Lunch Sessions SL4

Recent Progress and Future Directions at NICEATM-ICCVAM: Validation and Regulatory Acceptance of Alternative Test Methods that Reduce, Refine, and Replace Animal Use

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Summary

The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) promote the validation and regulatory acceptance of new, revised, and alternative test methods that are based on sound science and that will provide continued or improved protection of people, animals, and the environment while reducing, refining, and replacing the use of animals where scientifically feasible. NICEATM administers ICCVAM and provides scientific and technical support, including the conduct of high priority validation studies. ICCVAM has coordinated or contributed to the evaluation of 27 alternative test methods that have been accepted or endorsed by national and international authorities. In 2008, NICEATM-ICCVAM developed a Five-Year Plan in conjunction with Federal agencies that promotes the use of new science and technologies to develop alternative methods and expands international collaborations. Implementation of the Five-Year Plan is expected to advance alternative methods that will support improved safety assessments while reducing, refining, and replacing animal use.

Keywords: ICCVAM, NICEATM, regulatory acceptance, alternative test methods, safety testing

1 Introduction

In the United States, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) coordinates interagency issues on alternative toxicological test method development, validation, regulatory acceptance, and national and international harmonization. The committee is responsible both for coordinating Federal interagency technical evaluations of new, revised, and alternative safety testing methods and for forwarding test method recommendations to U.S. Federal agencies (USC, 2000). The National Institute of Environmental Health Sciences originally established ICCVAM in 1997 (NIEHS, 1997). In 2000, ICCVAM was established by law as a permanent interagency committee of the National Institute of Environmental Health Sciences under the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) (USC, 2000). ICCVAM is composed of representatives from the 15 U.S. Federal regulatory and research agencies that require, use, or generate toxicological testing data and information (Tab. 1).

NICEATM administers ICCVAM and provides scientific and operational support for ICCVAM activities. NICEATM also conducts and coordinates international validation studies to evaluate the usefulness and limitations of potential new alternative test methods. NICEATM and ICCVAM work together to evaluate and promote the validation and regulatory acceptance of scientifically valid toxicological test methods based on sound science that will protect human and animal health and the environment while reducing, refining (decreasing pain and distress), and replacing animal use where scientifically feasible (Stokes and Schechtman, 2007). NICEATM and ICCVAM provide an essential framework for translating advances in science and

Tab. 1: ICCVAM Member Agencies

ICCVAM Member Agencies	
Regulatory/Research	**Non-Regulatory/Research**
• Consumer Product Safety Commission • Department of Agriculture • Department of Interior • Department of Transportation • EnvironICCVAM Member Agenciesmental Protection Agency • Food and Drug Administration • Occupational Safety and Health Administration • Agency for Toxic Substances and Disease Registry	• Department of Defense • Department of Energy • National Cancer Institute • National Institute of Environmental Health Sciences • National Institute for Occupational Safety and Health • National Library of Medicine • National Institutes of Health, Office of the Director

technology into standardized and adequately validated safety testing methods that can be used to protect and advance public health. This paper summarizes recent progress and future plans of NICEATM and ICCVAM in advancing new, revised, and alternative safety testing methods.

2 Progress in the regulatory acceptance of alternative methods

Since its establishment in 1997, ICCVAM has coordinated or contributed to the evaluation of 27 alternative test methods that have been accepted or endorsed by national and international authorities. The types of safety testing and available alternatives are listed in Table 2.

The alternative methods accepted by Federal agencies include 17 non-animal methods and 10 methods that use fewer animals and/or provide for refinement by reducing or avoiding potential discomfort. Most of the *in vitro* methods do not require the use of animals when positive results are obtained. Recommendations for additional alternatives for allergic contact dermatitis (ICCVAM, 2009a, 2009b, 2009d) and ocular irritation are in development (ICCVAM, 2009c, 2009e).

3 The NICEATM-ICCVAM Five-Year Plan

In response to requests from the Appropriations Committees of the U.S. House of Representatives and U.S. Senate, ICCVAM and NICEATM prepared a five-year plan to describe goals and objectives for the years 2008 through 2012 (ICCVAM, 2008b). This plan was developed in conjunction with Federal agency program offices and describes how NICEATM and ICCVAM will promote the research, development, translation, validation, and regulatory acceptance of alternative test methods that reduce, refine, and replace the use of animals in testing while maintaining scientific quality and protecting human health, animal health, and the environment.

