

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 (sorted by Agency)¹

Type of Toxicity Testing	Agency	Status	Title	Type of Activity	Description (asterisk indicates entry has been edited for length)	Potentially Applicable 3Rs	Other Information
Chronic Toxicity/ Carcinogenicity	NIEHS	Complete Sep 2006 to Jul 2011	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- 05S1
Chronic Toxicity/ Carcinogenicity	NIEHS	Ongoing Jun 2010 to Nov 2012	Validation of a Rodent Mutagenicity Assay	Validate	Multi-lab validation of Pig-A mutation assay in erythrocytes and reticulocytes. Assay is based on presence of cell surface marker (GPI) that can be used to sort cells by FACS.	Refine	Grant 5R44ES018017- 01

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 ICCVAM agency. A list of ICCVAM agency representatives with contact information is available at http://iccvam.niehs.nih.gov/docs/about_docs/DetailRoster.pdf.

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Endocrine Active Substances	NIEHS	Ongoing Jun 2007 to Dec 2013	Validation of Human Vaginal Tissue Assay for Endocrine Disruptors	Validate	A validated human <i>in vitro</i> 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 estrogen by the tissue model and changes to tissue morphology and gene expression will be monitored as biomarkers of ED. A prediction model for ED will be finalized and the test method will undergo formal validation in a multi-center, GLP study. In addition, reproducibility of the assay method and adaptation of the method to a high throughput screen format will be investigated.	Reduce Replace	Grant 2R44ES015641-02
Genetic Toxicity	NIEHS	Complete Sep 2006 to Aug 2011	Reconstructed Skin Micronucleus Genotoxicity Assay	Validate	Validate <i>in vitro</i> 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

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Genetic Toxicity	NIEHS	Ongoing Apr 2012 to Mar 2013	Development of a Diversity Outcross ES Cell Panel for In Vitro Genetics of Toxicology	Res	This project will develop a panel of ES cells from the Diversity Outcross line of mice to simulate human genetic diversity in the responses to environmental toxicants. The long-term goal is a 600 ES cell line panel.	Replace	Grant 1R43ES021688-01
Genetic Toxicity	NIEHS	Ongoing Sep 2011 to Aug 2012	DNA Repair-on-a-Chip: Spatially Encoded Microwell Arrays	Develop	This Phase I develops a commercial version of the COMET-CHIP, an microwell/array version of the COMET assay for measuring DNA damage and repair in lab and human studies	Replace	Grant 1R43ES021116-01
Genetic Toxicity	NIEHS	Ongoing Jul 2012 to Dec 2012	Tools to Study Mammalian Mutagenesis	Develop	This Fast-Track project extends the PigA mutation assay to multi-well plates, optimizes scoring of Mutant RBCs and RETs using a magnetic bead column, and adapts the rodent assay to in vivo human studies. An important addition in the Phase II work is generation of mutation spectra in the mutant cells.	Replace	Grant 1R44ES21973
Ocular Toxicity	NIEHS	Ongoing Jan 2011 to Jun 2013	Human Corneal Model for Ocular Irritation Assay	Develop	Develops a human corneal full thickness model (epithelium, stroma and endothelium) to test chemicals and consumer products for ocular irritation	Replace	Grant 5R44ES020074-02
Ocular Toxicity	NIEHS	Ongoing Sep 2011 to Mar 2013	Novel Use of Confocal Microscopy on Cultured Porcine Corneas for Pre-Clinical Testing	Develop	"PORFOCAL" is a new in vitro assay for testing ocular irritants based on porcine cornea from the meat industry. Corneal tissue is imaged in an "optical histological" manner	Replace	Grant 1R43ES018016
Reproductive/ Developmental	NIEHS	Complete Apr 2010 to Mar 2011	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

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Reproductive/ Developmental	NIEHS	Ongoing May 2007 to Aug 2011	Genetically Diverse Embryonic Stem Cell Lines For Reproductive Toxicology	Res	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
Reproductive/ Developmental	NIEHS	Ongoing Aug 2011 to Jul 2012	Model of the Human Testis for Reproductive Toxicology	Develop	Develops an in vitro human Sertoli cell-based model of the testis for screening effects of reproductive toxicants (apoptosis and cell viability, initially).	Replace	Grant 1R43ES019824-01A1
Targeted Testing Areas	NIEHS	Ongoing	Monitoring Gene Expression Changes After Exposure to Toxicants in <i>Caenorhabditis elegans</i>	Res	Develop transgenic <i>C. elegans</i> lines with GFP reporter constructs to track pathway changes in response to toxic metals and other exposure	Reduce Refine	SBIR Contract

