use large numbers of animals and that can involve significant animal pain and distress. Currently, the four highest-priority testing areas are biologics, ocular toxicity, dermal toxicity, and acute toxicity. Other priorities include test methods for immunotoxicity, endocrine disruptor effects, pyrogenicity, reproductive and developmental toxicity, chronic toxicity and carcinogenicity, and the safety assessment of manufactured nanomaterials.

NICEATM and ICCVAM will continue to promote research, development, translation, and validation of alternative test methods by identifying critical knowledge and data gaps that need to be addressed in order to advance alternative methods for these and other evolving priority areas. ICCVAM will involve regulatory agencies, the scientific community, and other stakeholders in these activities, and distribute recommendations to stakeholder organizations with resources to carry out the recommended research, development and validation activities. ICCVAM and NICEATM will interact with stakeholders during the R&D process as well as during validation studies to facilitate the development of methods that are useful for regulatory testing purposes. Following receipt of validation studies, ICCVAM will evaluate the scientific validity of methods for regulatory testing purposes and provide recommendations to regulatory agencies on demonstrated usefulness and limitations.

Biologics Testing

Goal

Identify and promote research, development, translation, and validation activities for priority test methods that may further reduce, refine, and replace animals in regulatory testing for biologics.

Specific Objectives

- Recommend how *in vitro* test systems and earlier more humane endpoints can be used to further reduce, refine, and eventually replace animal use for vaccine potency and efficacy testing while ensuring the protection of human and animal health.
- Identify and prioritize future research initiatives necessary to advance development and validation of *in vitro* methods for vaccine potency and efficacy testing.
- Discuss how to promote the collection and submission of *in vitro* and *in vivo* test data in order to advance the development and validation of more predictive *in vitro* test methods and earlier more humane endpoints for vaccine potency testing.

Planned Activities for Implementation

1. Reactivate the ICCVAM Biologics Working Group (BWG)
    - ICCVAM agencies will be asked to nominate new members with expertise specific to vaccine potency (BWG previously constituted specifically to evaluate botulinum neurotoxin potency testing methods).

- Charge the BWG with developing a scientific workshop to 1) evaluate the state of the science for possible alternatives and 2) the use of humane endpoints in *in vivo* potency tests. Goals of the workshop include:
    - Recommend how *in vitro* test systems and earlier more humane endpoints can be used to further reduce, refine, and eventually replace animal use for vaccine potency testing while ensuring the protection of human and animal health.
    - Identify and prioritize future research initiatives necessary to advance development and validation of *in vitro* methods for vaccine potency testing.
    - Discuss how to promote the collection and submission of *in vitro* and *in vivo* toxicity test data to ICCVAM in order to advance the development and validation of more predictive *in vitro* test methods and earlier more humane endpoints for vaccine potency testing.

2008 to 2012 Accomplishments:

- March 2008: NICEATM and ICCVAM published "Report on the ICCVAM-NICEATM/ECVAM Scientific Workshop on Alternative Methods to Refine, Reduce or Replace the Mouse LD_{50} Assay for Botulinum Toxin Testing" which was cosponsored by NICEATM-ICCVAM and the European Centre for the Validation of Alternative Methods (ECVAM). Workshop participants identified methods that could be used in specific circumstances to reduce or refine the use of mice in the current mouse LD_{50} assay for botulinum toxin potency testing and identified additional development and validation efforts necessary for methods that might eventually replace the use of animals. The workshop participants also recommended best practices that could reduce and refine animal use required in the currently used animal test.
    - June 2011: Allergan received FDA approval for a fully *in vitro* cell-based assay to replace the mouse LD_{50} potency assay
- September 2010: *International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Vaccine Safety Testing: State of the Science and Future Directions* convened by NICEATM, ICCVAM, and their partners in the International Cooperation on Alternative Test Methods (ICATM) and reviewed the state of the science of alternative methods that are currently available for this purpose and developed recommendations for priority research needed to further advance alternative methods.
    - December 2011: Proceedings from this workshop (26 manuscripts) published in *Procedia in Vaccinology* (Kulpa-Eddy et al. 2011).
- October 2011: *International Workshop on Alternative Methods for Human and Veterinary Rabies Vaccine Testing: State of the Science and Planning the Way Forward* convened by NICEATM, ICCVAM, and their international partners and reviewed the available methods and approaches to reduce, refine, and replace animals used in rabies vaccine potency testing and developed an implementation strategy to achieve global acceptance and use of these alternatives.
    - Proceedings from this workshop were published in *Biologicals*.

- November 2012: *International Workshop on Alternatives to the Murine Histamine Sensitization (HIST) Test for Acellular Pertussis Vaccines: State of the Science and the Path Forward* convened by NICEATM, ICCVAM, and their international partners and reviewed the available methods and approaches to reduce, refine, and replace animals used in the murine histamine sensitization test and developed an implementation strategy to achieve global acceptance and use of these alternatives.

Planned Activities for Implementation

2. Evaluate *in vitro* Potency Tests for Leptospirosis vaccines developed by the U.S. Department of Agriculture (USDA)

   - Obtain data from an USDA/Michigan State University validation study on an *in vitro* potency test for selected Leptospirosis vaccines.

   - Conduct a formal evaluation of the usefulness and limitations of the test methods once the validation study is complete and submitted to ICCVAM.

2008 to 2012 Accomplishments:

- September 2012: *International Workshop on Alternative Methods for Leptospira Vaccine Potency Testing: State of the Science and the Way Forward* convened by NICEATM, ICCVAM, and their international partners and reviewed the available methods and approaches to reduce, refine, and replace animals used in human and veterinary Leptospira vaccine potency testing and developed an implementation strategy to achieve global acceptance and use of these alternatives.

Ocular Toxicity Testing

Goal

Two important goals in the area of ocular toxicity are to 1) identify and promote research, development, translation, and validation activities for test methods that can partially or fully replace the Draize rabbit eye test for the identification of substances that are potential ocular hazards and 2) implement procedures to avoid pain and distress where animals must still be used.

Specific Objectives

- Identify alternative test methods that can accurately predict the hazards associated with substances that cause reversible eye damage.
- Identify testing batteries that could be used to increase the accuracy for predicting all ocular hazard categories.
- Promote the inclusion of humane endpoints in current *in vivo* ocular toxicity tests.
- Promote the routine use of topical anesthetics and systemic analgesics in current *in vivo* ocular toxicity tests.

Planned Activities for Implementation

1a. Evaluate *in vitro* approaches for assessing the ocular irritation potential of antimicrobial cleaning products (both reversible and irreversible damage).

1b. Assess *in vitro* ocular toxicity test methods proposed for assessing reversible eye damage (vs. irreversible permanent eye damage).

1c. Evaluate testing strategies/batteries using multiple *in vitro* methods.

1d. Review the routine use of topical anesthetics and systemic analgesics for reducing pain and distress.

2a. For the bovine corneal opacity and permeability (BCOP) test method, evaluate relevance and reliability using an alternative corneal holder and using alternative vehicles for the test substance diluents.

2b. For the BCOP test method, evaluate the effect of modifying various test method protocol components (e.g., duration of test substance exposure) on accuracy and/or reliability.

3a. Promote the evaluation of ocular histopathology for its potential to improve test method predictivity.

3b. Create a reference atlas for the histopathology of chemically induced ocular lesions.

3c. Develop a standardized histological scoring system and revised hazard classification decision criteria for *in vivo* and *in vitro* test methods.

3d. Encourage the submission of *in vitro* testing results and histopathology specimens to NICEATM for characterization and to create a database for evaluation.

2008 to 2012 Accomplishments:

- Based on ICCVAM's reviews and recommendations, the bovine corneal opacity and permeability (BCOP) and isolated chicken eye (ICE) test methods can now be used worldwide in place of live animals for hazard identification of most substances that can cause severe and painful eye injuries resulting in temporary or permanent blindness. These are the first scientifically valid alternative methods to gain regulatory acceptance for ocular safety testing that do not use live animals.
- May 2009: Independent Scientific Peer Review Panel Meeting: *Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Strategies*
    - July 2009: Publication of the Peer Review Panel Report: "Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches"
- September 2009: OECD adoption of Test Guideline 437: "Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants" and TG 438: "Isolated Chicken Eye Test Method for Identifying Ocular Corrosives and Severe Irritants"

- September 2010: ICCVAM published four Test Method Evaluation Reports that recommended alternative methods and strategies to reduce animal use and to minimize or avoid unrelieved pain and distress during ocular safety testing:
  - "ICCVAM Test Method Evaluation Report on a Proposed *In Vitro* Testing Strategy for U.S. Environmental Protection Agency Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products"
  - "ICCVAM Test Method Evaluation Report: Recommendations for Routine Use of Topical Anesthetics, Systemic Analgesics, and Humane Endpoints to Avoid or Minimize Pain and Distress in Ocular Safety Testing"
  - "ICCVAM Test Method Evaluation Report: Current Validation Status of *In Vitro* Test Methods Proposed for Identifying Eye Injury Hazard Potential of Chemicals and Products"
  - "ICCVAM Test Method Evaluation Report: Recommendation to Discontinue Use of the Low Volume Eye Test for Ocular Safety"
- 2011: Federal agencies accepted or endorsed the following ICCVAM recommendations:
  - Pain management procedures that should always be used to avoid or minimize unrelieved pain and distress when *in vitro* methods do not provide sufficient eye safety information and it is necessary to use animals to meet regulatory safety testing requirements. These procedures include the routine use of topical anesthetics, systemic analgesics, and earlier humane endpoints.
  - An *in vitro* Cytosensor microphysiometer (CM) test method that can be used as a screening test to identify some types of substances that may cause permanent or severe eye injuries and to determine if some types of substances will not cause sufficient injury to require hazard labeling for eye irritation. The CM test method is the first accepted *in vitro* test method that can be used instead of animals to identify substances that do not require eye hazard labeling.
  - Four *in vitro* test methods for identifying substances with the potential to cause nonsevere eye injuries, and recommended studies to further characterize their usefulness and limitations.
  - The current validation status and additional studies for a non-animal *in vitro* testing strategy proposed to assess the eye irritation potential of antimicrobial cleaning products using the bovine corneal opacity and permeability (BCOP), CM, and EpiOcular™ (MatTek) test methods. The recommended studies will provide data necessary to support evaluation of the usefulness and limitations of the proposed testing strategy.
  - The validation status of the low volume rabbit eye test, and recommendations that it should not be used for prospective *in vivo* eye safety testing due to performance issues.
  - 2012: Revisions to OECD Test Guideline 405 being reviewed to include ICCVAM recommendations of the usefulness and limitations of routinely using topical anesthetics, systemic analgesics, and humane endpoints during *in vivo* ocular irritation safety testing

- January 2011: ICCVAM Workshop Series on Best Practices for Regulatory Safety Testing: *Assessing the Potential for Chemically Induced Eye Injuries*
- NICEATM and ICCVAM developed draft eye injury hazard classification criteria to support consumer product safety testing with 3 animals rather than the current 6 to 18 animals. The recommended classification criteria provide the same or greater level of eye injury hazard labeling as current requirements, while using 50% to 83% fewer animals.
- NICEATM and ICCVAM initiated collaborations with the Japanese Center for the Validation of Alternative Methods to review the validation status of a short time exposure model that uses cultured corneal cells to rapidly identify whether substances may pose an eye injury hazard.
- NICEATM and ICCVAM prepared a guidance document on histopathology from *in vitro* and *in vivo* models used for eye injury hazard testing. Collection of histopathology data will be used to determine whether this information can increase the accuracy of some *in vitro* test systems such as the BCOP, isolated chicken eye, and isolated rabbit eye.
    - 2011: OECD adopted GD 113: "Supplement to Test Guidelines 437 and 438: The Bovine Corneal Opacity and Permeability and Isolated Chicken Eye Test Methods: Collection of Tissues for Histopathological Evaluation and Collection of Data on Nonsevere Irritants"

Acute Toxicity Testing

Goal

Identify mechanism-based *in vitro* test systems and earlier, more humane endpoints that can be used to further reduce, refine, and eventually replace animal use for acute systemic toxicity testing, while ensuring the protection of human and animal health.

Specific Objectives

- Identify standardized procedures for collecting mechanistic information from acute oral toxicity testing to aid in developing batteries of predictive *in vitro* test methods that can further reduce and eventually replace animals for acute toxicity testing
- Identify more objective endpoints that could be used to define evident toxicity and their use to terminate a study.
- Explore opportunities to collaborate with the European Centre for the Validation of Alternative Methods (ECVAM) on the AcuteTox Project, which has the goal of developing an *in vitro* test strategy to completely replace *in vivo* testing of chemicals for acute toxicity
- Evaluate the applicability of the Up-and-Down Procedure (UDP) and the Fixed Dose Procedure (FDP) as ways to reduce animal use for acute dermal systemic toxicity and acute inhalation toxicity, where consistent with regulatory needs

Planned Activities for Implementation

1. Organize an international workshop to identify earlier, more humane endpoints and predictive batteries of *in vitro* test methods.
2. Work with stakeholders to promote the collection and submission of *in vitro* and *in vivo* toxicity test data to ICCVAM in order to advance the development and validation of more predictive *in vitro* test methods (or batteries of tests) and earlier, more humane endpoints for acute systemic toxicity testing.
3. Participate on an international Validation Management Group for a human hepatic biotransformation enzyme induction assay using HepaRG cells and cryopreserved human hepatocytes.

2008 to 2012 Accomplishments:

- February 2008: NICEATM, ICCVAM, and its international partners convened the *Workshop on Acute Chemical Testing: Advancing In Vitro Approaches and Humane Endpoints for Systemic Toxicity Evaluations* which concluded that systematic collection of mechanistic data from required *in vivo* studies could help identify predictive biomarkers of systemic toxicity. These biomarkers could be used as earlier, more humane endpoints to further reduce or avoid pain and distress in test animals. Participants also recommended ways to collect data to identify key toxicity pathways for acute oral systemic toxicity that could then be used to target the development of alternative predictive *in vitro* test methods.
    - March 2009: Publication of "Report on the ICCVAM-NICEATM/ECVAM/JaCVAM Scientific Workshop on Acute Chemical Safety Testing: Advancing *In Vitro* Approaches and Humane Endpoints for Systemic Toxicity Evaluations"
- Federal agencies accepted ICCVAM's February 2008 recommendation to always consider using one of two *in vitro* basal cytotoxicity test methods to estimate starting doses for acute oral systemic toxicity testing. Using these test methods in a weight-of-evidence approach for determining starting doses for *in vivo* studies can reduce animal use by up to an additional 50%.
- September 2009: OECD adoption of revision to Test Guideline 403: "Acute Inhalation Toxicity" and Test Guideline 436: "Acute Inhalation Toxicity – Acute Toxic Class Method" include these recommended reductions on the number of animals use per test
- NICEATM and ICCVAM prepared an OECD guidance document describing how to use two ICCVAM-recommended *in vitro* test methods to estimate starting doses for acute oral systemic toxicity tests. The tests can reduce animal use per test by up to 50%.
    - July 2010: OECD formally adopted GD 129: "Guidance Document on Using Cytotoxicity Tests to Estimate Starting Doses for Acute Oral Systemic Toxicity Tests".
- NICEATM and members of the ICCVAM Interagency Acute Toxicity Working Group participated on the validation management team for an international study to determine whether two types of cultured liver cells reliably predict drug metabolism and associated toxicity.

- NICEATM initiated development of an acute dermal systemic toxicity up-and-down procedure that is expected to reduce the number of animals needed to determine whether substances can be poisonous when they come in contact with the skin.

Dermal Toxicity Testing

Goal

The replacement of the rabbit skin test for corrosivity and irritation with alternative test methods that meet the requirements of U.S. regulators.

Specific Objectives

- Determine the usefulness and limitations of *in vitro* skin model systems for skin irritation testing.
- Determine how corrosive substances that have produced false negative results in *in vitro* corrosivity test methods will act in the *in vitro* dermal irritation test method protocols.