The plan identified four key challenges that NICEATM and ICCVAM will address in conjunction with agency programs. The first challenge is to identify priority toxicity testing areas for the next five years and to conduct and facilitate activities in those areas. Currently, the four highest priority areas are ocular toxicity, dermal toxicity, acute systemic toxicity, and biologics potency and safety testing. These are based on the potential for these tests to cause animal pain and distress and the fact that these are the most commonly conducted safety tests. Other priority areas include immunotoxicity, endocrine disruptors, pyrogenicity, reproductive and developmental, and chronic toxicity/carcinogenicity testing.

The second challenge is to identify and promote research initiatives that are expected to support future development of innovative alternative test methods. These new methods might incorporate techniques such as high throughput screening, computer modeling, informatics, and biomarkers.

The third challenge is for NICEATM and ICCVAM to foster the acceptance and appropriate use of alternative test methods through outreach and communication. This will be accomplished by sponsoring and participating in workshops, NICEATM-ICCVAM website communications, and the development and publication of standardized test method protocols.

Lastly, ICCVAM and NICEATM will develop partnerships and strengthen interactions with stakeholders to facilitate meaningful progress. These efforts are expected to facilitate research, development, translation, validation, and regulatory acceptance of alternative methods that will reduce, refine, and replace animal use while maintaining scientific quality and the protection of human health, animal health, and the environment.

In June 2009, ICCVAM and NICEATM released an Implementation Plan for the NICEATM-ICCVAM Five-Year Plan (ICCVAM, 2008b). This working document describes how ICCVAM and NICEATM are implementing the strategies outlined in the Five-Year Plan and includes goals, specific objectives, and planned activities for implementation. The Implementation Plan also describes NICEATM and ICCVAM accomplishments since February 2008 that relate to the goals and objectives outlined in the Implementation Plan.

NICEATM and ICCVAM recently created an alternative test methods milestones page on the NICEATM-ICCVAM website to provide a comprehensive overview of available alternative methods and a quick summary of current validation and evaluation activities (http://iccvam.niehs.nih.gov/methods/milestones.htm). The page provides a comprehensive summary of the status of ongoing and completed NICEATM-ICCVAM al-

Tab. 2: U,S. and international acceptance of alternative methods 1998-2009

Approved/Endorsed Alternative Safety Testing Method	Test Guidelines, Regulations, and Guidances	Other References
Acute Systemic Toxicity • Up and Down Procedure (UDP) • Fixed Dose Procedure (FDP) • Acute Toxic Class Method (ATC) • Acute Toxicity In Vitro Starting Dose Procedure, 3T3 cells • Acute Toxicity In Vitro Starting Dose Procedure, NHK cells	OECD TG 420 (OECD, 2001a) OECD TG 423 (OECD, 2001b) OECD TG 425 (OECD, 2008a) EPA OPPTS 870.1100 (EPA, 2002)	(ICCVAM, 2001a) (ICCVAM, 2001b) (ICCVAM, 2001c) (ICCVAM, 2006a) (Stokes et al., 2008)
Dermal Absorption • *In vitro* dermal absorption methods	OECD TG 428 (OECD, 2004a)	
Dermal Corrosivity and Irritation • CORROSITEX® Skin Corrosivity Test • EpiSkin™ Skin Corrosivity Test • EpiDerm™ Skin Corrosivity Test • SkinEthic™ Skin Corrosivity Test • Rat TER Skin Corrosivity Test	OECD TG 430 (OECD, 2004b) OECD TG 431 (OECD, 2004c) OECD TG 435 (OECD, 2006) 67/548 EEC	(ICCVAM, 1999a) (ICCVAM, 2002) (ICCVAM, 2004)
Dermal Phototoxicity 3T3 NRU Phototoxicity Test 3T3 NRU Phototoxicity Test: Application to UV Filter Chemicals	OECD TG 432 (OECD, 2004d) 67/548 EEC	(Spielmann et al. 1998)
Allergic Contact Dermatitis • Local Lymph Node Assay	OECD TG 429 (OECD, 2002) EPA OPPTS 870.2600 (EPA, 2003)	(Dean et al., 2001) (Haneke et al., 2001) (ICCVAM, 1999b) (ISO, 2002) (Sailstad et al., 2001)
Ocular Corrosivity and Irritation • Bovine Corneal Opacity and Permeability (BCOP) Test Method • Isolated Chicken Eye (ICE) Test Method	OECD TG 437 (OECD, 2009d) OECD TG 438 (OECD, 2009e)	(ICCVAM, 2006b) (ICCVAM, 2006c) (ICCVAM, 2006d) (ICCVAM, 2006e)
Pyrogenicity • Human Whole Blood/Interleukin-1B *In Vitro* Pyrogen Test • Human Whole Blood (Cryopreserved) Interleukin-1B *In Vitro* Pyrogen Test • Human Whole Blood/Interleukin-6 *In Vitro* Pyrogen Test • Human Peripheral Blood Mononuclear Cell/Interleukin-6 *In Vitro* Pyrogen Test • Monocytoid Cell Line Mono Mac 6/Interleukin-6 *In Vitro* Pyrogen Test	U.S. FDA (FDA, 2009) European Pharmacopoeia (EDQM, 2009a)	(ICCVAM, 2008a)
Vaccine Potency and Safety Testing • Use of Humane Endpoints in Animal Testing of Biological Products • Rabies Vaccine, Humane Endpoints • ELISA Test for Batch Potency Testing of Erysipelas Vaccines (refinement) • Relevance of the Target Animal Safety Test for Batch Safety Testing of Vaccines for Veterinary Use • ELISA Test for Batch Potency Testing of Human Tetanus Vaccines refinement • ToBI Test for Batch Potency Testing of Human Tetanus Vaccines refinement	9 CFR 117.4e 27 CFR 610.10 9 CFR 113.4 CVB Notice Number 04-09 (Hill, 2004) European Pharmacopoeia (EDQM, 2009b)	(Hendriksen et al., 1994) (Hendriksen, 2009) (Johannes et al., 2003) (Rosskopf-Streicher et al., 2001)