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Targeted Testing Areas (High-throughput screening)	NIEHS	Submitted 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	Use a quantitative nuclease protection assay to measure expression of mRNA and miRNA in liver cells for high-throughput screening	Reduce Replace	SBIR Contract

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Other (General toxicity testing)	NIEHS	Complete Jan 2011 to Dec 2011	Advanced GST Proteomics for Early Stage Organ-Specific Toxicity Screening	Develop Valid	Phase I: 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 these biomarkers, (c) employ animal models to validate these assays as a reliable way to detect organ-specific toxicity. Phase II: develop a comprehensive GST proteomics panel for high sensitivity organ-specific toxicology testing that has significant preclinical and clinical commercial applications.	Reduce Replace	Grant 1R43ES019037-01A1
Other (General toxicity testing)	NIEHS	Ongoing Jan 2011 to Dec 2012	Novel High Throughput Platform for Screening Cytochrome P450 Induction	Res Develop	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. Phase II: evaluate other inducible CYP P450s, expand the spectrum of nuclear receptors tested, and develop cell-based assays of relevant allelic variants of the xenobiotic-activated receptors.	Reduce Replace	Grant 1R43ES019807-01
Other (General toxicity testing)	NIEHS	Ongoing	An In Vitro Assay for Screening Chemicals Interacting with the Blood-Brain Barrier	Res Develop	This STTR Phase I projects develops an in vitro assay for evaluating the transport/permeability of drug compounds and toxicants across the blood-brain barrier. The approach isolates bovine brain microvessels and evaluates non-specific transport of chemicals.		Grant 1R41ES020659
Other (Airway toxicity)	NIEHS	Ongoing	Validation of an In Vitro Human Airway Model		3D in vitro human lung model		Grant 5R44ES014312-03

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Other (Hepatotoxicity)	NIEHS	Ongoing Jan 2011 to Dec 2012	Microfluidic Liver Array for Long Term <i>In Vitro</i> Hepatocyte Culture and Screening	Develop Validate	Complete development of a microfluidic liver array (MLA) platform for improved and lower cost <i>in vitro</i> 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 <i>in vitro</i> 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 <i>in vitro</i> and <i>in vivo</i> 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 4R44ES019035-03
Other (Hepatotoxicity)	NIEHS	Complete Feb 2010 to Jan 2012	TeamChip for High-throughput, Predictive Human Metabolism and Toxicology	Res	Develop TeamChip 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 chemical metabolism and toxicology. Provide pharmaceutical researchers with the information needed to predict the <i>in vivo</i> 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.	Reduce Replace	Grant 1R41ES018022-01
Other (Hepatotoxicity)	NIEHS	Ongoing	New In Vitro Human Liver Toxicity Bioassay System		This Phase II project develops a human 3D liver model using new matrix material (HuBiogel) and examines cell viability, cell morphology and biochemical endpoints (urea production, Phase 1 and 2 enzyme expression).		Grant 2R44ES018004

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Other (Hepatotoxicity)	NIEHS	Ongoing	Functionalizing Non-Useable Cryopreserved Human Hepatocytes into Useable Hepatic		This project develops cell-culturing methods to enable the use of stored/cryopreserved hepatocyte cultures by developing new culture media and approaches to attach cells (using ECM proteins) to plates for toxicology studies.		Grant 1R43ES020638
Other (Nephrotoxicity)	NIEHS	Complete Sep 2010 to Sep 2011	High Throughput Mitochondrial Nephrotoxicant Assay	Develop	Develop primary cultures of renal proximal tubular cells (RPTC) that exhibit <i>in vivo</i> 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	Complete Sep 2009 to 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	Complete Sep 2008 to Apr 2012	System for Comprehensive Tracking and Analysis of <i>C. elegans</i> Behaviors	Develop	Computerized microscope technology and software to track movement and behavior of <i>C. elegans</i> in toxicology studies. Introduction of this robust commercial software solution will significantly contribute to further replacement and reduction of conventional toxicology tests by alternative <i>in vivo</i> toxicology assays using <i>C. elegans</i> .	Reduce Replace	Grant 5R44ES017180-03
Other (Non-mammalian testing models)	NIEHS	Complete Sep 2008 to Aug 2012	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 <i>Brachydanio rerio</i>	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 <i>in vitro</i> 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