Planned Activities for Implementation

1a. Evaluate alternative dermal irritation test methods for their usefulness and limitations in U.S. regulatory testing.

1b. Assist in the development of an OECD Test Guideline for human skin model systems for skin irritation testing.

1c. Evaluate a combination (or battery) of *in vitro* test methods for evaluating skin corrosivity and irritation.

1d. Conduct a study to evaluate potential false negative corrosive chemicals in proposed *in vitro* dermal irritation assays.

2008 to 2012 Accomplishments:

- 2009 through 2012: Scientists from the ICCVAM Dermal Corrosivity and Irritation Working Group participated in OECD Expert Consultation meetings to evaluate several *in vitro* test methods useful for reducing the number of animals used for skin irritation testing.
- NICEATM and ICCVAM developed and submitted proposed revisions to two OECD test guidelines for *in vitro* test methods that can identify substances with the potential to cause skin burns. The revisions provide performance standards that can be used to validate similar test methods that may be more accurate, faster to perform, and less expensive.
- July 2010: Adoption of OECD Test Guideline 439: "*In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method"
- NICEATM completed a study to determine if revised procedures for reconstructed human skin models could increase the accuracy of the test methods for identifying whether chemicals can cause skin injuries. The results characterize limitations of the *in vitro* test

methods that will need to be addressed by other approaches or models in a non-animal integrated testing strategy.

- NICEATM completed a study to determine how *in vitro* skin irritation test methods classify corrosive substances incorrectly identified as noncorrosives by *in vitro* corrosivity test methods. These data will be used to ensure that any limitations associated with an *in vitro* testing strategy for skin corrosivity and irritation are adequately identified.

Dermal Sensitization Testing

Goal

Identify and promote research, development, translation, and validation activities for test methods that can reduce, refine, or replace the use of animals to determine the potential of chemicals and products to produce allergic contact dermatitis.

Specific Objectives

- Identify adequately valid test methods that can detect potential skin sensitizers without the requirement for radioactivity.
- Identify ways to reduce the number of animals required for skin sensitization testing
- Collect and review current murine local lymph node assay (LLNA) data to determine whether the applicability domain of the LLNA can be expanded
- Explore opportunities to collaborate with ECVAM on the Sens-It-Iv project, which has the goal of developing an *in vitro* testing strategy to replace animal tests currently used for the risk assessment of potential skin or lung sensitizers.

Planned Activities for Implementation

1a. Evaluate the validation status of the LLNA as a stand-alone assay for potency determination for classification purposes
1b. Evaluate the validation status of the LLNA for testing formulations, aqueous solutions, and metals
1c. Evaluate the validation status of modified LLNA protocols that do not use radioactivity
1d. Evaluate the validation status of the reduced LLNA (rLLNA) test method
1e. Develop test method performance standards for the LLNA that could be used to quickly and efficiently evaluate the usefulness and limitations of modified versions of the LLNA that are mechanistically and functionally similar to the traditional LLNA

2008 to 2012 Accomplishments:

- March 2008: Independent Scientific Peer Review Panel Meeting: *Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products: Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay*
    - May 2008: Publication of the Peer Review Panel Report: "Independent Scientific Peer Review Panel Report - Validation Status of New Versions and Applications of the

Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products"

- January 2009: Publication of "Recommended Performance Standards: Murine Local Lymph Node Assay"
- March 2009: Publication of "ICCVAM Test Method Evaluation Report - The Reduced Murine Local Lymph Node Assay: An Alternative Test Method Using Fewer Animals to Assess the Allergic Contact Dermatitis Potential of Chemicals and Products"

- April 2009: Independent Scientific Peer Review Panel Meeting: *Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products: Evaluation of the Updated Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay*
  - June 2009: Publication of the Peer Review Panel Report: "Updated Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products"
- June 2010: ICCVAM publishes three Test Method Evaluation Reports that recommended new versions and applications of the murine local lymph node assay (LLNA) that will further reduce animal use and expand the applicability of the LLNA for assessing the allergic contact dermatitis (ACD) hazard potential of chemicals and products:
  - "ICCVAM Test Method Evaluation Report on the Murine Local Lymph Node Assay: BrdU-ELISA, a Nonradioactive Alternative Test Method to Assess the Allergic Contact Dermatitis Potential of Chemicals and Products"
  - "ICCVAM Test Method Evaluation Report on the Murine Local Lymph Node Assay: DA, aA Nonradioactive Alternative Test Method to Assess the Allergic Contact Dermatitis Potential of Chemicals and Products"
  - "ICCVAM Test Method Evaluation Report on Using the Murine Local Lymph Node Assay for Testing Pesticide Formulations, Metals, Substances in Aqueous Solutions, and Other Products"
- 2010 and 2011: Federal agencies accepted or endorsed the following ICCVAM recommendations:
  - An updated LLNA protocol that achieves a 20% reduction in the number of required animals
  - Routine use of the reduced LLNA, when dose–response information is not required, to determine the ACD hazard potential of chemicals and products, enabling a 40% reduction in animal use for each test
  - Performance standards for the LLNA, which enable more rapid and efficient evaluation of the validity of new versions that are mechanistically and functionally similar to the LLNA
  - Two new "green" versions of the LLNA that do not require radioactive reagents and will allow use of the LLNA in nearly all laboratories worldwide

- NICEATM and ICCVAM forwarded proposals to update the OECD test guideline for the LLNA and to create two new test guidelines for the nonradiolabeled versions of the LLNA.
    - July 2010: OECD adopted TG 429: "Skin Sensitisation: Local Lymph Node Assay"; TG 442A: "Skin Sensitisation: Local Lymph Node Assay: DA"; and TG 442B: "442B: Skin Sensitisation: Local Lymph Node Assay: BrdU-ELISA", resulting in worldwide acceptance of these important methods.
- January 2011: ICCVAM Workshop on Best Practices for Regulatory Safety Testing: "Assessing the Potential for Chemically Induced Allergic Contact Dermatitis"
- June 2011: ICCVAM published the Test Method Evaluation Report: "Usefulness and Limitations of the Murine Local Lymph Node Assay for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans" which recommended that the LLNA may be used as a screening test to categorize substances as strong skin sensitizers.
    - 2012: Federal agencies accepted or endorsed these LLNA recommendations.
- 2012: NICEATM is evaluating multiple *in vitro* methods used in integrated testing strategies to reduce, refine, and replace animal use for identification of substances that may cause ACD.

Endocrine Disruptors Testing

Goal

Identify and promote research, development, translation, and validation activities for individual *in vitro* endocrine disruptor test methods, or batteries of these methods, that can reduce the numbers of animals needed to screen for chemicals that might interfere with the endocrine systems of humans or wildlife.

Specific Objectives

- Complete a joint international study with ECVAM and the Japanese Center for the Validation of Alternative Methods (JaCVAM) to evaluate the usefulness and limitations of an *in vitro* test method to identify estrogen-like chemicals that does not require the use of animals as donors for test components.
- Provide support in designing studies for the validation of the Certichem, Inc., MCF-7 Cell Proliferation Assay protocols for both the detection of estrogenic and anti-estrogenic activity.
- Increase involvement in OECD test guideline activities related to endocrine disruptors.

Planned Activities for Implementation

1a. Further standardize and optimize the agonist and antagonist protocols for the LUMI-CELL® estrogen receptor (ER) assay and test the 78 ICCVAM recommended substances for the validation of *in vitro* ER transactivation (TA) test methods, in three laboratories (one in Europe, one in Japan, and one in the United States), to evaluate test method reliability (intralaboratory repeatability, intra- and inter-laboratory reproducibility) and comparative performance against the NICEATM meta-data for ER active compounds.

1b. Use the results from the testing of the 78 ICCVAM recommended substances to develop a high quality *in vitro* ER TA database and performance standards that can be used to characterize the extent to which other *in vitro* endocrine disruptor test methods (or test method batteries) might be used to further reduce the requirements for animal use in the screening of potential endocrine disruptors.

2a. Provide comments on study design for the validation of the CertiChem, Inc., MCF-7 Cell Proliferation Assay protocols for both the detection of estrogenic and anti-estrogenic activity.

2b. Provide CertiChem, Inc., with coded samples of each of the compounds in the list of 53 ICCVAM recommended reference substances considered as the minimum for the validation of ER TA test methods.

2008 to 2012 Accomplishments:

- ICCVAM completed an evaluation of the *in vitro* LUMI-CELL® ER TA (BG1Luc ER TA) test method for its use as an initial screen to identify substances with the potential to induce or inhibit activation of the estrogen receptor. A draft international test guideline and performance standards were forwarded to the OECD, which received international review and acceptance in 2012.
- March 2011: Independent Peer Review Panel Meeting: *Evaluation of an In Vitro Estrogen Receptor Transcriptional Activation Assay for Endocrine Disruptor Chemical Screening*
    - May 2011: Publication of the Independent Scientific Peer Review Panel Report: "Evaluation of the LUMI-CELL ER® (BG1Luc ER TA) Test Method"
    - February 2012: ICCVAM published the Test Method Evaluation Report: "The LUMI-CELL® ER (BG1Luc ER TA) Test Method: An *In Vitro* Assay for Identifying Human Estrogen Receptor Agonist and Antagonist Activity of Chemicals"
    - August 2012: Federal agencies accepted or endorsed the ICCVAM recommendations
    - July 2012: Adoption of Revised OECD Test Guideline 455: "Stably Transfected Human Estrogen Receptor-α Transcriptional Activation Assay for Detection of Estrogenic Agonist-Activity of Chemicals"
    - 2012: BG1 Luc ER TA assay adapted for Tox21 High throughput screening and undergoing evaluation
- NICEATM completed its coordination of an international validation study to evaluate an *in vitro* test method that measures proliferation of cultured MCF-7 cells to identify substances with the potential to induce or inhibit activation of the estrogen receptor.

Challenge #2: Incorporating New Science and Technology

The second challenge is to identify and promote research incorporating new technologies that can be expected to support the future development of new test methods and approaches to reduce or eliminate the need for animals. While many of these approaches will require several years to develop and validate, some may be ready for use more quickly. To maximize the efficiency of this process, NICEATM and ICCVAM are working with Federal agencies and other stakeholders to link research and development activities to the standardization and validation of alternative test methods that may be used in regulatory testing.

ICCVAM Agencies have been surveyed for ongoing and planned research, development, translation, and validation activities relevant to test methods that reduce, refine, and replace the use of animals. A table of these activities is included as **Appendix A**. As part of this implementation plan, as appropriate, the role of ICCVAM working groups will be expanded to charge members with interacting with test method developers who would benefit from such consultation.

An ICCVAM Research and Development Working Group (RDWG) has been established to assist with implementation of activities relevant to incorporating new science and technology as outlined in the Five-Year Plan. The RDWG is specifically charged with aiding NICEATM and ICCVAM in identifying and promoting research incorporating new technologies that can be expected to support the future development of new test methods and approaches that will reduce, refine, and replace animal use in toxicity testing. NICEATM and ICCVAM seek to link research and development activities to the standardization and validation of alternative test methods that may be used in regulatory testing. Consultation and cooperation with NICEATM and appropriate ICCVAM Test Method Working Groups during test method development and validation is expected to maximize the value of such test methods and approaches to regulatory agencies. The RDWG will be asked to help identify test methods in the development phase for referral to appropriate Test Method Working Groups.

Nanomaterials Testing

Goal

Identify and promote research, development, translation, and validation activities for test methods that can reduce, refine, or replace the use of animals in regulatory safety testing for nanomaterials.

Specific Objectives

- Work with stakeholders to identify test methods that are considered to be most appropriate for nanomaterials
- Foster the development and evaluation of alternative methods for nanomaterials testing.
- Define the current and planned activities within ICCVAM agencies (or that are supported by ICCVAM agencies) that are relevant to nanomaterials testing and the use of alternative test methods

Planned Activities for Implementation

1. Assess the state of the science to determine if developing a scientific workshop to evaluate possible alternatives is warranted. Goals of such a workshop would be to:

- Identify and prioritize future research initiatives necessary to advance development and validation of *in vitro* methods for nanomaterials testing.
- Discuss how to promote the collection and submission of *in vitro* and *in vivo* toxicity test data to ICCVAM in order to advance the development and validation of *in vitro* test methods for nanomaterials testing.

2. Develop a one-day symposium to define the current planned activities with ICCVAM agencies that are relevant to nanomaterials testing and the use of alternative methods.

- ICCVAM agencies would be asked to identify current or new members with expertise specific to nanomaterials to each working group that could be involved in developing the proposed workshop.

3. Become engaged in OECD activities relevant to alternative test methods intended for safety testing of nanomaterials

- An ICCVAM and/or NICEATM representative will be proposed as a member of the OECD steering group that is reviewing existing alternative methods and making recommendations on test methods considered to be most appropriate for nanomaterials.

2008 to 2012 Accomplishments:

- The NICEATM-ICCVAM website includes a webpage that points to the federally funded research and other activities related to nanomaterials testing. NICEATM and ICCVAM are closely following progress in this area and will work with regulators and stakeholders to identify test methods that reduce, refine, and replace the use of animals for such testing requirements. Additional activities will be contingent on areas of need that are identified.
- The National Library of Medicine created a website on nanomaterials that contains extensive links for outreach on nanomaterials[4].
- The NIEHS website on NanoHealth and Safety contains information on the range of ongoing nanotechnology programs and initiatives studies with NTP, NIOSH, and FDA/NCTR[5].

High Throughput Screening

Goal

Identify batteries of rapid biochemical- or cell-based high throughput screening (HTS) assays that may reduce or replace the use of animals in toxicological tests.

Specific Objectives

- Facilitate the review of the usefulness and limitations of defined HTS approaches, and also assist in the identification of assays and endpoints that are relevant for alternative test methods that have already been adopted.

[4] http://sis.nlm.nih.gov/enviro/nanotechnology.html

[5] http://www.niehs.nih.gov/research/supported/programs/nanohealth/index.cfm

Planned Activities for Implementation

- Monitor progress in collaborations between research institutes within three ICCVAM Agencies (NIEHS [National Toxicology Program], EPA [National Center for Computational Toxicology], and NIH [Center for Chemical Genomics]) that will test a large number of compounds (~ 10,000) broadly characterizing and defining the chemical-biological space occupied by chemicals of toxicological concern. This collaboration will establish a spectrum of secondary and tertiary-screening assays to further define and characterize activities identified in initial high throughput screens. The goals of this interagency program are to:
    - Prioritize substances for further in-depth toxicological evaluation (to judiciously allocate efforts and resources to maximize public health impact).
    - Identify mechanisms of action for further investigation (e.g., disease-associated pathways).
    - Develop predictive models for *in vivo* biological response (predictive toxicology).
- Nominate substances identified by NICEATM/ICCVAM as reference compounds for the development of alternative test methods as well as other compounds that have been tested in various alternative test methods, in the standard *in vivo* toxicity tests, or in humans.

2008 to 2012 Accomplishments:

- ICCVAM and NICEATM are collaborating with member agency research initiatives such as the Tox21 collaboration that includes the EPA ToxCast and the NIEHS-NTP High Throughput Screening initiatives, as well as international partners, to speed the translation of research advances and new technologies into scientifically valid safety testing methods that will further reduce, refine, and replace animal use.
    - NICEATM nominated over 900 reference substances for use in this effort.
    - Promising methods and approaches that arise from this effort will be reviewed by ICCVAM, who will forward recommendations on appropriate use to Federal agencies.
    - NICEATM is participating on the Tox21 Pathways Steering Group that is charged with selecting assays the interrogate all of the relevant pathways of interest.
    - NICEATM nominated the LUMI-CELL ER assay for optimization to an HTS protocol at the NIH-National Chemical Genomics Center
    - NICEATM is collaborating with the European Union's ECVAM High Throughput Screening Unit at the Institute of Consumer Protection and Health to adapt alternative test methods to HTS

Challenge #3: Fostering Acceptance and Appropriate Use of Alternative Test Methods

Once regulatory authorities have accepted an alternative test method, ICCVAM will work to promote its use by broadly communicating the outcomes of ICCVAM review activities and/or workshops via the *Federal Register*, at national or international scientific meetings, via peer reviewed journal publications, and at training courses. Emphasis will also be placed on making the scientific community, including Institutional Animal Care and Use Committees (IACUCs), aware of new alternatives that are available for consideration in complying with the PHS Policy and Animal Welfare Act provisions, which state that such methods must be considered prior to testing in animals, where applicable.