ternative test method evaluation projects and projects to which NICEATM, ICCVAM, and agency scientists are contributing (ICCVAM, 2009f). The page also provides timelines for each project with links to more detailed information and relevant documents. Additional information about NICEATM and ICCVAM test method evaluation activities can be found at http://iccvam.niehs.nih.gov.

4 The international cooperation on alternative test methods

On April 27, 2009, a Memorandum of Cooperation for International Cooperation on Alternative Test Methods (ICATM) was signed by representatives of the United States, European Union, Canada, and Japan (ICATM, 2009). Signing the agreement were:

– Dr. Linda Birnbaum, Director, NIEHS and NTP, USA
– Dr. Masahiro Nishijima, Director, National Institute of Health Sciences, Japan
– Dr. Elke Anklam, Director, Institute of Consumer Protection and Health, JRC, European Commission
– Dr. David Blakey, Director, Health and Safety Bureau, Health Canada.

The agreement provides a framework for enhanced international cooperation, collaboration, and communication in three critical areas:

– Test method validation studies
– Independent peer review of the validation status of test methods

Development of harmonized formal test method recommendations for regulatory authorities

The initial participating validation organizations are the NICEATM and ICCVAM, the Japanese Center for the Validation of Alternative Methods (JaCVAM), the European Centre for the Validation of Alternative Methods (ECVAM), and the Environmental Health Science and Safety Bureau within Health Canada. The cooperation among these organizations facilitated by this agreement is expected to accelerate international adoption of scientifically valid alternative test methods (Blakey, 2009; Kojima, 2009; Kreysa, 2009; NIEHS, 2009; Stokes and Wind, 2009; Wind et al., 2009).

5 Alternative methods for allergic contact dermatitis: new versions and applications of the murine Local Lymph Node Assay

NICEATM and ICCVAM convened an international independent scientific peer review panel in 2008 and 2009 to review several new versions and applications of the murine Local Lymph Node Assay (LLNA), an alternative test method for assessing the allergic contact dermatitis potential of chemicals and products. ICCVAM originally evaluated the scientific validity of the LLNA in 1998 (ICCVAM, 1999b; Dean et al., 2001; Haneke et al., 2001; Sailstad et al., 2001). U.S. Federal agencies accepted the ICCVAM recommendations that the LLNA was a valid substitute for the current guinea pig test method used to assess the allergic contact dermatitis and that it could be used to evaluate most but not all types of substances. ICCVAM also concluded that the LLNA has many advantages over the traditional test methods, including using fewer animals, eliminating the potential discomfort that can occur from substances that are sensitizers, and providing dose-response information. The LLNA was subsequently incorporated into national and international test guidelines for the assessment of skin sensitization (ISO, 2002; OECD, 2002; EPA, 2003).