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Acute Systemic Toxicity	NIEHS-NTP	Complete	Mechanisms of Acute Systemic Toxicity and Lethality Workshop	Res	NICEATM and ICCVAM organized an international workshop on Advancing <i>In Vitro</i> Approaches and Humane Endpoints for Systemic Toxicity Evaluations. Workshop report published in 2009 (http://iccvam.niehs.nih.gov/methods/acutetox/Tox_workshop.htm)	Reduce Refine Replace	Workshop report published
Acute Systemic Toxicity	NIEHS-NTP	Ongoing	<i>In Vitro</i> Cytotoxicity of Mixtures	Valid	Determine 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 <i>in vitro</i> 3T3 NRU as they undergo mandatory <i>in vivo</i> safety testing by industry, where <i>in vivo</i> 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	<i>In Vitro</i> Methods for Detecting and Quantifying BoNT	Valid	In April 2011, NICEATM received a nomination for three <i>in vitro</i> methods 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™ <i>in vitro</i> 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

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Dermal Toxicity	NIEHS-NTP	Ongoing	Evaluation of EpiDerm™ and EPISKIN™ Dermal Irritation Assays for Identifying Corrosive Compounds Not Detected in <i>In Vitro</i> Corrosivity Assays	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 <i>in vitro</i> corrosivity tests, a prerequisite for consideration of these methods as a way to make dermal assessments without the use of any animals. This was endorsed by ICCVAM as a high priority activity.	Reduce Refine Replace	Testing phase to be complete Summer 2011
Endocrine Active Substances	NIEHS-NTP	Ongoing	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 <i>In Vivo</i> and <i>In Vitro</i> Comet Assays	Valid	Participate on Study Management Team with ECVAM in JaCVAM-sponsored international validation of (1) the <i>in vivo</i> Comet assay as a replacement for the currently accepted <i>in vivo</i> rat hepatocyte UDS assay, and (2) the <i>in vitro</i> Comet assay as a potential screening assay/replacement for the <i>in vivo</i> Comet assay	Reduce Refine Replace	Validation studies in progress: Phase IV of <i>in vivo</i> study completed Oct 2010; validation report expected in 2011 Phase III of <i>in vitro</i> study ongoing
Genetic Toxicity	NIEHS-NTP	Ongoing	<i>In Vitro</i> Cell Transformation Assays	Valid	Support ICCVAM commenting on the OECD draft report on the validation status of <i>in vitro</i> cell transformation assays as a screening test/replacement for cancer bioassays	Reduce Refine Replace	OECD currently considering future activities

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Genetic Toxicity	NIEHS-NTP	Complete	<i>In Vitro</i> Micronucleus Test	Valid	Support ICCVAM commenting on the validation status of the <i>in vitro</i> micronucleus test as an OECD test guideline, as an alternative to the <i>in vitro</i> chromosomal aberration test	Replace	TG 487 approved Dec 2009
Immuno-toxicity	NIEHS-NTP	Ongoing	Validation Status of the LLNA: <ul style="list-style-type: none"> • Reduced LLNA (rLLNA) • Non-radioactive tests • Performance standards • Potency Categorization 	Valid	Support the ICCVAM Evaluation of the validation status of alternative protocols and uses for the LLNA: <ul style="list-style-type: none"> • rLLNA • Non-radioactive LLNA versions • Developing performance standards • Potency categorization 	Reduce Refine Replace	Updated OECD 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 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 ICCVAM evaluation of 4 <i>in vitro</i> 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		Develop publicly available database of existing <i>in vivo</i> rabbit eye test data		Data entered as they are received