ICCVAM will collaborate with government and non-governmental organizations, where appropriate, to co-sponsor workshops. The objectives of these workshops will be to evaluate the state-of-the-science related to the development and validation of alternative toxicological test methods, and to identify high priority research, development, translation, and validation activities necessary to advance and characterize the usefulness of such methods. The results of these workshops will be broadly communicated to individuals and organizations that conduct such activities.

NICEATM-ICCVAM Website

Goal

- Provide user-friendly access to the latest information on validation processes and the most up-to-date status of the alternative test methods previously reviewed and those currently under review.
- Provide access to publicly available reference test method databases for use in the development and validation of alternative test methods.
- Promote active communication and outreach efforts with both government and non-government stakeholders.

Specific Objectives

- Use a combination of e-mail and website announcements to inform the public of the availability of newly published Federal Register notices, NICEATM documents, journal articles, and upcoming events

Planned Activities for Implementation

1a. Create agency websites dedicated to their specific activities associated with alternative test methods research, development, translation, and validation. NICEATM/ICCVAM will in turn provide a link on their website to these member agency websites.

1b. One or more lists of frequently asked questions (FAQs) will be developed to provide quick reference guides to broad issues related to the ICCVAM test method evaluation process, as well as more specific issues relevant to individual toxicity testing areas.

- The content for each website dedicated to alternative test method activities can include the following information:

- Currently Available Alternative Methods
- Validation and Acceptance Process for Alternative Methods
- Regulations and Applicability to Alternative Methods
- Alternative Methods Research, Development, and Translation Activities
- Frequently Asked Questions
- Resources

2. Create a web-based database of all test methods that have been reviewed or that are currently undergoing review.

2008 to 2012 Accomplishments:

- The NICEATM-ICCVAM website now includes a web-based database of all test methods that have been reviewed or that are currently under review, including their development, validation, evaluation, and regulatory acceptance status.
- The USDA has a website for the Animal Welfare Information Center (AWIC) spotlights activities in Alternatives in Toxicology which includes information about their ICCVAM activities[6]

[6] http://awic.nal.usda.gov/alternatives/alternatives-toxicology

Challenge #4: Developing Partnerships and Strengthening Interactions with ICCVAM Stakeholders

ICCVAM will also foster international collaboration by including experts from the international scientific community on workshops that review the state of the science for particular test method areas. Where appropriate, NICEATM and ICCVAM will also invite representatives from international organizations such as OECD and from OECD member countries to attend and participate in relevant NICEATM and ICCVAM-sponsored workshops, peer reviews, and other scientific activities. Similarly, to further ensure the development of scientifically valid international test guidelines, NICEATM and ICCVAM will seek to increase participation of its scientists in U.S. delegations to OECD test guideline meetings, expert consultations, and workshops.

Goal

Develop partnerships and strengthen interactions with ICCVAM stakeholders to promote research, development, translation, and validation activities for alternative test methods.

Specific Objectives

- Be more proactive in identifying research needs and promising methods that should be priorities for further development, translation, validation, or ICCVAM evaluation.
- Foster interagency collaboration among Federal research and regulatory agencies, including opportunities for test method validation activities.
- Strengthen international relationships with appropriate organizations to foster the validation and evaluation of alternative test methods.
- Foster international collaboration by including experts from the international scientific community on expert panels and workshops.

Planned Activities for Implementation

1. Collaborate with government and non-governmental organizations, where appropriate, to co-sponsor workshops. The objectives of these workshops will be to evaluate the state-of-the-science related to the development and validation of alternative toxicological test methods, and to identify high priority research, development, translation, and validation activities necessary to advance and characterize the usefulness of such methods. The results of these workshops will be broadly communicated to individuals and organizations that conduct such activities.

2008 to 2012 Accomplishments:

- February 2008: ICCVAM Symposium: *Celebrating Ten Years of Advancing Public Health and Animal Welfare With Sound Science - A Scientific Symposium Envisioning New Directions in Toxicology* included ICCVAM stakeholders and commemorated the tenth anniversary of the establishment of ICCVAM, presented the 2008-2012 Five-Year Plan, and how NICEATM and ICCVAM, in partnership with federal agencies, would promote the research, development, translation and validation of alternative test methods.
- February 2008: NICEATM, ICCVAM, and its international partners convened the *Workshop on Acute Chemical Testing: Advancing In Vitro Approaches and Humane Endpoints for*

Systemic Toxicity Evaluations which concluded that systematic collection of mechanistic data from required *in vivo* studies could help identify predictive biomarkers of systemic toxicity. These biomarkers could be used as earlier, more humane endpoints to further reduce or avoid pain and distress in test animals. Participants also recommended ways to collect data to identify key toxicity pathways for acute oral systemic toxicity that could then be used to target the development of alternative predictive *in vitro* test methods.

- September 2010: *International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Vaccine Safety Testing: State of the Science and Future Directions* convened by NICEATM, ICCVAM, and their partners in the International Cooperation on Alternative Test Methods (ICATM) and reviewed the state of the science of alternative methods that are currently available for this purpose and developed recommendations for priority research needed to further advance alternative methods.
    - December 2011: Proceedings from this workshop published in *Procedia in Vaccinology* (Kulpa-Eddy et al. 2011).
- January 2011: ICCVAM Workshop on Best Practices for Regulatory Safety Testing: "Assessing the Potential for Chemically Induced Eye Injuries"
- January 2011: ICCVAM Workshop on Best Practices for Regulatory Safety Testing: "Assessing the Potential for Chemically Induced Allergic Contact Dermatitis"
    - These workshops addressed current best practices for safety testing necessary to determine whether chemicals and products may cause eye injuries and allergic contact dermatitis (ACD).
- October 2011: *International Workshop on Alternative Methods for Human and Veterinary Rabies Vaccine Testing: State of the Science and Planning the Way Forward* convened by NICEATM, ICCVAM, and their international partners and reviewed the available methods and approaches to reduce, refine, and replace animals used in rabies vaccine potency testing and developed an implementation strategy to achieve global acceptance and use of these alternatives.
    - Proceedings from this workshop were published in *Biologicals*.
- September 2012: *International Workshop on Alternative Methods for Leptospira Vaccine Potency Testing: State of the Science and the Way Forward* convened by NICEATM, ICCVAM, and their international partners and reviewed the available methods and approaches to reduce, refine, and replace animals used in human and veterinary Leptospira vaccine potency testing and developed an implementation strategy to achieve global acceptance and use of these alternatives.
- November 2012: *International Workshop on Alternatives to the Murine Histamine Sensitization (HIST) Test for Acellular Pertussis Vaccines: State of the Science and the Path Forward* convened by NICEATM, ICCVAM, and their international partners and reviewed the available methods and approaches to reduce, refine, and replace animals used in the murine histamine sensitization test and developed an implementation strategy to achieve global acceptance and use of these alternatives.

Planned Activities for Implementation

2. Facilitate the international adoption of valid alternative test methods by providing standardized protocols that can be considered for adoption by international organizations (for example, the International Standards Organization [ISO], OECD, etc.). As appropriate, NICEATM and ICCVAM will provide comprehensive test method background review documents and the results of independent scientific peer reviews to facilitate the approval of these test methods by the international community.

2008 to 2012 Accomplishments:

- ICCVAM, in conjunction with stakeholders in the United States, the European Union (EU), and Japan, drafted an OECD Test Guidelines (TG) for the ICE and the BCOP test methods. The TGs were developed following an international peer review evaluation with contributions from ECVAM and JaCVAM. The TGs were formally adopted by the OECD Council and are now accepted by all 30 OECD member countries in accordance with OECD Mutual Acceptance of Data. The use of these TGs will reduce the use of rabbits for eye safety testing and eliminate such testing in animals of most substances likely to cause severe pain and discomfort.
- NICEATM, in conjunction with the ICCVAM interagency Acute Toxicity Working Group, drafted an OECD Guidance Document (GD) entitled In Vitro Neutral Red Uptake (NRU) Cytotoxicity Tests for Estimating Starting Doses for Acute Oral Systemic Toxicity Tests. The standardized protocols on which this draft GD is based were developed during a NICEATM/ICCVAM/ECVAM sponsored international validation study. OECD distributed the final GD in July 2010.
- NICEATM, in conjunction with the ICCVAM interagency Acute Toxicity Working Group, also evaluated and provided comments on a number of draft OECD documents between 2008 and 2012:
    - Test Guideline 223: Avian Acute Oral Toxicity Test, adopted by OECD in July 2010
    - Test Guideline 412: Subacute Inhalation Toxicity: 28-Day Study, accepted by OECD in September 2009
    - Test Guideline 413: Subchronic Inhalation Toxicity: 90-Day Study, accepted by OECD in September 2009
    - Draft Guidance Document on Histopathology for Inhalation Toxicity Studies, Supporting Test Guideline 412 (Subacute Inhalation Toxicity: 28-Day) and Test Guideline 413 (Subchronic Inhalation Toxicity: 90-Day), adopted by the OECD in June 2010 as Guidance Document 125
    - Draft Proposal for a Revised Test Guideline 403: Acute Inhalation Toxicity (OECD 2009e), accepted by OECD in September 2009
    - Comparison of Test Guideline 403 and C x t Protocols Via Simulation and for Selected Real Data Sets with Comments from the Expert Consultation Meeting in April 2008
    - Test Guideline 436: Acute Inhalation Toxicity – Acute Toxic Class (ATC) Method, accepted by OECD in September 2009

- Report on Biostatistical Performance Assessment of the Draft Test Guideline 436 Acute Toxic Class Testing Method for Acute Inhalation Toxicity
- OECD Environment, Health and Safety Publications Series on Testing and Assessment No. 39: Draft Guidance Document On Acute Inhalation Toxicity Testing, adopted by the OECD in July 2009
- Guidance Document 153 for the Derivation of an Acute Reference Concentration, adopted by the OECD in August 2011
- Draft Guidance Document on a the Threshold Approach for Acute Fish Toxicity Testing, adopted by the OECD in May 2010 as Guidance Document 126
- Proposal for a New Testing Strategy (Step-down approach) to Reduce the Use of Fish in Acute Aquatic Testing (supplement to OECD Test Guideline 203, Fish Acute Toxicity Test)

- NICEATM, in conjunction with the ICCVAM interagency Endocrine Disruptor Working Group, developed a new OECD test guideline for the BG1Luc ER TA test method, which is expected to be adopted by the OECD as Test Guideline 457 in 2012. NICEATM and the ICCVAM interagency Endocrine Disruptor Working Group were also active in developing a performance based test guideline for ER TA test methods, which will include BG1Luc ER TA. This updated version of Test Guideline 455, which was originally adopted in 2009, is also expected to be adopted by the OECD in 2012.
- NICEATM, in conjunction with the ICCVAM interagency Endocrine Disruptor Working Group, also evaluated and provided comments on a number of draft OECD documents between 2008 and 2012:
  - Test Guideline 441: Hershberger Bioassay in Rats: A Short-term Screening Assay for (Anti) Androgenic Properties, accepted by OECD in September 2009
  - Guidance Document 115 for the Weanling Hershberger Assay, accepted by OECD in November 2009
  - Test Guideline 455: Stably Transfected Human Estrogen Receptor-α _Transcriptional Activation Assay for the Detection of Estrogenic Agonist-Activity of Chemicals, originally adopted by OECD in 2009
- NICEATM and the ICCVAM interagency Genetic Toxicity Working Group evaluated and provided comments on two draft OECD documents:
  - Test Guideline 487: *In vitro* micronucleus assay
  - Draft Test Guideline for the Syrian hamster embryo cell transformation assay
- NICEATM and ICCVAM updated the OECD TG 429 for the LLNA and, in conjunction with stakeholders in Japan, developed new draft test guidelines for use of the nonradioactive LLNA: DA and LLNA: BrdU-ELISA. The updated LLNA OECD TG 429, TG 442A (LLNA: DA), and TG 442B (LLNA: BrdU-ELISA) were adopted by the OECD in July 2010.
- January 2011: NICEATM and ICCVAM convened two workshops on Best Practices for Regulatory Safety Testing. These workshops focused on Assessing the Potential for

Chemically Induced Eye Injuries, and Assessing the Potential for Chemically Induced Allergic Contact Dermatitis and were co-sponsored by the Society of Toxicology and the Society for Risk Analysis.

Planned Activities for Implementation

3. Work with other national and international validation organizations (for example, ECVAM and JaCVAM) to promote ICCVAM's validation and acceptance criteria, which have been substantially incorporated into OECD Guidance Document 34, and to consider other issues related to validation as they occur.

2008 to 2012 Accomplishments:

- April 2009: ICCVAM signed a Memorandum of Cooperation with ECVAM, JaCVAM, and Health Canada to establish the International Cooperation on Alternative Test Methods (ICATM) to strengthen cooperation, collaboration, and communications among national validation organizations on the scientific validation and evaluation of new alternative testing methods proposed for regulatory health and safety assessments.
- March 2011: This ICATM agreement was expanded to include the Korean Center for the Validation of Alternative Methods (KoCVAM).
- NICEATM and ICCVAM representatives are serving on the Validation Management Group for a prospective validation of Reconstructed Human Tissue models for identification of mild to moderate irritants and substances not labeled as ocular irritants.
- NICEATM and ICCVAM representatives are participating with ECVAM and JaCVAM in two Validation Management Groups for *in vitro* approaches to skin sensitization testing. They have provided comments on validation study design and chemical selection.
- NICEATM and ICCVAM representatives are involved in development of the validation study plan for the *in vivo* rodent and *in vitro* alkaline comet assay for detection of genotoxic carcinogens, the proposed protocol, and proposed list of reference substances, and ICCVAM has representatives on the Validation Study Management Team
- NICEATM and ICCVAM representatives provided comments to JaCVAM on their validation study plan and protocol for their validation study of the cell transformation assay as well as serving as providing liaison members to the Validation Study Management Team
- NICEATM and ICCVAM participants are providing input and guidance to an ECVAM Validation Study of a human hepatic biotransformation enzyme induction assay using cryopreserved HepaRG cells and cryopreserved human hepatocytes

Planned Activities for Implementation

4. Participate in the development of performance standards for international test guidelines.

2008 to 2012 Accomplishments:

- ICCVAM and NICEATM, in conjunction with ECVAM and JaCVAM developed internationally harmonized performance standards for the LLNA and submitted Special

Project Submission Forms (SPSFs) to OECD for updating OECD TG 429 (the LLNA) with these performance standards. The updated LLNA OECD TG 429, which included the performance standards, was adopted by the OECD in July 2010.

- ICCVAM and NICEATM also submitted SPSFs to OECD for updating OECD TG 430 (rat skin TER), and OECD TG 431 (Human skin model systems) with performance standards previously developed by ICCVAM
- Performance standards were developed for the BG1Luc ER TA test method. A new OECD test guideline for the BG1Luc ER TA, which includes these performance standards, is expected to be adopted by the OECD in 2012. These performance standards are also being used by an OECD expert group that is developing a performance based test guideline for ER TA test methods.

Planned Activities for Implementation

5. To further ensure the development of scientifically valid international test guidelines, NICEATM and ICCVAM will seek to increase participation of its scientists in U.S. delegations to OECD test guideline meetings, expert consultations, and workshops.

