



# UPDATE

## National Toxicology Program

U.S. Department of Health and Human Services

October 2012

Headquartered at the  
National Institute of Environmental  
Health Sciences • NIH-HHS

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## NIEHS launches five-year Strategic Plan

By Eddy Ball reprinted from *eFACTOR*, September 2012



NIEHS/NTP Director Linda Birnbaum (Photo courtesy of Steve McCaw)

With an Aug. 1 editorial in *Environmental Health Perspectives* (EHP), NIEHS/NTP Director Linda Birnbaum, Ph.D., officially launched the Institute's **2012-2017 Strategic Plan**.

In the **editorial**, Birnbaum made a point of emphasizing the collective ownership of the plan, and the input of hundreds of stakeholders, from scientists to public health advocates, that shaped its fresh vision and focused goals. As NIEHS prepares to celebrate its 50th anniversary in 2016, Birnbaum explained, the Institute has evolved to meet the new challenges of the environmental health sciences, building upon the accomplishments of the past.

"The NIEHS has come a long way in making environmental health research responsive to the needs and concerns of the American people — to make

environmental health part of the public health debate," Birnbaum wrote. "This continues to be a source of motivation and purpose for NIEHS staff and our research partners."

The strategic plan process was officially launched March 1, 2011 in a meeting at NIEHS with Deputy Director Rick Woychik, Ph.D., and Sheila Newton, Ph.D., director of the Office of Policy, Planning, and Evaluation, discussing online resources for contributing ideas for the plan. These included an interactive website called *Visionary Ideas*, where people could post their big-picture ideas about the strategic plan and vote to agree, disagree, or comment on ideas posted by others. ●



## Moving forward with alternative test methods

By Robin Mackar reprinted from *eFACTOR*, October 2012



Attending her last meeting of SACATM before her term expires, Gwendolyn McCormick, D.V.M., of Boehringer Ingelheim Pharmaceuticals Inc. provided input on how ICCVAM can better communicate its progress. (Photo courtesy of Michael Garske)

Reflecting on past achievements, moving forward, and establishing good metrics were some of the key themes that emerged during the annual meeting of the **Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)** Sept. 5-6 at NIEHS.

"ICCVAM has tripled progress in bringing alternative methods forward in the past five years," said Rear Adm. William Stokes, D.V.M., as he provided the advisory committee with an update on the efforts of the 15-member **Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)**, which his office, the **NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)**, supports. "We now have 58 alternative test methods that have been adopted and available for use, with 36 of those being *in vitro* tests that do not require animals."

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## ICCVAM successes



SACATM member Ochoa called for committee members to play a more active role in advising and assisting ICCVAM throughout the year. (Photo courtesy of Michael Garske)

NTP associate director John Bucher, Ph.D., cited the maturation of the program over the years, during his welcoming remarks. He highlighted the creation of the [International Cooperation on Alternative Test Methods](#) as an example of what can happen when agencies and countries work together to integrate and harmonize approaches. "The result is a much more efficient process for the acceptance of alternative methods across the globe," Bucher said. Representatives from the Republic of Korea, Japan,



Canada, and the European Union were on hand at the meeting to present updates on their organizations' efforts. Warren Casey, Ph.D., deputy director of NICEATM, also presented new findings on a method recently recommended by ICCVAM, LUMI-CELL<sup>®</sup> that has been specifically adapted for high-throughput or robotic toxicology testing by the National Center for Advancing Translational Sciences (NCATS). This method uses a human cell line, BG1, to identify potential endocrine disruptors without using animals. The NICEATM preliminary evaluation found that the high-throughput assay was as accurate as the manual one currently being used, and could test many substances, at different doses, in a short period of time. Casey noted that the assay was developed by an NIEHS grantee supported, in part, through the Superfund Research Program (see [text box](#)).

## Other Federal efforts

Staff from NIEHS, NIH, and EPA also presented some interesting talks, as they highlighted new research areas that might be incorporated into ICCVAM's new strategic planning efforts. Margaret Sutherland, Ph.D., of NIH, spoke about the use of the NIH Common Fund to develop 3-D tissue models to help predict drug safety; NIEHS program administrator Daniel Shaughnessy, Ph.D., highlighted projects funded by NIEHS through the [Small Business Innovation Research program](#); and Mary Manibusan, of the EPA, provided an update on the Endocrine Disruptor Screening Program for the 21st Century.

Stokes and other ICCVAM representatives elaborated on key accomplishments throughout the meeting, including progress in bringing 26 alternative methods forward for five of the six most commonly used tests to protect human health. If used, Stokes said, these tests could reduce animal use by 50 percent or more. ICCVAM has also contributed to the 14 alternative methods available for reducing and refining animal use for biologics and vaccine safety testing.