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Ocular Toxicity	NIEHS-NTP	Ongoing	Development of a Histopathology Atlas and Associated Decision Criteria for the <i>In Vivo</i> Rabbit Eye Test and for <i>In Vitro</i> Tests that use Intact Eyes or Corneas	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 <i>in vitro</i> ocular toxicity test method (rabbit, chicken, pig, bovine) and from eyes of rabbits used in <i>in vivo</i> tests. NICEATM will use the detailed reference atlas of chemically induced ocular 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.	Reduce Refine Replace	Draft Guidance Document submitted to OECD: Use of Histo-pathology as an additional endpoint in Ocular Safety Testing – Awaiting April 2011 WNT decision
Ocular Toxicity	NIEHS-NTP	Complete	PPDC Antimicrobial Project	Valid	Review 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
Pyrogenicity	NIEHS-NTP	Ongoing	Validation Status of <i>In Vitro</i> Pyrogenicity Tests using Human Cells	Valid	In April 2011, NICEATM received a nomination for an <i>in vitro</i> 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	<ol style="list-style-type: none"> 1. Targeted Research Grants 2. SBIRs (Devel and prevalidation) 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

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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.
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 <i>in Silico</i> , <i>In Vitro</i> , 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 <i>in silico</i> , <i>in vitro</i> , 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	

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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 (Nanoparticles)	NIH	Ongoing	Characterization/Bioinformatics-Modeling of Nanoparticle: Complement Interactions	Res Develop	Develop a standard series of assays to characterize the interactions of complement with lipid encapsulated nanoparticles. The results will be applied to a bioinformatics-modeling system to design and assess next generation nanoparticles for clinical use. Provide a proof-of-concept to study a broad array of nanostructure:biological interactions that are currently difficult to predict prior to human study.	Replace	1U01NS073457-01
Other (Mammalian Test Model)	NIH	Ongoing	Heart-Lung Micromachine for Safety and Efficacy Testing	Res Develop	Engineer biomimetic microsystems technologies that use living human cells cultured within three-dimensional microfluidic systems to replicate the complex physiological functions and mechanical microenvironment of the breathing lung and beating heart. Integrate these 'organ-on-chip' microdevices to produce a 'Heart-Lung Micromachine' that can provide quantitative real-time measures of the efficacy, bioavailability and safety of aerosol-based drugs, nanotherapeutics and other medical products on integrated lung and heart function.	Replace	1U01NS073474-01

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Other (Non-mammalian testing models)	NIH-NCRR	Project start/end Apr 2004-Mar 2014	A Resource Center for <i>Tetrahymena thermophila</i>	Res	Provide a centralized repository for genetically distinct strains of <i>Tetrahymena thermophila</i> , 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.	Replace	Project 5P40RR019688-07
Other (Non-mammalian testing models)	NIH-NCRR	Project start/end June 2003-May 2013	Preparation and Distribution of Adult Stem Cells	Res	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 <i>Aplysia</i>	Res	Provide research investigators with laboratory-reared <i>Aplysia californica</i> of known age and standardized environmental background, as well as their food source. Primary goal is to optimize and standardize <i>Aplysia</i> 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	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 (<i>Danio rerio</i>). 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	<i>Drosophila</i> Genomics Resource Center	Res	The <i>Drosophila</i> Genomics Resource Center (DGRC) collects and distributes reagents and materials essential for <i>Drosophila</i> 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

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Other (Non-mammalian testing models)	NIH-NCRR	Ongoing	Bloomington <i>Drosophila</i> Stock Center	Res	The center collects, maintains, and distributes ~23,000 genetically defined strains of <i>Drosophila melanogaster</i> with significant research value.*	Replace	Project RR007054
Other (Non-mammalian testing models)	NIH-NCRR	Ongoing	<i>Caenorhabditis</i> Genetics Center	Res	The <i>Caenorhabditis</i> Genetics Center (CGC) acquires, maintains, and distributes genetic stocks and information about stocks of the small free-living nematode <i>Caenorhabditis elegans</i> . The CGC maintains a searchable strain database accessible from the CGC website. This site also provides general information about <i>C. elegans</i> 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 <i>Xenopus</i> Resource Center	Res	Two species of <i>Xenopus</i> are commonly used by biologists, <i>Xenopus laevis</i> and <i>Xenopus tropicalis</i> . <i>Xenopus laevis</i> 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 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 <i>in vitro</i> assays for more predictive, mechanistically-based methods than those used with current animal testing.	Reduce, Replace	Intramural activity number ZIA HG200319