2008 to 2012 Accomplishments:

- NICEATM and ICCVAM representatives participated in an OECD Expert Consultation during the development of the aforementioned TGs for ICE and BCOP.
- NICEATM and ICCVAM representatives are participating as members of an OECD Expert Working Group on a draft TG for human skin model systems and will host an Expert Consultation meeting in June 2009
- NICEATM and ICCVAM provided nominations of independent experts to serve on an ESAC peer review panel for the cell transformation assay
- NICEATM and ICCVAM provided nominations of independent experts to serve on an ESAC peer review panel of four cell function-based *in vitro* methods (fluorescein leakage, neutral red release, cytosensor microphysiometer and red blood cell haemolysis test methods) for identification of mild to moderate irritants and substances not labeled as ocular irritants.
- In 2011, NICEATM provided nominations of independent experts to serve on an ESAC peer review panel to review the potential usefulness of DPRA and the KeratinoSens assay in a testing strategy to identify potential skin sensitizers.
- In 2011, NICEATM provided nominations of independent experts to serve on an ESAC peer review panel to review the usefulness of the 3T3 NRU assay in identifying substances with LD50 > 2000 mg/kg (i.e., nontoxic substances).

Planned Activities for Implementation

6. Invite representatives from international organizations such as OECD and from OECD member countries to attend and participate in relevant NICEATM and ICCVAM-sponsored workshops, peer reviews, and other scientific activities.

2008 to 2012 Accomplishments:

- *2008*: ICCVAM recommended five *in vitro* pyrogenicity test methods measuring cytokine release from human cells as replacements for the rabbit test, subject to product-specific validation, to detect endotoxin contamination in parenteral drugs. These recommendations were finalized following consideration of conclusions and recommendations from an independent peer review panel that included members from five different countries.
- 2008: ICCVAM completed reviews of the rLLNA and LLNA performance standards and subsequently forwarded recommendations to Federal agencies. These recommendations were finalized following consideration of conclusions and recommendations from an independent peer review panel that included members from eight different countries. In 2009, this Panel also reviewed additional data relevant to the applicability domain of the LLNA and three non-radiolabeled LLNA methods.
- May 2009: An independent peer review panel that included members from six different countries reviewed the validation status of several methods relevant to ocular safety testing. Their report was published in June 2009.
- March 2011: An independent peer review panel that included members from six different countries reviewed the validation status of the LUMI-CELL® ER assay for its ability to identify substances with *in vitro* estrogen agonist and antagonist activity. The Panel report was published in May 2011.

Planned Activities for Implementation

7. Engage interested stakeholders in assessing how to efficiently meet Federal peer review requirements, and seek input on ways to streamline processes that will not compromise transparency, scientific rigor, or the opportunity for stakeholder participation.

2008 to 2012 Accomplishments:

- January 2011: NICEATM and ICCVAM convened two workshops on Best Practices for Regulatory Safety Testing. These workshops focused on Assessing the Potential for Chemically Induced Eye Injuries, and Assessing the Potential for Chemically Induced Allergic Contact Dermatitis and were co-sponsored by the Society of Toxicology and the Society for Risk Analysis

Appendix A Ongoing and Planned Research, Development, Translation, and Validation Activities in ICCVAM Member Agencies Relevant to Test Methods That Reduce, Refine, And Replace The Use Of Animals[1]

Color coding: Green = New activity; Orange = Activity previously reported is being continued; Pink = Activity has been discontinued; White = No update provided as of May 19, 2011

Agencies are listed in the following order: NIEHS; NLM; other NIH Institutes and Centers reporting relevant activities; other ICCVAM member agencies reporting relevant activities

Type of Toxicity Testing	Agency	Status	Title	Type of Activity	Description (asterisk indicates entry has been edited for length)	Poten-tially Applic-able 3Rs	Other Information
Chronic Toxicity/ Carcino-genicity	NIEHS	Ongoing	Predictive Gene Sets For Chemically-Induced Liver Cancer	Res	Rodent 3D liver models for drug and toxicant testing. Uses gene expression profiles in addition to cell survival for endpoints.	Replace	Grant 5R44ES012618-05
Endocrine Active Substances	NIEHS	Project start/end dates Jun 2007 - Dec 2013	Validation of Human Vaginal Tissue Assay for Endocrine Disruptors	Validate	A validated human *in vitro* method to identify endocrine disruptors (ED) is an area of great importance. This research project will validate an organotypic EpiVaginal tissue model for Tier 1 screening of chemicals with endocrine disrupting potential. Phase I research will validate an organotypic vaginal-ectocervical (EpiVaginal™) tissue model for use in identifying ED. A battery of 75 model compounds with known ED activity will be selected from the revised ICCVAM list of recommended substances. The production of estrone by the tissue model and changes to tissue morphology and gene expression will be monitored as biomarkers of ED. A prediction model for ED with be finalized and the test method will undergo formal validation in a multi-center, GLP study. In addition, reproducibility of the	Reduce Replace	Grant 2R44ES015641-02

[1] Information in this table was provided to NICEATM by ICCVAM principal agency representatives in response to a request for updates on agency research, development, translation, and validation activities that relevant to the NICEATM-ICCVAM Five Year Plan. Questions about specific activities listed in this table should be directed to the principal agency representative of the appropriate

Type of Toxicity Testing	Agency	Status	Title	Type of Activity	Description (asterisk indicates entry has been edited for length)	Poten-tially Applic-able 3Rs	Other Information
					assay method and adaptation of the method to a high throughput screen format will be investigated.		
Genetic Toxicity	NIEHS	Project start/end dates Sept 2006 - Aug 2011	Reconstructed Skin Micronucleus Genotoxicity Assay	Validate	The long term goal of the proposal is to validate an *in vitro* test method to accurately determine human skin genotoxicity. In Phase 1, a standardized protocol, a pre-screen cytotoxicity assay, and a prediction model were defined and tested. A previously published method was improved to increase the sensitivity of the assay for detecting genotoxins requiring metabolic activation. In addition, long term reproducibility studies utilizing tissue from multiple donors showed highly reproducible results. Phase 2 will further build on Phase 1 results to optimize the assay method, automate scoring, expand the database of materials tested, demonstrate interlaboratory reproducibility, and adapt the assay to a high throughput format. These studies will lay the groundwork for formal validation and regulatory acceptance of the assay.	Replace	Grant 5R44ES015002-03
Reproduct-ive/ Develop-mental	NIEHS	Ongoing	A Novel Analytical Assay for Predictive Embryotoxicity Using Human Embryonic Stem Cells	Res	Uses human embryonic stem cell lines to screen for toxicant effects on p53 expression as an initial marker.	Reduce Replace	Grant 1R43ES017997-01
Reproduct-ive/ Develop-mental	NIEHS	Project start/end dates May 2007 - Aug 2011	Genetically Diverse Embryonic Stem Cell Lines For Reproductive Toxicology	Res	Project will develop mouse embryonic stem cell lines for toxicity testing to assess the impact of genetic background on reproductive toxicity. A panel of approximately 100 genetically distinct ES lines will be tested with a panel of reference compounds, toward the development of a system to define, map and identify the genetic components of cellular response to environmental burden.	Reduce Replace	Grant 5R44ES015646-03
Targeted Testing Areas	NIEHS	Ongoing	Monitoring Gene Expression Changes After Exposure to Toxicants in	Res	Develop transgenic *C. elegans* lines with GFP reporter constructs to track pathway changes in response to toxic metals and other exposure	Reduce Refine	SBIR Contract

Type of Toxicity Testing	Agency	Status	Title	Type of Activity	Description (asterisk indicates entry has been edited for length)	Poten-tially Applic-able 3Rs	Other Information
			Caenorhabditis elegans				
Targeted Testing Areas (High-throughput screening)	NIEHS	Sub-mitted to Tox 21 April 15, 2011	Endocrine Disruptors of Oxytocin Signaling	Res	**Biological Pathway:** Oxytocin receptor signaling through Gq stimulated calcium release **Relevance of the pathway/target to Tox21:** Oxytocin is a neuropeptide in the brain that is implicated in regulation of social behaviors. It signals through G protein-coupled receptors that stimulate Gq, which activates phospholipase C, an enzyme that hydrolyzes phosphatidylinositol bis-phosphate into the soluble second messenger 1,4,5 IP3 which binds to and opens calcium channels in the endoplasmic reticulum membrane to allow calcium to diffuse into the cytosol. **Subsequent validation for any compounds that you identify:** Confirm preliminary compounds at the single cell level in the confocal microscope. Investigate the mechanism of action by further experiments. Test whether the compound disrupts oxytocin's ability to stimulate synaptic plasticity in brain slices of hippocampus.	Reduce Replace	Assay Source: Loren L Looger, Howard Hughes Medical Institute, Janelia Farm Research Campus, Ashburn, Virginia, USA. Tian, L. et al., Nature Methods 6 (12), 875 - 881 (Dec 2009) Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators
Targeted Testing Areas (High-throughput screening)	NIEHS	Ongoing	Novel HTS for Gap Junctional Communication	Res	Develop a high-throughput screening assay to measure gap junction communication and effects from toxicant exposures	Reduce Replace	SBIR Contract
Targeted Testing Areas (High-throughput screening)	NIEHS	Ongoing	qNPA Metabolism HTS Assay	Res	Uses a quantitative nuclease protection assay to measure expression of mRNA and miRNA in liver cells for high-throughput screening	Reduce Replace	SBIR Contract
Other (General toxicity testing)	NIEHS	Project start/end dates Jan 2011 -	Advanced GST Proteomics for Early Stage Organ-Specific Toxicity Screening	Develop Valid	The goal of Phase I is to develop (a) highly specific antibodies capable of distinguishing between three GSTA isoforms, namely GSTA1-1/2-2, GSTA3-3 and GSTA4-4 and (b) ultrasensitive immunoassays for	Reduce Replace	Grant 1R43ES019037-01A1

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		Dec 2011			these biomarkers. In addition, in Phase I we will (c) employ animal models to validate these assays as a reliable way to detect organ-specific toxicity. The long-term goal (Phase II) involves development of a comprehensive GST proteomics panel for high sensitivity organ-specific toxicology testing that has significant preclinical and clinical commercial applications.		
Other (General toxicity testing)	NIEHS	Project start/end dates Jan 2011 - Dec 2011	Novel High Throughput Platform for Screening Cytochrome P450 Induction	Res Develop	During Phase I, develop platforms for evaluation of CYP1A2, CYP3A4 and CYP2B6 transcriptional regulation using reporter gene assays in human hepatic cell lines. These CYP P450 induction platforms will be available for toxicological screening of drugs early in the drug discovery process. During Phase II, extend the studies to evaluate other inducible CYP P450s, expand the spectrum of nuclear receptors tested, and to further develop cell-based assays of relevant allelic variants of the xenobiotic-activated receptors.	Reduce Replace	Grant 1R43ES019807-01
Other (Hepato-toxicity)	NIEHS	Project start/end dates Jan 2011 - Dec 2012	Microfluidic Liver Array for Long Term *In Vitro* Hepatocyte Culture and Screening	Develop Validate	The goal of this proposal is to complete development of a microfluidic liver array (MLA) platform for improved and lower cost *in vitro* toxicity screening targeting the human liver. This will lead to the commercialization of a product with widespread application in the biopharmaceutical and chemical safety industry as an *in vitro* alternative to animal testing. In order to commercialize this technology, it is necessary to more fully validate the long term biologic functions of human hepatocytes cultured in the MLA, and compare with the best current *in vitro* and *in vivo* data. This will ensure that the MLA is rigorously tested against industry relevant benchmarks to maximize the commercial utility of the novel technology.	Reduce Replace	Grant 4R44ES019035-02
Other (Hepato-toxicity)	NIEHS	Project start/end dates Feb 2010 -Jan	TeamChip for High-throughput, Predictive Human Metabolism and	Res	The TeamChip is being developed to mimic the first-pass metabolism of the human liver and to predict enzyme-specific hepatotoxicity, providing for high-throughput analysis of systematic drug candidate and	Reduce Replace	1R41ES018022-01

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		2012	Toxicology		chemical metabolism and toxicology. The ultimate goal is to provide pharmaceutical researchers with the information needed to predict the *in vivo* metabolism of drug candidates, and thus help to decide which compounds are brought forward for lead optimization and the ultimate development of better and safer drugs. Furthermore, this research is relevant to the prioritization of industrial and environmental chemicals in terms of their safety and use.		
Other (Nephro-toxicity)	NIEHS	Project start/end dates Sept 2010-Aug 2011	High Throughput Mitochondrial Nephrotoxicant Assay	Develop	Grantee has developed primary cultures of renal proximal tubular cells (RPTC) that exhibit *in vivo* levels of aerobic metabolism, are not glycolytic, and retain higher levels of differentiated functions. In conjunction, a new technology (Seahorse Extracellular Flux Analyzer) is used to measure cell metabolism in real time. The long-term goal of this proposal is to merge the RPTC model and the Seahorse technology to develop a quantitative high-throughput assay to measure the effects of toxicants on renal mitochondrial function. This assay system will identify nephrotoxicants with mechanism-based criteria for assessment of new drugs, consumer products, and environmental agents. The final results of the proposed research will be a quantitative high-throughput assay that can assess new drugs, consumer products, and environmental agents for their potential to cause kidney damage in humans.	Reduce Replace	Grant 1R43ES019378-01
Other (Non-mammalian testing models)	NIEHS	Project start/end dates Sept 2009 - Jun 2011	High-Content Analysis Tools for Developmental Toxicity Screens in Zebrafish	Res	To date, the quantitative evaluation of zebrafish assays is performed semi-manually at best, which constitutes a significant bottleneck in terms of workflow and screening cost. In Phase I research, we will specialize software algorithms that will enable automated quantitative assessment of various toxicity endpoints, thus allowing the screening of a large number of potentially toxic compounds.	Reduce	Grant 1R43ES017590-01

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Other (Non-mammalian testing models)	NIEHS	Project start/end dates Sept 2008 – Apr 2012	System for Comprehensive Tracking and Analysis of *C. elegans* Behaviors	Develop	Computerized microscope technology and software to track movement and behavior of *C. elegans* in toxicology studies. Introduction of this robust commercial software solution will significantly contribute to further replacement and reduction of conventional toxicology tests by alternative in vivo toxicology assays using *C. elegans*.	Reduce Replace	Grant 5R44ES017180-03
Other (Non-mammalian testing models)	NIEHS	Ongoing	Phase 2 SBIR: Zebrafish Cytochrome P450 Assays for Assessing Drug Metabolism	Res	Screening assay in zebrafish for effects of toxicants on Cyp P450 induction - transgenic human fluorogenic substrates.	Reduce	Grant 5R44ES017366-04
Other (Non-mammalian testing models)	NIEHS	Ongoing	Moderate Throughput Non-invasive Toxicity Assays in *Brachydanio rerio*	Res	Develop an imaging system based on optical coherence tomography (Doppler-OCT) to evaluate morphological and physiological changes in zebrafish embryos exposed to toxicants.	Reduce Refine	SBIR Contract
Other (Toxicology database)	NIEHS	Ongoing	Integrated Prediction Systems to Support Environmental Science	Valid	Develops software and a user-friendly interface to integrate chemical and toxicology databases	Reduce Refine Replace	SBIR Contract
Acute Systemic Toxicity	NIEHS-NTP	Complete	Monitor and Collaborate with ECVAM on the ACuteTox Project	Res Devel Trans Valid	NICEATM and the ICCVAM/ATWG will monitor progress and provide input for ECVAM's ACuteTox Project to develop *in vitro* tests and other methods necessary to achieve accurate acute oral hazard classification in order to further reduce and potentially replace animals for this purpose. The ECVAM project implements recommendations from the 2000 ICCVAM Workshop on this topic.	Reduce, Replace	Final report pending (May 2011)
Acute Systemic Toxicity	NIEHS-NTP	Ongoing	Up-and-Down Procedure for Acute Dermal Toxicity	Res Devel Trans Valid	NICEATM is collecting acute dermal toxicity data for use in computer simulations for future validation.	Reduce Refine Replace	FOIA submitted to EPA requesting data - responses ongoing
Acute Systemic Toxicity	NIEHS-NTP	Complete	Mechanisms of Acute Systemic Toxicity and Lethality Workshop	Res	NICEATM and ICCVAM organized an international workshop on Advancing In Vitro Approaches and Humane Endpoints for Systemic Toxicity Evaluations. Workshop report published in 2009	Reduce Refine Replace	Workshop report published