Based on a nomination in 2007 from the U.S. Consumer Product Safety Commission, NICEATM and ICCVAM developed LLNA performance standards and evaluated the validation status of the following new versions and applications of the LLNA:

– Three modified non-radioactive versions of the traditional LLNA:
  • LLNA: BrdU-ELISA (Takeyoshi et al. 2001; OECD 2009b)
  • LLNA: BrdU-Flow Cytometry (MB Research Laboratories)
  • LLNA: DA (Idehara et al. 2008; OECD 2009a)
– The reduced LLNA (rLLNA; also referred to as the LLNA limit dose procedure)
– Use of the traditional LLNA to test mixtures, metals, and aqueous solutions
– Use of the LLNA for potency categorization

NICEATM and ICCVAM evaluated the new versions and applications in conjunction with ECVAM and JaCVAM. This included the development of internationally harmonized performance standards for the LLNA that can be used to more rapidly and efficiently determine the validity of nonradioactive and other modified versions of the LLNA (ICCVAM, 2008a, 2008b). The evaluations included two meetings of an international independent scientific peer review panel (Panel) in 2008 and 2009 (ICCVAM, 2009c).

The Panel agreed with ICCVAM's recommendations that the available data and test method performance support the use of two of the non-radioactive modified versions of the LLNA to identify substances as potential skin sensitizers and nonsensitizers, with certain limitations. The Panel concluded that the LLNA could be used to test any chemical or product for allergic contact dermatitis potential, including pesticides and substances such as fragrances and dyes, unless there are unique physiochemical properties associated with these materials that might affect their ability to interact with immune processes. The Panel also endorsed ICCVAM's revised protocol for the LLNA and draft LLNA performance standards and a reduced LLNA procedure.

ICCVAM submitted a revised Test Guideline (TG) 429 to the Organization for Economic Cooperation and Development (OECD) in June 2009 that includes the ICCVAM updated LLNA protocol, the reduced LLNA procedure, and harmonized performance standards (OECD, 2009c). The revised protocol provides for a 20% reduction in animal use, and the reduced protocol can decrease animal use by an additional 40%. ICCVAM, in collaboration with JaCVAM, also submitted draft OECD Test Guidelines for the LLNA:DA and the LLNA:BrdU-

ELISA, the two non-radioactive methods endorsed by the Panel (OECD, 2009a, 2009b).

NICEATM and ICCVAM are also evaluating the application of *in vitro* methods and integrated decision strategies to the regulatory safety assessment of allergic contact dermatitis hazards. This includes validation of two *in vitro* cell culture-based methods, the human cell line activation test (h-CLAT) (Sakaguchi et al., 2006), and the Myeloid U937 Skin Sensitization Test (MUSST) assay (Ovigne et al., 2008), and a chemistry-based assay, the direct peptide reactivity assay (DPRA) (Gerberick et al., 2007). An ECVAM/JACVAM Study Management Team that includes ICCVAM and NICEATM liaisons is coordinating the validation studies. NICEATM is evaluating the integration of various types of physical-chemical properties and *in vitro* data with uncertain *in vivo* test results to determine decision algorithms that can be used for regulatory safety decisions. NICEATM and ICCVAM will also recommend *in vitro* assays that may help increase the accuracy of *in vitro* predictions of allergic contact dermatitis for inclusion in high throughput screening (HTS). NICEATM will evaluate the HTS results to identify useful assays.

6 Ocular toxicity test method evaluation activities

Ocular safety testing is one of ICCVAM's top four priorities because it is one of the four most common safety tests required and therefore involves a significant number of animals. Such testing also has the potential to cause significant unrelieved pain and distress when chemicals and products cause eye damage (ICCVAM, 2008b). In 2008, two ICCVAM-recommended *in vitro* safety testing methods, the bovine corneal opacity and permeability (BCOP) and isolated chicken eye (ICE) test methods, were accepted by U.S. Federal agencies for identifying substances with the potential to cause severe or permanent damage to eyes without using live animals. The OECD Council formally adopted TGs for these test methods in 2009, with the BCOP described in TG 437 and the ICE in TG 438 (OECD, 2009a, 2009b). These methods can now be used worldwide to identify substances that may cause severe or irreversible eye damage. Positive results can be used for hazard classification without the need to use live animals, thereby avoiding the pain and distress that may have resulted if animals had been required.