Warren Casey, Ph.D., deputy director of NICEATM, also presented new findings on a method recently recommended by ICCVAM, LUMI-CELL<sup>®</sup> that has been specifically adapted for high-throughput or robotic toxicology testing by the National Center for Advancing Translational Sciences (NCATS). This method uses a human cell line, BG1, to identify potential endocrine disruptors without using animals. The NICEATM preliminary evaluation found that the high-throughput assay was as accurate as the manual one currently being used, and could test many substances, at different doses, in a short period of time. Casey noted that the assay was developed by an NIEHS grantee supported, in part, through the Superfund Research Program (see [text box](#)).

## Upcoming Events

November 28-29, 2012

NICEATM-ICCVAM  
Alternatives to the Pertussis Test  
NIH  
Natcher Conference Center  
Bethesda, MD

December 11-12, 2012

NTP Board of Scientific Counselors  
NIEHS  
111 TW Alexander Drive  
Research Triangle Park, NC

June 25-26, 2013

NTP Board of Scientific Counselors  
NIEHS  
111 TW Alexander Drive  
Research Triangle Park, NC

September 24-25, 2013

Scientific Advisory Committee on  
Alternative Toxicological Methods  
NIEHS  
111 TW Alexander Drive  
Research Triangle Park, NC

<http://ntp.niehs.nih.gov/go/calendar>



Bucher and Stokes surprised ICCVAM Chair Jodie Kulpa-Eddy, D.V.M., of the U.S. Department of Agriculture (USDA), with a plaque, certificates of appreciation, and flowers for her exceptional work with ICCVAM over the years. Kulpa-Eddy is ending her tenure on ICCVAM and will be taking on new responsibilities at USDA. (Photo courtesy of Michael Garske)

ICCVAM Vice-chair Joanna Matheson, Ph.D., of the U.S. Consumer Product Safety Commission, followed up by describing the four key strategic opportunities and the ongoing transformation of safety testing presented in the NICEATM-ICCVAM five-year plan.

### Need for metrics

SACATM members, including Chair Steven Niemi, D.V.M., from Massachusetts General Hospital; Steven Hansen, D.V.M., from the American Society for the Prevention of Cruelty to Animals; Ricardo Ochoa, D.V.M., Ph.D., from Pre-Clinical Safety Inc.; and others, called for more metrics and quantifiable goals to determine the real impact that ICCVAM is making. “We need to start counting our accomplishments in measurable ways, as soon as possible,” Niemi said. He and others requested that ICCVAM find ways to determine baseline use of animals in safety testing and research so, a year from now, they can determine the progress made. They also called for regulatory agencies to start actively promoting the alternative methods that ICCVAM has already brought forward. ●

*(Robin Mackar is the news director in the NIEHS Office of Communications and Public Liaison.)*


## Superfund research plays key role in cell bioassay development

Recent approval of an alternative cell bioassay for endocrine disruptors by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) highlights a success story in translating basic research findings to worldwide applications.

This assay, called the LUMI-CELL® ER or BG1Luc estrogen receptor transactivation assay (BG1LucER TA) can now be used with confidence by governments around the world as a test for endocrine disruptors in various types of samples. The history of the assay development sheds light on how basic research at the Superfund Research Program (SRP) leads to successful development of products and outcomes that impact environmental health.

In the mid-1990s, there was a critical need for rapid, inexpensive, and high-throughput methods for detection of endocrine disruptors, primarily xenoestrogens, that could be used as future screening assays by the U.S. Environmental Protection Agency (EPA). With funding from an SRP grant to the University of California, Davis, Michael Denison, Ph.D., and Jane Rogers, Ph.D., took advantage of the receptor-dependent mechanism of estrogen action to produce a recombinant human ovarian carcinoma (BG1) cell line, referred to as BG1Luc4E2 cells, to meet this need.

The recombinant cells contain a stably transfected estrogen-responsive firefly luciferase reporter gene that responds to estrogenic chemicals by inducing luciferase activity in a time-, dose-, chemical-, and estrogen receptor-dependent manner. This new bioassay is rapid, inexpensive, and sensitive. Most importantly, it provides a new screening bioassay for activators and inhibitors of the estrogen-receptor signaling pathway.

This research led to a subsequent collaboration between a small biotechnology company, Xenobiotic Detection Systems, and Denison to further develop and optimize the bioassay for commercial screening purposes. ICCVAM made its recommendation in 2012 (see [story](#) ) , and the assay is now being used by the U.S. EPA, NTP, and in screening of the Tox21 10K library.

For additional information, see studies by Denison and Rogers published in [2000](#)  and [2002](#). 



## Stokes inducted as Board Certified Environmental Scientist

By Eddy Ball reprinted from *eFACTOR*, October 2012

NTP center director [Rear Adm. William Stokes, D.V.M.](#), was selected as one of the inaugural 21-member class of Board Certified Environmental Scientists (BCESs). The announcement appeared in *Environmental Engineer*, the quarterly publication of the American Academy of Environmental Engineers (AAEE), which bestows the prestigious certification on environmental professionals.