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					(http://iccvam.niehs.nih.gov/methods/acutetox/Tox_workshop.htm)		
Acute Systemic Toxicity	NIEHS-NTP	Ongoing	*In Vitro* Cytotoxicity of Mixtures	Valid	Determine the usefulness of the 3T3 NRU test method for reducing and refining the use of animals for the acute oral systemic toxicity testing of chemical mixtures 1. Collect acute historical oral LD50 values for mixtures from standardized acute oral toxicity test methods with rats (provided by regulatory agencies and/or chemical manufacturers). 2. Prospectively test mixtures using *in vitro* 3T3 NRU as they undergo mandatory *in vivo* safety testing by industry, where in vivo data will be made publicly available	Reduce Refine Replace	Encourage Industry to submit data – no data received as of May 2011
Biologics/ Vaccines	NIEHS-NTP	Complete	Botulinum Toxin Workshop Report	Res	NICEATM published a workshop report detailing the discussions and output from the meeting (http://iccvam.niehs.nih.gov/methods/biologics/botulinum.htm)	Reduce Refine Replace	Workshop report published
Biologics/ Vaccines	NIEHS-NTP	Ongoing	*In Vitro* Methods for Detecting and Quantifying BoNT	Valid	In April 2011, NICEATM received a nomination for three in vitro methods proposed for identifying BoNT and/or quantifying BoNT potency for one or more BoNT serotypes. The nomination is for potential validation studies or other necessary activities to demonstrate the usefulness of these methods for these purposes.	Reduce Replace	Nomination pending final prioritization
Dermal Toxicity	NIEHS-NTP	Complete	Evaluation of EpiDerm™ and EPISKIN™ Dermal Irritation Assays for Classifying Dermal Irritants	Valid	NICEATM will support the ICCVAM Evaluation of EpiDerm™ and EPISKIN™ in vitro dermal irritation assays for predicting US (i.e., EPA and FHSA) and GHS hazard classifications for dermal irritants, and the independent scientific peer review, and development of ICCVAM recommendations for agencies	Reduce Refine Replace	OECD TG 439 adopted 2010
Dermal Toxicity	NIEHS-NTP	Ongoing	Evaluation of EpiDerm™ and EPISKIN™ Dermal Irritation Assays for Identifying Corrosive	Res Devel Trans Valid	ICCVAM has concluded that an evaluation is needed of the EpiDerm™ and EPISKIN™ dermal irritation assays for their utility in identifying corrosive substances that are false negatives in *in vitro* corrosivity tests, a pre-requisite for consideration of these methods as a way to	Reduce Refine Replace	Testing phase to be complete Summer 2011

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			Compounds Not Detected in *In Vitro* Corrosivity Assays		make dermal assessments without the use of any animals. This was endorsed by ICCVAM as a high priority activity.		
Endocrine Active Substances	NIEHS-NTP	Complete	International Validation Study of the LUMI-Cell ER TA Assay	Valid	Joint Validation Study with ECVAM and JaCVAM to validate the LUMI-Cell ER TA assay for detecting agonists and antagonists; draft recommendations and performance standards under consideration	Reduce Refine Replace	Peer Review Panel report published May 2011
Endocrine Active Substances	NIEHS-NTP	Ongoing	International Validation of the CERI ER TA Assay for Detecting Antagonists	Valid	JaCVAM requested that NICEATM consider participating in an international validation study of the CERI ER TA Antagonist assay, after OECD has finished peer review of the agonist assay.	Reduce Refine Replace	Validation study in progress
Endocrine Active Substances	NIEHS-NTP	Ongoing	Validation of the CertiChem MCF-7 ER TA Assay	Valid	Joint Validation Study with JaCVAM and KoCVAM to validate the CCi MCF-7 assay for detecting agonists and antagonists	Reduce Refine Replace	Testing phase complete Spring 2011; data analyses ongoing
Genetic Toxicity	NIEHS-NTP	Ongoing	International Validation of the *In Vivo* and *In Vitro* Comet Assays	Valid	Participate on Study Management Team with ECVAM in JaCVAM-sponsored international validation of (1) the *in vivo* Comet assay as a replacement for the currently accepted *in vivo* rat hepatocyte UDS assay, and (2) the *in vitro* Comet assay as a potential screening assay/replacement for the *in vivo* Comet assay	Reduce Refine Replace	Validation studies in progress: Phase IV of *in vivo* study completed Oct 2010; validation report expected in 2011 Phase III of *in vitro* study ongoing
Genetic Toxicity	NIEHS-NTP	Ongoing	*In Vitro* Cell Transformation Assays	Valid	Support ICCVAM commenting on the OECD draft report on the validation status of *in vitro* cell transformation assays as a screening test/replacement for cancer bioassays	Reduce Refine Replace	OECD currently considering future activities
Genetic Toxicity	NIEHS-NTP	Complete	*In Vitro* Micronucleus Test	Valid	Support ICCVAM commenting on the validation status of the *in vitro* micronucleus test as an OECD test guideline, as an alternative to the *in vitro* chromosomal aberration test	Replace	TG 487 approved Dec 2009
Immuno-	NIEHS-	Ongoing	Validation Status of	Valid	Support the ICCVAM Evaluation of the validation	Reduce	Updated OECD

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toxicity	NTP		the LLNA: • Reduced LLNA (rLLNA) • Non-radioactive tests • Performance standards • Potency Categorization		status of alternative protocols and uses for the LLNA: • rLLNA • Non-radioactive LLNA versions • Developing performance standards • Potency categorization	Refine Replace	TG 429; New OECD TG 442A/B adopted by OECD in 2010. Agency acceptance 2011; Potency recommendations currently being transmitted to agencies (May 2011)
Ocular Toxicity	NIEHS-NTP	Complete	Topical Anesthetics/ Systemic Analgesics and the Draize Eye Test	Valid	Support the ICCVAM evaluation of using topical anesthetics, systemic analgesics, and humane endpoints in the rabbit eye test to eliminate or reduce pain and distress associated with this procedure	Refine	Agency acceptance 2011; proposed update to OECD TG 405 - Awaiting April 2011 WNT decision
Ocular Toxicity	NIEHS-NTP	Complete	Detection of Mild/ Moderate Eye Irritants Using BCOP, IRE, ICE, or HET-CAM	Valid	Support the ICCVAM evaluation of 4 *in vitro* ocular toxicity test methods for identifying mild/moderate eye irritants by preparing comprehensive background review documents; Recommendations for further optimization and studies	Reduce	Recommendations endorsed by agencies 2011
Ocular Toxicity	NIEHS-NTP	Ongoing	ALTTOX		Development of a publicly available database of existing *in vivo* rabbit eye test data		Data entered as they are received
Ocular Toxicity	NIEHS-NTP	Ongoing	Development of a Histopathology Atlas and Associated Decision Criteria for the *In Vivo* Rabbit Eye Test and for *In Vitro* Tests that use Intact Eyes or	Devel Trans	NICEATM (in partnership with ECVAM and JaCVAM) will create an international working group to facilitate the collection of reference micrographs of chemically induced ocular lesions in excised corneas and enucleated eyes used in an *in vitro* ocular toxicity test method (rabbit, chicken, pig, bovine) and from eyes of rabbits used in *in vivo* tests. NICEATM will use the detailed reference atlas of chemically induced ocular	Reduce Refine Replace	Draft Guidance Document submitted to OECD: Use of Histo-pathology as an additional endpoint in Ocular Safety Testing –

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			Corneas		lesions to create a standardized scoring system for the evaluation of these lesions. Decision criteria for the BCOP, ICE, and IRE test methods will be revised to utilize histological endpoints as a component for hazard classification.		Awaiting April 2011 WNT decision
Ocular Toxicity	NIEHS-NTP	Complete	PPDC Antimicrobial Project	Valid	Review of non-animal methods and approaches for determining the eye irritation potential of antimicrobial cleaning product formulations; recommendations for further studies	Reduce Refine Replace	Recommendations endorsed by agencies 2011
Pyrogen-icity	NIEHS-NTP	Ongoing	Validation Status of *In Vitro* Pyrogenicity Tests using Human Cells	Valid	In April 2011, NICEATM received a nomination for an *in vitro* pyrogenicity test method that uses cryopreserved human blood cells intended as replacements for the rabbit pyrogen test. This method is one of five methods that were previously reviewed and recommended to agencies for identifying Gram-negative endotoxin. The nomination is for validation studies to demonstrate the usefulness of this method for identifying non-endotoxin pyrogens.	Reduce Replace	Nomination pending final prioritization
Targeted Testing Areas	NIEHS-NTP	Future Possible Activities	1. Targeted Research Grants 2. SBIRs (Devel and prevalidaton) 3. Validation Contracts 4. NICEATM validation studies	Res Devel Trans Valid	NICEATM works with ICCVAM to organize Workshops, Scientific Symposia and Expert Panels to identify high priority research, development, translation, and validation activities considered necessary to advance alternative test methods for specific toxicity endpoints. These are potential mechanisms available to carry out high priority activities.	Reduce Refine Replace	Reports of Workshops, Symposia, and Expert Panels
Targeted testing areas (High-throughput screening)	NIEHS-NTP	Ongoing	Tox21 Phase 1	Res	Using a quantitative HTS (qHTS) approach, the NIH Chemical Genomics Center (NCGC) screens compounds for activity in biochemical- and cell-based assays. Endpoints assessed included phenotypic readouts, apoptosis, membrane integrity, mitochondrial toxicity, gene tox and cell signaling. Concurrently, EPA's NCCT screened 309 unique compounds across more than 500 biochemical- and cell-based assays in their ToxCast™ program. These compounds were also tested in NTP's "WormTox" lab.*	Reduce, Replace	Work being conducted in partnership with FDA and EPA.

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Targeted testing areas (High-throughput screening)	NIEHS-NTP	Near future (begin mid-2011)	Tox21 Phase 2	Res	In the next phase, the "Tox21" partners will test an expanded library of more than 10,000 unique compounds. The initial focus will be on nuclear receptors and stress response pathways, with data being used to assess data reproducibility. Approximately 700 of these compounds will be tested in Phase II of EPA's ToxCast™ program. The NTP, EPA, FDA, and NCGC will establish a full spectrum of secondary and tertiary screening assays to further define and characterize activities identified in initial high throughput screens.*	Reduce, Replace	Work being conducted in partnership with FDA and EPA.
Other – Informatics	NLM	Ongoing	Access to Information on *in Silico*, *In Vitro*, and Improved (Refined) Animal Testing Methods, Along With Information on the Testing Strategies Incorporating These Methods and Other Approaches	Other	The NLM is the world's largest biomedical library and its resources are accessible for free by global users. NLM is developing an enhanced version of its ALTBIB® Web portal to provide better access to information: a) on *in silico*, *in vitro*, and improved (refined) animal testing methods, b) about which methods have been validated or are in the process of being validated, and c) on the testing strategies incorporating these methods and other approaches.	Reduce Refine Replace	
Ocular Toxicity	NIH	Project start/end: Sept 2010-Aug 2012	Replacement Ocular Battery (ROBatt)	Dev Valid	Development and prevalidation of the Replacement Ocular Battery (ROBatt), a tiered testing strategy consisting of a battery of four alternative ocular irritancy assays: the Bovine Corneal Opacity and Permeability Assay (BCOP), the Chorioallantoic Membrane Vascular Assay (CAMVA), the Porcine Corneal Reversibility Assay (PorCORA) and the Porcine Confocal Assay (PorFocal). ROBatt is intended to replace regulatory mandated acute ocular irritation testing using the Draize Rabbit Eye test, and could significantly reduce the number of rabbits used in the toxicological assessment of consumer products, chemicals, and raw materials.*	Reduce, Replace	Grant 1U01NS073481
Other (Non-mammalian	NIH-NCRR	Project start/end	A Resource Center for *Tetrahymena*	Res	This resource provides a centralized repository for genetically distinct strains of *Tetrahymena*	Replace	Project 5P40RR019688-

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testing models)		Apr 2004-Mar 2014	*thermophila*		*thermophila*, a ciliated protozoan that has served as a key model for studies of eukaryotic cellular and molecular biology for more than 50 years. The stock center will: 1) collect, annotate, and store experimentally useful cell lines; 2) establish a database that will provide essential information about these strains to the community at-large; and 3) supply actively growing cultures to researchers around the world.		07
Other (Non-mammalian testing models)	NIH-NCRR	Project start/end June 2003-May 2013	Preparation and Distribution of Adult Stem Cells	Res	The overall objective is to establish a center for preparation, quality testing, and distribution to multiple investigators of the adult human, rat, and mouse bone marrow stromal cells (also known as mesenchymal stem cells), referred to as MSCs.	Replace	Project RR017447-08
Other (Non-mammalian testing models)	NIH-NCRR	Project start/end May 1996-Mar 2014	National Resource for *Aplysia*	Res	This resource provides research investigators with laboratory-reared *Aplysia californica* of known age and standardized environmental background, as well as their food source. Primary goal is to optimize and standardize *Aplysia* used by NIH investigators.	Replace	Project RR010294-15
Other (Non-mammalian testing models)	NIH-NCRR	Project start/end Sept 2009-Sept 2011	Zebrafish International Resource Center	Res	The objective of the Zebrafish International Resource Center is to provide a central repository for materials and information about zebrafish research, as well as a stock center for wild-type and mutant strains of zebrafish (*Danio rerio*). Materials and zebrafish strains are distributed to the research community.	Replace	Project RR012546-13
Other (Non-mammalian testing models)	NIH-NCRR	Project start/end Apr 2002-Mar 2013	*Drosophila* Genomics Resource Center	Res	The Drosophila Genomics Resource Center (DGRC) collects and distributes reagents and materials essential for *Drosophila* genomics research, including large clone sets, common transformation vectors, cell lines, and DNA microarrays. It also tests emerging genomics technologies and provides users with guidance in the use of resources.	Replace	Project 5P40RR017093-08
Other (Non-mammalian testing	NIH-NCRR	Ongoing	Bloomington *Drosophila* Stock Center	Res	The center collects, maintains, and distributes ~23,000 genetically defined strains of *Drosophila melanogaster* with significant research value.*	Replace	Project RR007054

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models)							
Other (Non-mammalian testing models)	NIH-NCRR	Ongoing	*Caenorhabditis* Genetics Center	Res	The *Caenorhabditis* Genetics Center (CGC) acquires, maintains, and distributes genetic stocks and information about stocks of the small free-living nematode *Caenorhabditis elegans*. The CGC maintains a searchable strain database accessible from the CGC website. This site also provides general information about *C. elegans* and links to key websites of use to scientists, including WormBase and WormBook.*	Replace	Project RR072097
Other (Non-mammalian testing models)	NIH-NCRR	Ongoing	National *Xenopus* Resource Center	Res	Two species of *Xenopus* are commonly used by biologists, *Xenopus laevis* and *Xenopus tropicalis*. *Xenopus laevis* has been used for many years to investigate the early period of embryonic development due to the rapid development of functional organs after fertilization. The role of genes in development can be assayed by injecting a tiny amount of an mRNA encoding the gene of interest into an early embryo, then once again allowing the embryo to grow into a tadpole.*	Replace	Project RR072097
Targeted testing areas (High throughput screening)	NIH-NHGRI	Ongoing	Toxicology in the 21st Century project (Tox21)	Res	In collaboration with the EPA, the FDA, and the NTP, the NIH Center for Translational Therapeutics is a key partner in the Toxicology in the 21st Century project (Tox21). Tox21 is an initiative designed to predict the toxicity of chemicals on human health and the environment. This is accomplished by research, development, validation, and translation of new and innovative test methods that characterize key steps in toxicity pathways; included in this is the development of in vitro assays for more predictive, mechanistically-based methods than those used with current animal testing.	Reduce, Replace	Intramural activity number ZIA HG200319
Targeted testing areas (High throughput	NIH-NHLBI	Project start/end Aug 2009-July 2011	Generation of HESC Reporter Lines Using Improved Gene Targeting Technology	Res	The investigator is generating hESC lines using improved gene targeting technology. The reporter lines will allow real-time measurement of the activity of tissue-specific promoters for use in high-throughput screens and *in vivo* studies.	Replace	Grant R21HL092489