NICEATM and ICCVAM organized an international independent scientific peer review panel that met in May 2009 to evaluate nine alternative methods and strategies for ocular safety testing. These included:

– The routine use of topical anesthetics, systemic analgesics, and humane endpoints to avoid and minimize pain and distress during *in vivo* ocular irritation testing (ICCVAM, 2009g).
– The use of the BCOP, the Cytosensor® Microphysiometer (CM), the ICE, the isolated rabbit eye, and the hen's egg test – chorioallantoic membrane test methods for identifying moderate and mild ocular irritants and for identifying substances that do not cause sufficient eye injury to require ocular hazard labeling (non-labeled category) (IIVS, 2008; ICCVAM, 2009h, 2009i, 2009j).
– The *in vivo* low volume eye test (ICCVAM, 2009k).
– Non-animal testing strategies using the BCOP, CM, and/or EpiOcular™ test methods to assess the eye irritation potential of antimicrobial cleaning products and to determine their appropriate U.S. Environmental Protection Agency ocular hazard classification (ICCVAM, 2009l).

Highlights of the Panel's conclusions and recommendations include:

– Topical anesthetics and systemic analgesics should routinely be used prior to all *in vivo* ocular irritancy testing. The Panel recommended an enhanced protocol of specific pain-relieving drugs and schedule of administration to effectively avoid or minimize discomfort.
– The BCOP and CM test methods could be used as screening tests to identify some products and substances that would not require hazard labeling for eye irritation. These methods will now be recommended for use in a "bottom-up testing approach" to identify substances that are not expected to cause sufficient injury to require classification as an ocular irritation hazard.
– Proposed non-animal testing strategies using three *in vitro* test methods to assess the eye irritation potential of antimicrobial cleaning products for EPA ocular hazard classification and labeling purposes appear promising. The Panel recommended that studies should be conducted to further characterize the *in vitro* test methods and that testing strategies should be designed in coordination with ICCVAM.

ICCVAM also provided recommendations to an ECVAM Scientific Advisory Committee peer review of four cell function-based assays proposed for the identification of substances that can cause mild to moderate ocular irritation and to identify substances that do not require ocular hazard labeling (Cytosensor® Microphysiometer, Flourescein Leakage, Neutral Red Release, Red Blood Cell Hemolysis).

Information on the NICEATM and ICCVAM ocular toxicity test method evaluations can be found on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/ocutox/ocutox.htm. The report from the peer panel meeting is currently available with ICCVAM test method evaluation reports, including final ICCVAM test method recommendations, to be available in late 2009.

In conjunction with ECVAM, ICCVAM is currently evaluating two other *in vitro* test methods for ocular irritation testing that are proposed for identifying substances that do not cause classifiable ocular irritation. An ECVAM-led SMT with NICEATM and ICCVAM liaison members is currently planning validation studies for these two methods, Epiocular™ and SkinEthic™ .

7 Acute systemic toxicity test method evaluation activities

Acute systemic toxicity testing is one of the top four NICEATM-ICCVAM priorities because it is the most common safety test performed worldwide and because of the potential for significant pain and distress to animals when substances produce toxic

effects (ICCVAM, 2008b). In 2008, ICCVAM forwarded recommendations on the use of two *in vitro* test methods for estimating starting doses for acute oral systemic toxicity studies (ICCVAM, 2006a). ICCVAM recommended that these test methods should always be considered before using animals for acute oral toxicity testing and that the methods should be used where determined appropriate. These recommendations were accepted by U.S. Federal agencies, and data from the test methods should now be used in a weight-of-evidence approach for determining starting doses for *in vivo* studies. Using these *in vitro* methods where appropriate is expected to reduce the number of animals required for each acute toxicity study.

A draft guidance document describing use of these two *in vitro* test methods for estimating starting doses for acute oral systemic toxicity tests has been prepared and forwarded to OECD for consideration (OECD, 2009f). In addition, the ICCVAM Acute Toxicity Working Group has provided comments on five new and revised OECD Test Guidelines and Guidance Documents for assessment of acute toxicity hazards (OECD, 2008b, 2008c, 2008d, 2009g, 2009h). These additional methods are expected to further reduce animal use for acute systemic toxicity testing.