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Targeted testing areas (High throughput screening)	NIH-NHLBI	Project start/end Aug 2009-July 2011	Generation of HESC Reporter Lines Using Improved Gene Targeting Technology	Res	Generate 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 <i>in vivo</i> studies.	Replace	Grant R21HL092489
Targeted testing areas (High throughput screening)	NIH-NHLBI	Project start/end Aug 2009-May 2012	New Strategies and Screening Methods for Metalloproteinase Inhibition	Res	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	Develop toxicity screens for drugs using cardiomyocytes derived from human fibroblasts.	Replace	Contract HHSN268200900 044C
Other (Cardio-vascular)	NIH-NHLBI	Project start/end Sept 2007-July 2010	Human Embryonic Stem Cell-Derived Cardiomyocytes for <i>In Vitro</i> Drug Screening	Res	Develop human embryonic stem cell-derived cardiomyocytes for drug safety screening. The cells would be used <i>in vitro</i> 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	Develop a cell-based assay 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

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Other (Cardio-vascular)	NIH-NHLBI	Project start/end July-Dec 2010	Optimizing the Action Potential of Stem Cell-derived Human Cardiomyocytes for Cardiac Safety Screening	Res	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 or replace animal toxicity studies.	Reduce, Replace	Grant R43 HL104948
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	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 (Respirator y)	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 (Respirator y/ 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	Characterize 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

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Biologics/ Vaccines	NIH- NIAID and FDA	Ongoing	Assessing Safety of Cell Substrates and Vaccine Components	Dev	Develop 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 <i>Transmissible Spongiform Encephalopathy</i> (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
Biologics/ Vaccines	NIH- NIAID	Contract funded Oct 2005- Oct 2010	Duke University Multi-scale Systems Immunology for Adjuvant Development (MSI)	Res	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	Test 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

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Acute Systemic Toxicity	NIH-NIAID	Project start/end Feb 2009-July 2011	Accessory Toxin-Mediated Evasion of Innate Immunity During <i>V. cholerae</i> Infection	Research	Address the pathogenesis of <i>Vibrio vulnificus</i> , 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 supplement Grant R21AI072461S1
Acute Systemic Toxicity/ High Throughput Screening	NIH-NIAID	Project start/end July 2008-Jun 2011	<i>A. C. elegans</i> 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, <i>Candida albicans</i> . Common laboratory roundworms, called <i>C. elegans</i> , 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 <i>ex-vivo</i> 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 latently infected cells.	Reduce Replace	Contact: Karl Salzwedel, NIAID Project Link on REPORTer Published in Methods Grant 5R01AI087508-02

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Neurotoxicity	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 screening)	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 further reduces the need for animals by permitting each animal to serve as its own control.*	Reduce	Contact: Susanna Weiss, NIAID
Other (Non-mammalian testing models)	NIH-NIAID	Complete	Novel 3-D Mucosal Model to Study <i>S. typhi</i> 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 <i>Gastroenterology</i> – 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

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Targeted Testing Areas (High throughput screening)	NIH-NIAMS	Project start/end July 2008-Jun 2011	Matrix-Induced Myogenesis & Pharmacology 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 cellular platform.	Replace	Grant 1R43AR060111-01
Immunotoxicity	NIH-NIAMS	Project start/end Dec 2004-Aug 2011	<i>In Vitro</i> 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	<i>Drosophila</i> 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 <i>ex vivo</i> genetically corrected dystrophic mouse iPSC, to assess whether these cells have <i>in vivo</i> 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

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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 induced pluripotent stem cells (iPSC) lines.*	Replace	Grant 5P30AR057220-02
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 <i>ex vivo</i> , thus greatly reducing the number of animal-based experiments.*	Reduce, Replace	Grant 5R21EB008844-02

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Neurotoxicity	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
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 <i>in vivo</i> 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

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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 <i>in silico</i> approach has the potential to greatly reduce or even eliminate the need for <i>in vivo</i> 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 <i>in vivo</i> . 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
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

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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 <i>in vivo</i> . 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 toxicity testing/ high-throughput screening)	NIH-NIBIB	Project start/end May 2010-Apr 2012	High-Throughput Vibrational Cytometry	Res	A non-invasive flow cytometer system, capable of providing chemically specific information, is proposed 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.*	Reduce Refine Replace	Grant 1R21EB011703-02
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