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screening)							
Targeted testing areas (High throughput screening)	NIH-NHLBI	Project start/end Aug 2009-May 2012	New Strategies and Screening Methods for Metalloproteinase Inhibition	Res	This project seeks to discover selective metalloprotein inhibitors and develop a facile method by which the selectivity of these inhibitors can be rapidly assessed. The investigator will test the new metalloprotein inhibitors using cell-based screening methods.	Replace	Grant R21HL094571-02
Other (Cardio-vascular)	NIH-NHLBI	Funded for FY2009 only	Drug Safety Assessment in IPS-Derived Cardiomyocytes	Res	The investigator is developing toxicity screens for drugs using cardiomyocytes derived from human fibroblasts.	Replace	Contract HHSN268200900044C
Other (Cardio-vascular)	NIH-NHLBI	Project start/end Sept 2007-July 2010	Human Embryonic Stem Cell-Derived Cardiomyocytes for *In Vitro* Drug Screening	Res	This is a proposal to develop human embryonic stem cell-derived cardiomyocytes for drug safety screening. The cells would be used in vitro assays related to drug effects on ion channels known to have important roles in cardiac rhythmicity, particularly drug-induced QT prolongation.	Replace	Grant R43 HL086271
Other (Cardio-vascular)	NIH-NHLBI	Project start/end July 2006-May 2011	Notch Function in Myocardial Development and Homeostasis	Res	A cell-based assay is being developed to perform a chemical screen to find potential drug candidates capable of repressing activity of a protein called CSL. In addition to avoiding subjecting mice to a drug-candidate screen, a cell-based assay would greatly reduce the cost and time required of a drug screen.	Replace	Grant 5R01HL83463
Other (Cardio-vascular)	NIH-NHLBI	Project start/end Aug 2009-Jun 2014	Percutaneous Mitral Valve Repair: A Validated Fluid-Structure Interaction Model	Res	This interdisciplinary project combines mechanical modeling, computational fluid dynamics, computer simulations, imaging data, and clinical observations. To assess the effects of pathology and proposed mitral valve surgical repair, it will utilize a computational model in which pathologic or surgical alterations can be assessed systematically.	Reduce, Replace	Grant 1 R01 HL092926-01A2
Other (Cardio-vascular)	NIH-NHLBI	Project start/end July-Dec 2010	Optimizing the Action Potential of Stem Cell-derived Human Cardiomyocytes for Cardiac Safety	Res	The investigator will develop stem-cell derived human cardiomyocytes that can be used to predict cardiac outcomes in pre-clinical toxicity studies of proposed drugs. This screening process will decrease the risk of adverse cardiac events in clinical trials, improve cost efficiency in pharmaceutical development, and reduce	Reduce, Replace	Grant R43 HL104948

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			Screening		or replace animal toxicity studies.		
Other (General toxicity testing)	NIH-NHLBI	Project start/end Aug 2010-Jan 2012	A New Toxicity Screen to Assess Mitochondrial DNA Content and Protein Synthesis	Res	The investigator will develop a new screening assay, and associated software, that will measure toxicity by assessing mitochondrial DNA depletion and inhibition of mitochondrial protein synthesis. Successful development will allow earlier identification of toxicity during the drug screening process and reduce or replace animal toxicity studies.	Reduce, Replace	Grant R43 HL105061
Other (Respiratory)	NIH-NHLBI	Project start/end June-Nov 2009	Detection of Fine Aerosols Using a Novel Aerosol Sampler	Res	Develop a personal exposure monitor for particulate matter in the ultrafine to 10 micron size range. The device will collect ambient air in the wearer's breathing zone and provide samples for analysis. The device will aid in the elucidation of the relationships between particulate exposure and adverse health outcomes.*	Replace	Grant R43 HL096248
Other (Respiratory/ Biomarkers)	NIH-NHLBI	Project start/end Sept 1997-Apr 2011	A Breath Test for Lung Cancer	Res	Develop a breath test for volatile organic compounds (VOCs) that is sensitive for primary carcinoma of the lung. VOCs are products of oxidative stress and, in lung cancer, appear to have an accelerated catabolic rate such that their altered concentrations in breath can be used as a biomarker for disease. Development of a test for early primary pulmonary carcinoma biomarkers will reduce the cost, patient burden, and potentially morbidity and mortality.	Reduce Refine	Grant R44-HL070411; results reported in journal articles
Biologics/ Vaccines	NIH-NIAID	Contract funded FY 2007-2010	Assessing Safety of Cell Substrates and Vaccine Components	Dev	This contract supports the characterization of new cell substrates and tests them for safety. Efforts to develop, characterize, and validate assays for the detection of novel or latent/occult adventitious agents are also supported.	Reduce	Project N01AI40100-6-0-1
Biologics/ Vaccines	NIH-NIAID and FDA	Ongoing	Assessing Safety of Cell Substrates and Vaccine Components	Dev	This IAA supports the development of assays and standards for detection of unknown and/or latent viruses; assessment of the oncogenicity of cellular DNA; assessment of the potential of cell substrates to propagate *Transmissible Spongiform Encephalopathy* (TSE) agents; and the development of a rapid and reliable assay for the detection of mycoplasma and other contaminating bacteria.	Reduce	Interagency activity with NIH-NIAID and FDA

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Biologics/ Vaccines	NIH-NIAID	Contract funded Oct 2005-Oct 2010	Duke University Multi-scale Systems Immunology for Adjuvant Development (MSI)	Res	The main goal of this contract is to develop mathematical models for early screening of vaccine adjuvants. The model is built on data of T and B cell activation, germinal center formation, and antibody production in response to various adjuvant/antigen combinations. The model is under development and is not designed to include possible toxic effects of adjuvants, but could be adapted by other users once it is made available to the research community.	Reduce, Replace	Contract number HHSN266620050 0019C
Other (Biomarkers of Toxicity)	NIH-NIAID	Project start/end Sept 2007-July 2012	Risk Factors for the Development of Lactic Acidosis and Pancreatitis Among HAART-Treated Adults in Botswana	Res	BMI and nucleoside analogue reverse transcriptase inhibitors treatments are associated with the higher risk of having a lactic acidosis event in HIV patients. Other ongoing analyses will assess host genetic risk factors associated with the development of lactic acidosis and pancreatitis. Identification of host factors associated with mitochondrial toxicity in HAART therapy may significantly inform public policy in the region.*	Replace	Grant 1K23 AI073141 - Extramural
Acute Systemic toxicity	NIH-NIAID	Project start/end Apr 2009-Mar 2014	Optimization of Small-Molecule Inhibitors of Shiga and Ricin Toxins	Research	This study tests the efficacy of small molecules in reducing the toxicity of Shiga and Ricin toxins, using a Zebrafish model. Because of similarities and presence of toxicity-related genes in Zebrafish, large numbers of molecules may be efficiently screened. Only promising small molecule candidates will be tested in rodents, reducing the total number of animals used for each group of compounds.	Reduce, Refine	Contact: Shahida Baqar, NIAID Grant U01AI082120
Acute Systemic Toxicity	NIH-NIAID	Project start/end Feb 2009-July 2011	Accessory Toxin-Mediated Evasion of Innate Immunity During *V. cholerae* Infection	Research	This study seeks to address the pathogenesis of *Vibrio vulnificus*, the leading cause of seafood-associated death in the United States, using a mouse model. Recovery Act funding helped purchase an animal imager to minimize the use of animals for this and affiliated projects—reducing animal usage by 80%. In addition, since the progress of pathogenicity could be imaged from the onset of infection, resolution data were greatly improved over the progression of disease in time and locality.	Reduce Refine	Contact: Robert Hall, NIAID ARRA supplément Grant R21AI072461S1

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Acute Systemic Toxicity/ High Throughput Screening	NIH-NIAID	Project start/end July 2008-Jun 2011	A *C. elegans* High-Throughput Assay for the Identification of New Antifungal Agents	Research	An assay was developed to assess the antifungal activity and toxicity of compounds against the fungal disease, *Candida albicans*. Common laboratory roundworms, called *C. elegans*, were used in this assay. A high throughput screen on the toxicity of the various compounds was performed and those compounds that killed the nematodes were not developed further.	Reduce	Contact: Rory Duncan, NIAID Grant R01AI075286-01A2
Acute Systemic Toxicity/ High Throughput Screening	NIH NIAID	Project start/end Sept 2009-Aug 2014	Identifying Novel Anti-Infectives by High Throughput Screening in Whole Animals	Research	High throughput screening of potential anti-infective compounds will be performed in non-mammal models (e.g. nematodes and fruit flies) to increase the likelihood of identifying drugs that will work in humans. This project bypasses the current bottleneck of toxicity/efficacy testing by eliminating toxic compounds (e.g. those that kill the nematodes).	Replace Reduce	Contact: Zuoyu Xu, NIAID Grant R01AI085581-02
Biologics	NIH-NIAID	Project start/end Feb 2010-Jan 2015	An *ex-vivo* model of HIV latency and reactivation using primary memory cells	Research	This study will use a novel cell-based system to address the mechanisms that HIV uses to establish latency in an infected cell, and subsequently, become reactivated. This approach is still under development. It is hoped that it eventually may reduce the number of monkeys or humanized mice used to evaluate these mechanisms. Knowledge from these areas will be applicable in future translational studies that will seek compounds mimicking or antagonizing these pathways, with the ultimate goal of destroying lately infected cells.	Reduce Replace	Contact: Karl Salzwedel, NIAID Project Link on REPORTer Published in Methods Grant 5R01AI087508-02
Neurotox-icity	NIH-NIAID	Project start/end May 2009-Apr 2011	Development of a Highly Sensitive Cell-Based Assay for Botulinum Neurotoxin	Research	A cell-based assay was developed for specific, sensitive and quantitative detection of botulinum toxins as well as serum antibodies and inhibitors against the toxin. This test provides an alternative to using mice in research. Fewer animals will have to be used to quantify the toxin's potency and mechanisms of action.	Reduce	Contact: Marian Wachtel, NIAID ; Grant R21AI082826
Targeted Testing Areas (High throughput	NIH-NIAID	Ongoing intramural activity	Integrated Research Facility (IRF) at Frederick	Research	The IRF incorporates hospital-type imaging modalities into the biocontainment environment. This innovation reduces the number of animals needed for a study by allowing sequential evaluation of tissues on a single animal over the duration of the infection. The approach	Reduce	Contact: Susanna Weiss, NIAID

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screening)					further reduces the need for animals by permitting each animal to serve as its own control.*		
Other (Non-mammalian testing models)	NIH-NIAID	Complete	Novel 3-D Mucosal Model to Study *S. typhi* Immunity	Research	This project developed and characterized a three-dimensional (3-D) model of human small intestinal epithelium. The model allowed for the investigation of the effects of enteric pathogens on intestinal epithelial cells, other intestinal mucosal cells, and cellular components of the human immune system. This innovative model had many morphological and functional similarities to animal tissues. In particular, preliminary data showed the usefulness of this system in studying the early effects of the bacterium that causes typhoid fever. These models might eventually serve as a means to reduce the number of animals used in research, or eventually replace them entirely.	Reduce Replace	Contact: Melody Mills, NIAID Salerno-Gonçalves et al. accepted for publication in *Gastroenterology* – April 2011 Grant N01AI30028
Other (Non-mammalian testing models)	NIH-NIAID	Ongoing	Vascularized Organotypic Model of the Human Intestinal Mucosa	Research	This project aims to optimize an existing 3-D model of the human intestinal mucosal epithelium. Specifically, it will develop blood vessel-like conduits that would more closely mimic the human gastrointestinal environment. Then, the usefulness of this system will be assessed by exposing the system to various enteric pathogens.	Reduce Replace	Contact: Melody Mills, NIAID Grant U19AI82655
Targeted Testing Areas (High throughput screening)	NIH-NIAMS	Project start/end July 2008-Jun 2011	Matrix-Induced Myogenesis & Pharmaco-Screens of MSCS	Res	Researchers are developing tissue-mimetic cell culture models of normal and diseased muscle formation, with the goal of screening an NIH library for drug candidates that direct matrix-coupled myogenesis of stem cells. Existing tissue-culture matrixes will be adapted to low and high throughput screening formats.*	Replace	Grant 5R21AR056128-02
Targeted Testing Areas (High throughput screening)	NIH-NIAMS	Project start/end July 2010-Jun 2011	Discovery of Inhibitors of PTH-WNT Signaling Synergy in Bone Cells	Res	DiscoveryBioMed, Inc., (DBM) has developed a light-based assay, high-throughput screening (HTS) friendly bioassay that it seeks to 'multiplex' with additional light-based endpoints in cellular lysates and in the supernatant collected before cell lysis to monitor a series of endpoints relevant to osteoporosis drug discovery. The screening program utilizes a mammalian bone cell line as the biologically relevant	Replace	Grant 1R43AR060111-01

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					cellular platform.		
Immuno-toxicity	NIH-NIAMS	Project start/end Dec 2004-Aug 2011	*In Vitro* Tissue Model of Psoriasis	Res	Investigators are developing a full-thickness 3-dimensional immuno-competent psoriatic tissue model that pharmaceutical companies and the academic institutions working in the field of psoriasis can use for high-throughput screening of potential immunosuppressive agents.	Replace	Grant 5R44AR052982-03 (ARRA)
Other (General toxicity testing)	NIH-NIAMS	Project start/end July 2010-Jun 2012	*Drosophila* as a Model for Emery-Dreifuss Muscular Dystrophy	Res	Researchers developed a fruit fly model of Emery-Dreifuss muscular dystrophy (EDMD) for whole organism drug screens.	Replace	Grant 1RC1AR058118-01
Other (Muscular disease)	NIH-NIAMS	Project start/end Jan 2010-Jan 2012	Examining The Therapeutic Potential of IPS Cells in Duchenne Muscular Dystrophy	Res	A novel method is being used to generate muscle progenitors from mouse ES cells, both to wild-type mouse induced pluripotent stem cells (iPSC) and *ex vivo* genetically corrected dystrophic mouse iPSC, to assess whether these cells have *in vivo* regenerative potential. Researchers will also investigate the mechanisms controlling muscle differentiation in human ES cells to apply this knowledge to human iPSC obtained from patients with Duchenne muscular dystrophy.*	Replace	Grant 1RC1AR058118-01
Other (Muscular disease)	NIH-NIAMS	Project start/end Sept 2009-Aug 2011	FSHD IPS Cells: Modeling Disease Mechanisms, Genetic Correction and Cell Therapy	Res	To investigate the disease mechanism of fascioscapulohumeral muscular dystrophy (FSHD) and potential cell therapy, researchers derived iPS cells from myoblast cultures of cells from FSHD patients and unaffected individuals. FSHD-affected cells are now being used to better understand of this disease and its potential genetic therapy.*	Replace	Grant 5RC2AR058919-02
Other (Muscular disease)	NIH-NIAMS	Project start/end Aug 2009-Jun 2014	Histology and Clinical Repository Core	Res	Facility at the University of Minnesota Muscular Dystrophy Clinic will provide researchers with tissue or cultured cells for detailed analysis of muscle structure and function. Research on clinically defined specimens will allow fibroblasts to be studied directly, differentiated into myoblasts, or used to generate	Replace	Grant 5P30AR057220-02