In February 2008, NICEATM and ICCVAM, in collaboration with ECVAM and JaCVAM, organized an International Workshop on Acute Chemical Safety Testing: Advancing *In Vitro* Approaches and Humane Endpoints for Systemic Toxicity Evaluation. The workshop was based on prior ICCVAM and expert panel recommendations that standardized procedures to collect information pertinent to an understanding of toxicity mechanisms should be included in future *in vivo* rat acute oral toxicity studies. The workshop participants recommended ways to collect data to identify key toxicity pathways for acute systemic toxicity so this mechanistic information can be used to target the development of predictive *in vitro* alternative test methods. The workshop also recommended that systematic collection of mechanistic data from required *in vivo* studies would help identify predictive biomarkers of systemic toxicity that could be used as earlier, more humane endpoints during in vivo tests to further reduce or avoid pain and distress (ICCVAM, 2009e).

More than one hundred people from seven countries attended the workshop, representing U.S. Federal agencies, academia, industry, international organizations, and the animal welfare community. Attendees participated in breakout group discussions to address questions for the following topics:

- Identifying key pathways leading to acute systemic toxicity
- Improving current acute systemic toxicity injury and toxicity assessments
- Identifying earlier humane endpoints for acute systemic toxicity testing
- Applying *in vivo* mode of action and mechanistic information to the development and validation of *in vitro* methods for assessing acute systemic toxicity
- Increasing industry involvement in test method development, validation, and use

NICEATM and ICCVAM are participating in the Validation Management Group of an ECVAM metabolism validation study. The goal of the study, the *ECVAM Validation Study in the Field of Toxicokinetics and Metabolism: Provision of a Standard for Human Hepatic Metabolism and Toxicity by Assessing as an Indicator Biotransformation Enzyme Induction using HepaRG Cells and Cryopreserved Human Hepatocytes*, is to develop a standard *in vitro* test system for human hepatic metabolism and metabolism-mediated toxicity and assess the potential for cytochrome P450 induction at clinically relevant doses. Issues being evaluated include 1) reliability of the test systems, 2) phenotypic stability of the cells, 3) within/between laboratory reproducibility, 4) transferability, and 5) predictivity for assessing *in vivo* human induction.

A high-throughput screening program at the NIH NCGC recently evaluated 13 other cytotoxicity test methods for predicting acute oral toxicity in humans (Xia et al., 2008). The generation of high-quality cytotoxicity data on a library of 1408 known compounds using HTS demonstrates the potential of this methodology to profile a much broader array of assays and compounds, which, in aggregate, may be valuable for prioritizing compounds for further toxicologic evaluation, identifying compounds with particular mechanisms of action, and potentially predicting *in vivo* biological response. NICEATM and ICCVAM will continue to identify candidate test methods for inclusion in the HTS that may help increase the accuracy of *in vitro* methods for predicting *in vivo* acute systemic toxicity.

8 Evaluations of endocrine disruptor screening methods

In 2002, an independent expert panel review concluded that there were no adequately validated *in vitro* estrogen receptor (ER) or androgen receptor based *in vitro* test methods available for screening of chemicals with potential endocrine disruptor activity. Based on the Panel's conclusions and recommendations, ICCVAM developed recommendations for minimum procedural standards and a list of 78 reference substances that should be used to standardize and validate such test methods. ICCVAM then invited the nomination of *in vitro* test methods that met the published recommendations for validation studies. Xenobiotic Detection Systems (XDS), Inc. subsequently nominated their LUMI-CELL® ER assay for the detection of ER agonists and antagonists to ICCVAM for validation. LUMI-CELL® is a stably transfected transcriptional activation assay (STTA) that uses a human cell line with human ERs. A joint NICEATM-ECVAM-JaCVAM international validation study of the LUMI-CELL® assay using the 78 recommended reference substances is currently being conducted in laboratories in Italy, the U.S., and Japan. An independent scientific peer review panel will evaluate the results of the study in spring 2010. CertiChem, Inc. also nominated its MCF-7 cell proliferation assay for validation studies. Like the LUMI-CELL® assay, it uses a human cell line that identifies human estrogen receptor agonist and antagonist activity. An interlaboratory validation study of the CertiChem assay is planned. Data from these validation studies will be used to develop OECD test guidelines and test method performance standards for inclusion in the test guidelines.