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Other (General toxicity testing/ high-throughput screening)	NIH-NIBIB	Project start/end May 2010-Apr 2012	Compact High-Performance Microfluidic-Based Flow Cytometer	Dev	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	Build models of primary human systems to serve as close mimics of <i>in vivo</i> complexity.	Replace	Grant 5R01 EB 10246-02
Dermal Irritation	NIH-NICHD	Project start/end Sept 2005-Aug 2012	Validation of a Human <i>In Vitro</i> Vaginal Irritation Test	Res	Develop a human reconstructed tissue based system that may provide a sensitive and validated assay method for screening chemicals/formulations that may potentially irritate the vagina. Developing this approach could reduce the use of laboratory animals.*	Reduce	Grant 5 R44HD050023-03
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

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Targeted Testing Areas (High-throughput screening)	NIH-NIDCD	Project start/end Apr 2003-Jun 2013	Genetics of Zebrafish Hair Cell Toxicity	Res	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	Develop noninvasive assays to test the viability, composition and metabolic activity of grafted <i>Ex-vivo</i> 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	Develop human iPS cells from skin biopsies obtained from individuals with craniometaphyseal dysplasia (CMD) and normal subjects and optimize protocols for efficient differentiation of these cells into osteoblasts. Use cells for studies of CMD mechanisms, and for safety and efficacy screening of therapeutics for CMD. Such screens would minimize or eliminate animal testing.*	Reduce, Replace	Grant R21 DE019892
Immuno-toxicity	NIH-NIDDK	Ongoing 2006-2012	Friendly Immunosuppression for Endocrine Regeneration	Res	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

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Other (General toxicity testing)	NIH-NIDDK	Phase 2 Ongoing 2007-2012	Nuclear Receptor Signaling Atlas (NURSA)	Res	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	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 Palpator™ 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 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.*	Reduce	Grant 5R44GM087784-03

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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
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

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Other (Hepatotoxicity)	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	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 <i>in vitro</i> and <i>in vivo</i> 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 preliminary dose-range finding toxicity studies are conducted by a variety of methods. Wherever possible, ICCVAM-approved methods (modified up-and-down procedure, <i>in vitro</i> predictive models) are used for accurate prediction of dose levels before required toxicology assays.*	Reduce Refine	Contract N01-271200900018C-1-0-1
Acute systemic toxicity	NIH-NIMH	Ongoing	Preclinical Development of HIV-1 Vif Antagonists	Res	Use human peripheral blood monocyte cells (PBMCs) for <i>in vitro</i> testing of HIV drug cytotoxicity, thereby avoiding the exposure of people infected with HIV to these compounds.	Replace	Grant 5U19MH081836-04

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Neurotoxicity	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 <i>in vitro</i> assay of HIV-1 neurotoxins, using monocytes isolated from healthy donors. The monocytes are first cultured <i>in vitro</i> , 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	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
Neurotoxicity	NIH - NINDS	Near-future	Engineering Form and Function in Neuronal Networks	Res	Develop the ability to design and implement robust <i>in vitro</i> 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	
Neurotoxicity	NIH - NINDS	Ongoing	Neural Cell Based Assays Derived from Human ES Cells	Res	Develop 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 for pharmacological and toxicological studies.	Reduce, Replace	
Other (Adaptive Clinical Trial Design)	NIH - NINDS	Ongoing	Accelerating Drug And Device Evaluation Through Innovative Clinical Trial Design	Res Devel	Illustrate and explore how best to use adaptive clinical trial designs to improve the evaluation of drugs and medical devices.	Reduce, Replace	1U01NS073476-01
Acute Systemic Toxicity	ATSDR	Ongoing	Predictive Toxicity Methods	Res Devel Valid	Use 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).