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					induced pluripotent stem cells (iPSC) lines.*		
Other (Muscular disease)	NIH-NIAMS	Project start/end Apr 2010-Mar 2012	Transplantability of Induced Pluripotent Stem Cells for Skeletal Tissues	Res	Researchers are assessing migration, engraftment, and differentiation of cells derived from induced pluripotent stem cells (iPSC) into skeletal tissues following systemic transplantation. They anticipate the results will provide a platform for future investigations of iPSC cells in musculoskeletal tissue repair and regeneration.	Replace	Grant 5R21AR059383-02
Biologics/ Vaccines	NIH-NIBIB	Project start/end Jul 2008-Jun 2011	A New Experimental Platform to Study Biofilms: Microfluidic-DHM	Res	This grant will develop a new experimental platform for the study of biofilms, or bacterial consortia living on surfaces. Our approach is to integrate microfluidics and digital holographic microscopy to create a powerful new platform for biofilm studies. If this new platform is successful, many interventional strategies can be extensively studied *ex vivo,* thus greatly reducing the number of animal-based experiments.*	Reduce, Replace	Grant 5R21EB008844-02
Neuro-toxicity	NIH-NIBIB	Project start/end Sept 2007-May 2012	Microfluidic Patch Clamp Chips for Multi-Unit, High Throughput Recordings	Res	Ion channels play key roles in all known brain functions. We propose to develop a patch clamp chip design for monitoring multiple cells on the surface of brain slices. We will use the device to investigate the propagation of neuronal signals across developing cortical networks. This project deals with development of a platform that enables investigation of drug effects on ion channels, studies which could identify side effects prior to clinical testing.	Reduce, Refine	Grant 5R01EB007526-03
Ocular toxicity	NIH-NIBIB	Project start/end Aug 2008-Jul 2013	A Virtual Tissue Simulator for Biomedical Optics	Res	This work aims to provide a tool to realistically simulate the impact of tissue properties and organization on light-tissue interactions. This tool will enable researchers to examine the impact of tissue transformations on optical signals and thereby provide critical guidance for improved design of optical probes/instrumentation used for therapeutic/diagnostic applications. The availability of such a tool will decrease the use of animal models in assessing the effectiveness of such devices under development.	Reduce, replace	Grant 5K25EB007309-03

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Targeted Testing Areas (Computer modeling)	NIH-NIBIB	Project start/end Sept 1997-Aug 2013	Biomedical Simulations Resource	Res	Investigators are developing models for pharmacokinetic and pharmacodynamic systems analysis of drug therapies in multiple clinical applications and to examine the influence of genetic factors on drug kinetics and dynamics (pharmacogenetics), to better quantify intersubject differences in drug action, and to estimate in vivo drug potency when the drug target is itself subject to endogenous regulation.	Reduce	Grant 5P41EB001978-26
Targeted Testing Areas (Computer modeling)	NIH-NIBIB	Project start/end Dec 2003-Aug 2011	A 3-D Interactive Atlas of the Hand and Wrist Joints	Res	The digital anatomical libraries of small joints produced from this project will help to further the understanding and treatment of the musculoskeletal system. This grant aims to produce useful models of the hand and wrist joints. Having a library on hand of these joints gives developers the ability to test the safety of a product over a range of shapes and sizes of joints.	Replace	Grant 5R44EB003067-04
Other (Cardio-vascular)	NIH-NIBIB	Project start/end Sept 2007-Aug 2011	Optimizing Cardiovascular Devices Thrombogenicity for Eliminating Anticoagulation	Dev	A thrombogenicity predictive technology for blood contacting cardiovascular (CVS) devices will be developed. This research will develop a predictive capability to anticipate potential thrombus formation stemming from implanted cardiovascular devices, as well as a rational means to redesign such devices to avert blood clots. This *in silico* approach has the potential to greatly reduce or even eliminate the need for *in vivo* animal testing to screen prototype cardiovascular implants.*	Reduce, Replace	Grant 5R01EB008004-03
Other (Cardio-vascular)	NIH-NIBIB	Project start/end Sept 2009-Aug 2011	Resistance to Aortic Endograft Migration: Comparative Effectiveness of FDA Approved Devices	Res	This study provides a novel longitudinal approach to study how effectively approved aortic endograft devices function and perform *in vivo*. Although this study is conducted in humans, if the approach proves viable, it offers an excellent approach to follow the function of implants in animals over time, thus greatly reducing the need to sacrifice animals at intermediate time points.	Reduce	Grant 5RC1EB011443-02

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Other (Computer modeling)	NIH-NIBIB	Complete	Molecular Modeling of Bioactive Agent Release from Structural Biomaterials	Res	Polymers used in implanted medical devices such as artificial arteries may host bacteria, or support fungal growth. To counter this, agents such as antibiotics that leach out over time are incorporated into the polymer matrix. This work which models the polymer-agent interactions to predict and optimize the leaching rate, will lead to a minimization of the animal testing required to ensure safety and effectiveness.	Reduce, Replace	Grant 1R15EB012297-01
Other (General toxicity testing/ computer modeling)	NIH-NIBIB	Project start/end Jul 2006-Apr 2011	Optimizing Coordinated Combination Drug Therapy	Res	This laboratory has developed parametric and especially nonparametric (NP) population modeling software to capture these relationships with statistical consistency and precision. This work should greatly improve understanding and control of combination and interacting drug relationships, and the quality and precision of combination drug therapy for patients who must receive potentially toxic drugs.	Reduce	Grant 5R01EB005803-04
Other (General toxicity testing/ high-throughput screening)	NIH-NIBIB	Project start/end Sept 2004-Feb 2015	Raman Flow Cytometry for Diagnostics and Drug Delivery	Dev	This project aims to significantly increase the analysis capabilities of flow cytometry by incorporating Raman spectral analysis capabilities. The result of this work will be a significant new tool for the highly multiparameter analysis of cell systems to help understand, diagnose, and prevent disease. Flow cytometry has the potential to be used for evaluation of preclinical toxicity of drugs through development of assays that provide information on cell functions.	Reduce Refine Replace	Grant 2R01EB003824-07
Other (General toxicity testing/ high-throughput screening)	NIH-NIBIB	Project start/end Sept 2009-Aug 2011	High Throughput Screening in Human 3D Spheroids of Epithelial, Endothelial Culture Systems	Res	Whilst 2D monolayers of human cell lines are routinely utilized for high throughput screening, the observed effects are rarely recapitulated *in vivo*. By generating modular 3D tissue culture models comprising multiple cell types a more physiological environment can be created. Comparison of engineered 3D co-cultures of normal vs. cancerous cells will thus facilitate rapid evaluation of the safety and efficacy of drug candidates in a more applicable cellular context.	Replace	Grant 5RC1 EB 11780-02
Other (General	NIH-NIBIB	Project start/end	High-Throughput Vibrational	Res	A non-invasive flow cytometer system, capable of providing chemically specific information, is proposed	Reduce Refine	Grant 1R21EB011703-

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toxicity testing/ high-throughput screening)		May 2010-Apr 2012	Cytometry		and will be developed and validated. The proposed technology has potential applications in pathology, immunology, toxicology, and pharmacology, and could also be used for evaluation of preclinical toxicity of drugs through development of assays that provide information on cell functions.*	Replace	02
Other (General toxicity testing/ high-throughput screening)	NIH-NIBIB	Project start/end Feb 2009-Jan 2011	High-Throughput Analysis of Cell Response to Chemical Libraries	Res	The overall goal of the proposed research is to develop a microarray for the high-throughput analysis of cell behavior in response to chemicals in their microenvironment. This proposal aims to make a significant impact on the ability to screen and understand cellular behavior and to result in a significant scientific impact. This project deals with development of a platform that enables cell assays with the potential for identifying potential drug toxicities.	Reduce Refine Replace	Grant 5R21EB009196-02
Other (General toxicity testing/ high-throughput screening)	NIH-NIBIB	Project start/end May 2010-Apr 2012	Compact High-Performance Microfluidic-Based Flow Cytometer	Dev	In this program, we will develop and demonstrate a rugged, fluidic-chip-based, multi-parameter flow cytometer that is functionally appropriate for POC applications and capable of the performance dictated by clinical diagnostic requirements. Flow cytometry has the potential to be used for evaluation of preclinical toxicity of drugs through development of assays that provide information on cell functions.	Reduce Refine Replace	Grant 1R21EB011662-01
Other (Hepato-toxicity)	NIH-NIBIB	Project start/end Jul 2008-Jun 2010	Development of 3D Micro-scale Engineered Tissue Model Systems for Drug Discovery	Res	Development of new porous scaffolds to facilitate 3D culture of liver. These constructs will be used for candidate drug screening.	Replace	Grant R21 EB 8573-03
Other (Non-mammalian testing models)	NIH-NIBIB	Project start/end Sept 2009-Aug 2014	Perfused 3D Tissue Surrogates for Complex Cell-Cell Communication Systems	Res	The goal of this project is to build models of primary human systems to serve as close mimics of *in vivo* complexity.	Replace	Grant 5R01 EB 10246-02
Dermal Irritation	NIH-NICHD	Project start/end Sept	Validation of a Human *In Vitro* Vaginal Irritation	Res	Researchers are developing a human reconstructed tissue based system that may provide a sensitive and validated assay method for screening	Reduce	Grant 5 R44HD050023-03

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		2005-Aug 2012	Test		chemicals/formulations that may potentially irritate the vagina. Developing this approach could reduce the use of laboratory animals.*		
Targeted Testing Areas (High-throughput screening)	NIH-NIDCD	Project start/end June 2010-May 2015	Screens for Modulators of Hair Cell Regeneration	Res	High throughput screen for drug molecules that protect ears from ototoxic drugs in zebrafish.	Replace	Grant 1R01DC011269-01
Targeted Testing Areas (High-throughput screening)	NIH-NIDCD	Project start/end Apr 2009-Mar 2014	Identifying and Characterizing Chemical Modulators of Hair Cell Death	Res	Candidate otoprotective and ototoxic drugs will be identified with a high throughput screen using the zebrafish lateral line.	Replace	Grant 5K08DC009631-03
Targeted Testing Areas (High-throughput screening)	NIH-NIDCD	Project start/end Apr 2003-Jun 2013	Genetics of Zebrafish Hair Cell Toxicity	Res	This research will characterize five zebrafish mutants that are not susceptible to ototoxic drugs, identify molecular pathways involved in hair cell death, screen for genes and drugs that alter the response to aminoglycosides in zebrafish, and determine the degree that these findings can be extended to mammals.	Replace	Grant 5R01DC005987-08
Other (General toxicity testing)	NIH-NIDCR	Project start/end Sept 2009-May 2013	Non-invasive Assessment of Tissue Engineered Human Oral Mucosa	Res	The goal of this activity is to develop noninvasive assays to test the viability, composition and metabolic activity of grafted *Ex-vivo* Produced Oral Mucosa Equivalent (EVPOME). Noninvasive Raman spectroscopy will be used to examine the viability and function of engineered EVPOME and identify markers of abnormal EVPOME. This integrative approach can provide a useful platform for safety testing of engineered human oral mucosa that does not involve animal testing.*	Replace Refine	Grant R01 DE019431
Other (General toxicity testing)	NIH-NIDCR	Project start/end Sept 2009-Aug 2011	Development of Induced Pluripotent (iPS) Cells to Study Craniometaphyseal Dysplasia in Humans	Res	Human iPS cells will be developed from skin biopsies obtained from individuals with craniometaphyseal dysplasia (CMD) and normal subjects and optimize protocols for efficient differentiation of these cells into osteoblasts. Cells will then be used for studies of CMD	Reduce, Replace	Grant R21 DE019892

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					mechanisms, and for safety and efficacy screening of therapeutics for CMD. Such screens would minimize or eliminate animal testing.*		
Immunotoxicity	NIH-NIDDK	Ongoing 2006-2012	Friendly Immunosuppression for ENDocrine Regeneration	Res	Project will develop several advanced experimental models to test the impact of select immunosuppressive agents on mouse and human beta cell regeneration. The results of these experiments will provide important information for the design of clinical trials and the design for future immunosuppressive agents for the treatment of type 1 diabetes.	Reduce, Replace	Grant U01-DK-072473
Other (General toxicity testing)	NIH-NIDDK	Phase 2 Ongoing 2007-2012	Nuclear Receptor Signaling Atlas (NURSA)	Res	Goal is to better understand the structure, function and role in disease of nuclear receptors. Many of the targets of NR action include genes that code for proteins with roles in detoxification of drugs and toxins. Assays based on binding to NRs and measurement of effects on targets specific to cellular detoxification could represent a powerful analytical tool in drug and product safety testing.	Reduce, Replace	Grant U19-DK-062434; extramural
Other (Nephrotoxicity/ Biomarkers)	NIH-NIDDK	Ongoing, multiple projects	Development of Novel Biomarkers for Preclinical Testing of Agents for Treatment of Renal Toxicity	Res/Dev	The overall goal of these studies is to develop and validate urinary and serum biomarkers of acute kidney injury that will identify the onset and severity of kidney injury at an earlier stage than is currently possible. The FDA recently qualified a set of markers for pre-clinical testing and evaluation of kidney toxicity. The group is now extending their work to test if these markers can be used to monitor renal toxicity in early phase clinical studies.*	Reduce, Replace	Grants R01-DK-081695; R01-DK-072381; R01-DK-073462; R01-DK-075976
Targeted testing areas (High-throughput screening)	NIH-NIGMS	Project start/end Jun 2009-Nov 2011	Engineered Tissue-Based, High-Throughput Compound Profiling	Res	A novel high-throughput screening platform will measure drug-induced changes in the physiological properties of engineered tissues. Phase I focuses on completing the development of the PalpatorTM screening system and obtaining feedback from academic and industrial collaborators. Phase II focuses on scaling up the engineered tissue-based screening system to make it amenable to high-throughput applications in industry. This highly efficient Palpator	Reduce	Grant 5R44GM087784-03

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					screening system will be used to profile the effects of 50 commonly prescribed cardiovascular drugs on engineered heart tissues. To further validate the engineered tissue model, a library of compounds with known cardiovascular effects will be screened using the Palpator system. The combination of the engineered tissue models and the Palpator screening device will accelerate drug discovery and reduce the need (and associated costs) of extensive animal studies.*		
Targeted testing areas (High-throughput screening)	NIH-NIGMS	Project start/end Jul 2006-Oct 2012	Chemical Address Tags: A Cheminformatic & Image Data Management And Analysis Plan	Res	A new generation of microscopic imaging instruments known as "high content screening" or "HCS" systems has been developed. HCS instruments can provide preclinical, human cell-based data to complement animal studies in predictive toxicology. As a high-throughput platform, HCS systems can be used to screen large collections of small molecules in physiologically-relevant assays. To incorporate HCS technology into standard biomedical research practice, a cheminformatic and image data management and analysis plan will be developed to study the subcellular localization of fluorescent, small molecules in living cells.*	Reduce	Grant 5R01GM078200-05
Other (Computer modeling)	NIH-NIGMS	Project start/end Jun 2007-May 2012	Building and Validating Location Proteomics Databases	Res	Current approaches for measuring the effects of drugs are not able to address the large number of potential targets that these drugs may have. This project will determine through automated fluorescence microscopy and machine learning the subcellular location of thousands of proteins in NIH 3T3 cells. This approach could increase the efficiency of work being done through the extensive NIH-supported Molecular Libraries Screening Centers Network. The proposed work will use a sophisticated probabilistic model and an active learning approach to demonstrate how such effects can be learned without measuring all possible combinations of drugs and targets.*	Reduce, Replace	Grant 3R01GM075205-04S1