9 Dermal safety assessment test method activities

In 2004, ICCVAM established performance standards for *in vitro* test methods for skin corrosion (ICCVAM, 2004). The performance standards were based on four *in vitro* test methods evaluated by ICCVAM for the identification of substances with the potential to cause skin corrosion. The standards can be used to evaluate the reliability and accuracy of other test methods that are based on similar scientific principles that measure or predict the same biological or toxic effect. NICEATM and ICCVAM have submitted revisions to OECD TG 430 (rat skin transcutaneous electrical resistance assay) and TG 431 (human skin model systems) that incorporate the ICCVAM-recommended performance standards (ICCVAM, 2004; OECD, 2009i, 2009j). ICCVAM scientists have also been participating in OECD Expert Consultation meetings to evaluate several *in vitro* skin irritation assays for their inclusion in a draft TG.

NICEATM is currently conducting a study to determine how *in vitro* dermal irritation test methods (i.e., EpiDerm™, EPISKIN™, and SkinEthic™) will classify corrosive substances incorrectly identified as non-corrosives by *in vitro* corrosivity test methods. Current false negative rates for *in vitro* corrosivity assays range from 12% to 21% (ICCVAM, 1999a). This study will also confirm the extent to which a new procedure to identify substances that directly reduce MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) will reduce the false negative rate in corrosivity assays. A completely non-animal testing strategy for dermal irritation and corrosion must be able to accurately identify corrosive substances since these substances can cause permanent injuries and even death from severe chemical burns.

10 Genetic toxicity test method evaluation activities

The ICCVAM Genetic Toxicity Working Group (GTWG) recently provided comments on cytotoxicity evaluation procedures for a draft OECD Test Guideline 487 for the *in vitro* micronucleus test (OECD, 2007). The GTWG also provided comments on the proposed study plan, protocol, and reference substances for a JaCVAM-led international validation study of the *in vivo* rodent comet assay and nominated experts for an ESAC Peer Review Panel on a cell transformation assay. Similarly, the GTWG has provided technical comments on a planned JaCVAM study of a cell transformation assay.

11 Pyrogen test method evaluation activities

ICCVAM forwarded recommendations on five *in vitro* test methods proposed for assessing potential pyrogenicity of pharmaceuticals and other products to Federal agencies in November 2008 (ICCVAM, 2008a). All applicable Federal agencies, including the U.S. FDA, accepted or endorsed the ICCVAM recommendations (ICCVAM, 2009m). These test methods were also recently adopted by the European Pharmacopoeia Commission during its 133rd session in March 2009 for implementation into the European Pharmacopeia in 2010 (EDQM, 2009a). These methods should now be considered prior to conducting *in vivo* pyrogenicity testing and should be used where determined appropriate for specific testing situations. The availability of these test methods may reduce the number of animals required for pyrogenicity testing.

12 Biologics test method evaluation activities

Biologics potency and safety testing is one of the four highest ICCVAM-NICEATM priorities because it is required by multiple agencies and can require large numbers of animals that may experience significant pain and distress during testing (ICCVAM, 2008b). NICEATM and ICCVAM, in conjunction with ECVAM and JaCVAM, are organizing an international workshop on alternative methods to reduce, refine, and replace the use of animals in vaccine potency and safety testing. The workshop is scheduled for September 14-16, 2010, at the William H. Natcher Conference Center on the main campus of the NIH in Bethesda, MD. More information about the workshop will be posted at http://iccvam.niehs.nih.gov/methods/biologics/biologics.htm as it is available. NICEATM and ICCVAM are also planning an evaluation of U.S. Department of Agriculture/Michigan State University study of *in vitro* potency tests for Leptospirosis vaccines.

13 Conclusions

Since its establishment, ICCVAM has contributed to the evaluation of 27 alternative test methods that have now been accepted or endorsed by national and international authorities. Numerous other test methods are expected to be adopted for regulatory use over the next few years as NICEATM and ICCVAM implement their Five-Year Plan. In addition, many new test methods are expected to emerge from NICEATM-ICCVAM collaborations with member agencies to conduct research, development, translation, and validation efforts relevant to alternative methods. These efforts will integrate scientific advances and new technologies into new test methods and strategies. Implementation of the International Cooperation on Alternative Test Methods is also expected to result in collaborations that will expedite the adoption of alternative methods that will support improved safety assessments while reducing, refining, and replacing animal use.

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