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Targeted Toxicity Testing	ATSDR	Ongoing	Development of Methods for Mixtures Toxicity Evaluation	Res Devel	Conduct 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	Incorporate biomarkers data to develop improved computational models (such as PBPK) for risk assessment	Reduce Refine Replace	<u>Toxicol. Letters</u> 198: 44-48 (2010).
Acute Systemic Toxicity	DOD-MRICD	Ongoing	Cell and Tissue Models Used in Development of Medical Chemical Countermeasures	Res Devel	Use a variety of human cell and tissue models <i>in vitro</i> 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	Use mouse embryonic stem cells 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 <i>in vivo</i> screens.	Reduce Replace	
Acute Systemic Toxicity	DOD-MRICD	Ongoing	Animal Reduction and Refinement Procedures for Development of Medical Countermeasures to Nerve Agent Exposure	Res Devel	(1) Use statistical methods 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 <i>ex vivo</i> assays (e.g., brain slice preparation) as a refinement for looking at mechanisms of action of nerve agents and response to therapeutic compounds. (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.	Reduce Refine Replace	

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Acute Systemic Toxicity	DOD-MRICD	Future	<i>In Vivo</i> Imaging	Res	Develop <i>in vivo</i> 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 <i>in vivo</i> 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 labor hours.*	Reduce	

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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	<i>In vitro</i> 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 <i>in vitro</i> models will provide insight & focus for <i>in vivo</i> 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 <i>in vivo</i> 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 <i>in vivo</i> studies.*	Reduce	
Other (Non-mammalian testing models)	DOD-MRICD	Future	Non-standard Animal Models for Medical Chemical Countermeasure Development	Res	Use zebrafish 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 proceed with the revised protocol.*	Reduce	

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Targeted Testing Areas (High throughput screening/ computer modeling)	EPA-ORD/NCCT	Ongoing	ToxCast	Res Devel	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. ToxCast 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	Continue to develop assays to evaluate various toxicity endpoints in fish and amphibians.	Replace	

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Aquatic toxicity	EPA-ORD/NHEERL	Ongoing and future	Development of Amphibian Metamorphosis Assay	Res Devel	Continue to participate in the validation of an assay to evaluate amphibian metamorphosis.	Replace	
Targeted testing areas	EPA-ORD/NHEERL	Ongoing and future	Development of Mammalian Assays	Res Devel	Continue to develop assays to evaluate various human health toxicity endpoints in rodents.	Refine	
Biologics/Vaccines	FDA	Ongoing	<i>In Vitro</i> Assays of Vaccine Efficacy and Correlates of Protection for Vaccines for Intracellular Pathogens	Res	1) Develop <i>in vitro</i> tissue culture assay that measures the ability of T cells from mice sublethally infected with <i>Mycobacterium tuberculosis</i> (<i>M. tb.</i>), and thus immune, to reduce intracellular bacterial growth when co-cultured with <i>M. tb.</i> -infected macrophages. 2) Develop <i>in vitro</i> tissue culture assay that measures the ability of T cells from mice sublethally infected with <i>Francisella tularensis</i> 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 <i>In Vivo</i> : New Endpoints to Eliminate Death and Suffering of Animals for Evaluation of Therapeutic Agents, Passive Immunity and Prophylactic Vaccines	Res Devel	Develop method that uses recombinant vaccinia that expresses 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	

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Biologics/ Vaccines	FDA	Ongoing	Development of <i>In Vitro</i> Quantitative Assays to Be Used as Vaccine Potency Release Criteria to Replace <i>In Vivo</i> Animal Immunogenicity Assays	Devel	Develop a set of <i>in vitro</i> quantitative assays to measure the levels of transcription and translation that should be sensitive to loss of potency and be predictive of <i>in vivo</i> immunogenicity.	Replace	
Reproductive/ Developmental	FDA	Ongoing	Development of High Throughput, <i>In Vitro</i> Systems for Identifying Potential Developmental Toxicants	Res Devel	Develop 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 <i>Caenorhabditis elegans</i> (<i>C. elegans</i>) 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 <i>C. elegans</i>	Replace	
Biologics/ Vaccines	USDA	Ongoing	Development of Quantitative Assay and Physicochemical Correlates of Biological Activity for <i>Clostridium haemolyticum</i> beta toxin (phospholipase C)	Res	Identify a protective immunogen and develop an <i>in vitro</i> potency test for <i>C. haemolyticum</i> bacterin-toxoid.	Replace	

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Biologics/ Vaccines	USDA	Ongoing	Develop <i>in vitro</i> 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	
Biologics/ Vaccines	USDA	Near-future	Develop an <i>in vitro</i> rabies potency test	Res	Develop an <i>in vitro</i> assay for rabies vaccines, potentially in conjunction with FDA and CDC.	Replace	