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Other (General toxicity testing)	NIH-NIGMS	Project start/end Jul 2004-Apr 2011	Mitochondrial Dysfunction In HAART: Point of Care Tests	Dev	Improved earlier detection of serious side effects of Highly Active Anti-Retroviral Therapy (HAART) used to treat HIV/AIDS could reduce risk to patients and avoid onset of clinical symptoms. A set of dipstick immunoassay tests are now being used in drug safety screening to identify mitotoxic side effects of new therapeutic drugs. These tests could also be used to monitor similar disturbances known to occur in many other diseases, and also to screen new therapeutic drugs for similar toxic effects and avoid their use in patients.*	Reduce, Replace	Grant 9R42GM093388-02A2
Other (Hepato-toxicity)	NIH-NIGMS	Project start/end Mar 2010-Feb 2012	Microfluidic Liver Array for Drug Metabolite Profiling	Res	Drug metabolite profiling using primary human hepatocytes has gained more importance in the past decade as it has become recognized that drug metabolism is closely related to drug safety. CellASIC is developing a microfluidic liver array (MLA) system that will allow more accurate prediction of the adverse effects of new drug compounds on human liver prior to clinical and animal studies. Key benefits include safer drugs, reduced cost, more clinically relevant data at an earlier stage, reduced reliance on animal testing, and improved understanding of toxicity mechanisms.*	Reduce	Grant 1R43GM090466-01
Acute Systemic Toxicity	NIH-NIMH	Ongoing	Toxicological Evaluation of Novel Ligands Program	Trans Valid	This program will advance the discovery of biomarkers by accelerating the development and application of novel ligands for PET, SPECT, and MRI imaging in humans by providing toxicology and safety assessment of promising, target-selective compounds, including limited assessment of novel psychoactive agents for clinical research and as potential therapeutics. The program will also provide access to toxicology consultation services and support for in vitro and in vivo toxicity testing for promising lead compounds from NIMH-relevant ligand and therapeutics development projects. The ability to collect biomarker data during early animal efficacy studies may reduce the need for preliminary toxicity screening (e.g., dose-range finding studies). As part of this program	Reduce Refine	Contract N01-271200900018C-1-0-1

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					preliminary dose-range finding toxicity studies are conducted by a variety of methods. Wherever possible, ICCVAM-approved methods (modified up-and-down procedure, in vitro predictive models) are used for accurate prediction of dose levels before required toxicology assays.*		
Acute systemic toxicity	NIH-NIMH	Ongoing	Preclinical Development of HIV-1 Vif Antagonists	Res	This project is using human peripheral blood monocyte cells (PBMCs) for in vitro testing of HIV drug cytotoxicity, thereby avoiding the exposure of people infected with HIV to these compounds.	Replace	Grant 5U19MH081836-04
Neurotox-icity	NIH-NIMH	Ongoing	Anti-HIV Neuroimmunomodulatory Therapy with Neurokinin-1 (NK-R) Antagonists: Neurotoxicity Sub-project	Res	The neurotoxic factor(s) released from HIV-1-infected cells hinder brain cell repair, and may cause severe brain cell damage, especially in the basal ganglia and hippocampus. This project employs an in vitro assay of HIV-1 neurotoxins, using monocytes isolated from healthy donors. The monocytes are first cultured in vitro, then treated with or without drug, and then infected with HIV.	Replace	Grant 5U01MH090325-02
Targeted testing areas (High-throughput screening)	NIH-NIMH	Ongoing	Macrophage Targeted Therapy for HAD and HIV Disease	Res	This project has been designed to provide information not only on potential limitations or liabilities of 80 drug candidates for HIV-associated dementia, but also on possible candidates' non-target activity. Drug targets in the high-throughput panel include transmembrane and soluble receptors, ion channels, and monoamine transporters.	Reduce, Replace	Grant 5U19MH081835-05
Neurotox-icity	NIH - NINDS	Near-future	Engineering Form and Function in Neuronal Networks	Res	Development of the ability to design and implement robust in vitro neural circuits on biochips that allows activity monitoring. Such an approach would allow neural circuits to be used as a test bed for neuroactive drug and toxin testing	Reduce	
Neurotox-icity	NIH - NINDS	Ongoing	Neural Cell Based Assays Derived from Human ES Cells	Res	Development of kits containing the reagents to propagate and reliably differentiate an improved embryonic stem cell line (WA09) primary cultures of neurons and glial cells, key cells in nervous system. The expected outcome is that researchers will have increased access to human cells of the nervous system	Reduce, Replace	

Type of Toxicity Testing	Agency	Status	Title	Type of Activity	Description (asterisk indicates entry has been edited for length)	Poten-tially Applic-able 3Rs	Other Information
					for pharmacological and toxicological studies.		
Acute Systemic Toxicity	ATSDR	Ongoing	Predictive Toxicity Methods	Res Devel Valid	Uses available computational tools and approaches to evaluate toxicity of chemicals for screening and prioritizing chemicals for further research and analysis.	Reduce Refine Replace	SAR and QSAR in Environ. Res. Vol 21: 603-618 (2010).
Targeted Toxicity Testing	ATSDR	Ongoing	Development of Methods for Mixtures Toxicity Evaluation	Res Devel	The Agency conducts hypothesis driven research to evaluate toxicity of chemical contaminants and their mixtures through cooperative agreements with research institutions and other federal agencies.	Reduce Refine Replace	Environmental Tox Pharma Vols 16, 18
Other (Biomarkers of Toxicity)	ATSDR	Ongoing	Use of Biomarkers Data (Microarray) in Computational Models	Res Devel	Incorporation of biomarkers data to develop improved computational models (such as PBPK) for risk assessment	Reduce Refine Replace	Toxicol. Letters 198: 44-48 (2010).
Acute Systemic Toxicity	DOD-MRICD	Ongoing	Cell and Tissue Models Used in Development of Medical Chemical Countermeasures	Res Devel	A variety of human cell and tissue models used *in vitro* to (a) examine the effects of chemical warfare agents and efficacy of therapeutics, (b) look for biomarkers of exposure, and (c) elucidate molecular pathways of injury.*	Replace	
Acute Systemic Toxicity	DOD-MRICD	Ongoing	Embryonic Stem Cell-Based Assay for Effects of Botulinum Neurotoxin and Therapeutics	Res Devel	Mouse embryonic stem cells are being used to generate neurons in culture that are responsive to botulinum neurotoxin (BoNT). The model is used to elucidate mechanisms of action and screen therapeutics, replacing the need for using animals in BoNT mouse lethal assays. Animal use is thus only needed for validation of the most promising compounds, rather than used for high-throughput *in vivo* screens.	Reduce Replace	
Acute Systemic Toxicity	DOD-MRICD	Ongoing	Animal Reduction and Refinement Procedures for Development of Medical Countermeasures to Nerve Agent	Res Devel	(1) Statistical methods are used to reduce animal use in our LD_{50} studies. We use sequential instead of up-down methods; this has resulted in fewer animals. (2) We collect multiple measures from the same animal. (3) We use *ex vivo* assays (e.g., brain slice preparation) as a refinement for looking at mechanisms of action of nerve agents and response to therapeutic compounds.	Reduce Refine Replace	

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			Exposure		(4) Blood assays are used to look at immunological response to agents. (5) We are conducting meta-analysis of data and conducting PBPK/PD, QSAR, and other computer modeling simulations to reduce animal usage and predict toxicity and human response to xenobiotics. (6) Swine are being used as a lower-order replacement for African green monkeys in sulfur mustard wound healing studies.		
Acute Systemic Toxicity	DOD-MRICD	Future	*In Vivo* Imaging	Res	The Institute will develop *in vivo* imaging technologies to follow pathological progression after CWA exposure and the efficacy of potential therapeutics over time in individual animal subjects. This allows for a reduction of the number of experimental groups that are required in *in vivo* studies, since data on specific experimental end points can be collected from a single group of subjects at several different time points.*	Reduce	
Acute Systemic Toxicity	DOD-MRICD	Ongoing	Tissue Sharing	Res Devel	Tissue sharing is practiced to provide stem cells and other tissues for use in other research projects.	Reduce	
Acute Systemic Toxicity	DOD-MRICD	Ongoing	Development of a Single New Fixation Technique Suitable for Molecular, Protein and Histological Evaluation in the Tissue of the Same Animal: Potential for Decreasing Animal Use and Cost While Increasing Data Power	Res Devel	Traditional fixation methods with formaldehyde or formalin coupled with paraffin processing are not compatible with modern techniques to investigate RNA, DNA and proteins (i.e. macromolecules). Recently, a new protocol for fixing and processing tissue--universal molecular fixative (UMFIX)--has been developed that preserves both macromolecules and tissue cytoarchitecture so same animal tissues can be assayed using multiple techniques. This proposal aims to test the use of UMFIX with a rapid tissue processing technique to process GD-damaged brains for multiple macromolecular assays and histology. In addition, this study will compare the quality of these data to data gathered following traditional processing for each assay. If successful, these techniques could be further developed and implemented here at MRICD as a way to reduce animal usage, resource expenditure and	Reduce	

Type of Toxicity Testing	Agency	Status	Title	Type of Activity	Description (asterisk indicates entry has been edited for length)	Poten-tially Applic-able 3Rs	Other Information
					labor hours.*		
Acute Systemic Toxicity	DOD-MRICD	Future	Animal Reduction and Refinement Procedures for Development of Medical Countermeasures to Nerve Agent Exposure - Future	Res	Early endpoints and early removal criteria are being formulated to minimize pain and distress.	Refine	
Ocular Toxicity	DOD-MRICD	Future	Corneal Cell Models for Development of Therapeutics for Vesicant Injury	Res	*In vitro* models of both corneal epithelial tissues and endothelial tissues are being developed to analyze the pathological progression of chemical warfare agent injury and the efficacy of candidate therapeutics. Development of *in vitro* models will provide insight & focus for *in vivo* work and reduce the number of animals required in these studies. Cell-culture expansion of cells derived from corneas will increase the number of distinct experimental samples that can be analyzed by over 10-fold compared to the number of eyes that would be required in an *in vivo* study. These models will be developed using mouse corneal cells, opening the possibility of using a variety of mutant strains to gain an initial evaluation of potential targets for therapeutic intervention prior to the initiation of *in vivo* studies.*	Reduce	
Other (Non-mammalian testing models)	DOD-MRICD	Future	Non-standard Animal Models for Medical Chemical Countermeasure Development	Res	Zebrafish will be used as a lower-order species to examine the toxicology of nerve agents and examine efficacy of potential therapeutic agents. Transgenic mice will be used to help elucidate mechanisms of action and validate therapeutic targets.	Replace	
Acute Systemic Toxicity	DOI	Cancelled	Revised Test Protocol for Evaluation of Candidate Nontoxic Shot and Shot Coatings	Res	A three-tiered toxicological protocol was developed and approved that evaluates the hazard of candidate nontoxic shot and shot coatings used in hunting. The proposed activity entailed modifying the existing protocol to include solubility testing in tier one of the protocol. The DOI Office of the Solicitor opted not to	Reduce	

Type of Toxicity Testing	Agency	Status	Title	Type of Activity	Description (asterisk indicates entry has been edited for length)	Poten-tially Applic-able 3Rs	Other Information
					proceed with the revised protocol.*		
Targeted Testing Areas (High throughput screening/ computer modeling)	EPA-ORD/ NCCT	Ongoing	ToxCast	Res Devel	EPA-ORD/NCCT will continue to develop a toolbox (ToxCast) for prioritizing chemicals for toxicology evaluation, providing computational models that will define bioactivity profiles of chemicals using a variety of high throughput high content screening assays. The ToxCast project started with a proof of concept effort that collected data on 300 pesticides. Algorithms will be developed to match the bioactivity data to known toxicological phenotypes. NCCT is currently evaluating 1000 chemicals in the full program, and are extending the analysis of endocrine related endpoints to an additional 1000 chemicals. If the preliminary phases are successful, the project will proceed to an implementation phase where bioactivity profiles of chemicals in need of toxicological evaluation will be obtained and recommendations for testing priorities will be provided as the final outcome.	Reduce Refine Replace	
Targeted testing areas (High throughput screening)	EPA-ORD/ NCER	Ongoing	Developing HTP Assays for Predictive Modeling of Reproductive/ Developmental Toxicity Modulated Through the Endocrine System or Pertinent Pathways in Humans and Species Relevant to Ecological Risk Assessment	Res Devel	EPA, as part of its Science to Achieve Results (STAR) program, is seeking applications for research in development of high-throughput assays for use in analyzing chemicals or mixtures of chemicals to explain how exposure can be causally related to adverse, apical outcomes related to development and reproduction. These applications can address toxicity modulated by chemical effects on the endocrine system or via a variety of other pathways. Assay systems of interest are those relevant to humans and other species relevant to human health and/or ecological risk assessment.	Reduce Refine Replace	
Aquatic toxicity	EPA-ORD/ NHEERL	Ongoing and future	Development of Fish and Amphibian Assays	Res Devel	EPA-ORD/NHEERL will continue to develop assays to evaluate various toxicity endpoints in fish and amphibians.	Replace	
Aquatic toxicity	EPA-ORD/ NHEERL	Ongoing and future	Development of Amphibian Metamorphosis	Res Devel	EPA-ORD/NHEERL will continue to participate in the validation of an assay to evaluate amphibian metamorphosis.	Replace	

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			Assay				
Targeted testing areas	EPA-ORD/ NHEERL	Ongoing and future	Development of Mammalian Assays	Res Devel	EPA-ORD/NHEERL will continue to develop assays to evaluate various human health toxicity endpoints in rodents.	Refine	
Biologics/ Vaccines	FDA	Ongoing	*In Vitro* Assays of Vaccine Efficacy and Correlates of Protection for Vaccines for Intracellular Pathogens	Res	1) Development an *in vitro* tissue culture assay that measures the ability of T cells from mice sublethally infected with *Mycobacterium tuberculosis* (*M. tb*.), and thus immune, to reduce intracellular bacterial growth when co-cultured with *M. tb*.-infected macrophages. 2) Development of an *in vitro* tissue culture assay that measures the ability of T cells from mice sublethally infected with *Francisella tularensi*s LVS (LVS) co-cultured with LVS-infected macrophages to reduce intracellular bacterial growth.	Reduce, Refine	
Biologics/ Vaccines	FDA	Ongoing	Evaluation of Vaccinia Replication and Dissemination *In Vivo*: New Endpoints to Eliminate Death and Suffering of Animals for Evaluation of Therapeutic Agents, Passive Immunity and Prophylactic Vaccines	Res Devel	Development of a method that uses a recombinant vaccinia, expressing the reporter genes B-galactosidase (B -Gal) or luciferase, to follow vaccinia dissemination to internal organs in normal animals and in several knockout mouse strains.	Reduce Refine Replace	
Biologics/ Vaccines	FDA	Ongoing	Development of *In Vitro* Quantitative Assays to Be Used as Vaccine Potency Release Criteria to Replace *In Vivo* Animal Immunogenicity	Devel	Development of a set of *in vitro* quantitative assays to measure the levels of transcription and translation that should be sensitive to loss of potency and be predictive of *in vivo* immunogenicity.	Replace	

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			Assays				
Repro-ductive/ Develop-mental	FDA	Ongoing	Development of High Throughput, *In Vitro* Systems for Identifying Potential Developmental Toxicants	Res Devel	Development of high throughput, mechanistically-based assays that can be used for prioritizing potential teratogens for further testing, elucidation of their mechanisms of action, and using mechanism of action information to determine if relationships exist between an agent's chemical structure and the potential to cause birth defects.	Replace	
Other (General toxicity testing)	FDA	Ongoing	Validation of the Predictive Performance of *Caenorhabditis elegans* (*C. elegans*) as a New Animal Model in Toxicity Testing and Investigation of Host-Pathogen Interactions	Valid	Validating short term toxicity assays utilizing growth, maturation, reproduction and survivability as endpoints of toxicity in *C. elegans*	Replace	
Biologics/ Vaccines	USDA	Ongoing	Development of Quantitative Assay and Physiochemical Correlates of Biological Activity for *Clostridium haemolyt*icum beta toxin (phospholipase C)	Res	Identification of a protective immunogen and development of an in vitro potency test for *C. haemolyticum* bacterin-toxoid.	Replace	
Biologics/ Vaccines	USDA	Ongoing	Development of in vitro assays for measuring the relative potency of leptospiral bacterins containing serovars pmona, canicola, grippotyphosa and icterohaemorrhagiea	Valid	Validation of the leptospira bacterin ELISA potency test.	Replace	

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Biologics/ Vaccines	USDA	Near-future	Development of an in vitro rabies potency test	Res	Development of an in vitro assay for rabies vaccines, potentially in conjunction with FDA and CDC.	Replace	

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