“These highly qualified individuals were accepted into the Academy by unanimous vote during our spring Board of Trustees’ meeting,” wrote AAEE Immediate Past President Brian Flynn. “Their admission signals the beginning of our process to recognize the distinct and vital talents of environmental professionals: engineers and scientists working together to protect the environment today and our legacy tomorrow.”

Stokes serves as director of the [NTP Interagency Center for the Evaluation of Alternative Toxicological Methods \(NICEATM\)](#), and executive director of the [Interagency Coordinating Committee on the Validation of Alternative Methods](#), which provide scientific support and coordinate interagency initiatives for advancing new safety testing methods, including those that can replace, reduce, and refine the use of animals in toxicity testing. In addition to his AAEE certification, Stokes holds certification as a Diplomat of the American College of Laboratory Animal Medicine. He studied environmental and biomedical engineering at the University of Louisville’s J.B. Speed Scientific School, before attending the College of Veterinary Medicine at Ohio State University.

“This honor reflects well on Bill’s achievements in environmental public health and the promotion of alternative testing methods,” said NIEHS/NTP Director Linda Birnbaum, Ph.D. “I am gratified to see that his accomplishments are being recognized by his selection as a member of the first group of BCESs.”

### A new kind of certification

The BCES certification represents a broadening by AAEE in the range of its validation services. The idea of expanding the Academy’s certification mission to include environmental scientists arose from discussions in the early spring of 2010, which led to its implementation in November 2011. AAEE certifications are internationally recognized as premium credentials that are awarded to experienced professionals who have demonstrated expertise in one or more areas of specialization.

The basic premise was that, since most of the organizations that employ Academy members utilize environmental engineers and environmental scientists on multidisciplinary teams to solve environmental problems, it would be useful for AAEE to certify both. In this way, the Academy can offer the users of environmental services and environmental employers a full range of professional certification services.

The inaugural class of BCESs reflects the range of members — scientists working in the academic, government, foundation, and private sectors as biologists, chemists, geologists, hydrologists, and toxicologists — dedicated to protecting environmental public health. Stokes, who is the only veterinarian, and U.S. Environmental Protection Agency toxicologist Bruce Macler, Ph.D., are sole federal scientists in the new class of BCESs.

Like their engineering colleagues, BCESs must have at least eight years of professional experience and demonstrate high ethical integrity, before they can be considered as candidates by the AAEE Admissions Committee. Following approval as a candidate, in most cases, an aspiring board-certified environmental professional must stand for written and oral examinations. ●



Stokes is the sole member of the U.S. Public Health Service to become a BCES. (Photo courtesy of Steve McCaw)



## NTP fellow wins 1st Place Young Investigator Award

By Eddy Ball reprinted from eFACTOR, August 2012

NTP visiting fellow [Xiaohua Gao, M.D., Ph.D.](#), was the winner of the 1st Place Young Investigator Award for research presented at the [Society of Toxicologic Pathology \(STP\) 31st Annual Symposium](#) June 24-28 in Boston. Her abstract, titled "Effects of Cadmium on Receptor Tyrosine Kinase (RTK) Phosphorylation, MAPK Activation, and Estrogen Receptor (ER) Alpha and Beta Binding *In Vitro*," was selected from a pool of all the young investigator submissions for the meeting.



"I'm very lucky to be working here," Gao said. "I feel that Dr. Dixon's mentoring has contributed greatly to advancing my career as a physician scientist specializing in toxicologic pathology." (Photo courtesy of Steve McCaw)



After completing her doctor of veterinary medicine and doctor of philosophy degrees, Dixon served as a postdoctoral fellow at The Rockefeller University before joining NIEHS in 1987. She earned certification as a Diplomate in the American College of Veterinary Pathologists in 1987. (Photo courtesy of Steve McCaw)

Gao is a member of the Molecular Pathogenesis Group headed by [Darlene Dixon, D.V.M., Ph.D.](#), of the NTP Laboratory. A physician with a Ph.D. in toxicology, now in the fourth year of her fellowship, Gao has successfully applied her knowledge of medicine and toxicology to understanding the molecular basis of disease and deciphering the pathogenesis of changes induced by environmental toxicants. In addition to her STP award, Gao has been the first author on [two papers](#) published by the group in peer-reviewed journals, as well as another one now under review, and a co-author on four others.

"I'm very proud of Dr. Gao's accomplishment in winning this award," Dixon said. "I think it acknowledges Xiaohua's outstanding work as a research toxicologist in training and reflects very favorably on the quality of the [NTP postdoctoral training programs](#)."

Posters were evaluated and scored for experimental design, clarity, data interpretation, quality of visual aids, and impact of research by a panel of international investigators from the STP Career Development and Outreach Committee, as well as the Annual Symposium Poster Committee. Gao was also evaluated and scored for her presentation of the data.

The award-winning research is part of work in the Dixon group on understanding the pathogenesis of toxicant-induced changes in the uterus and the role of toxicants in uterine disease. In the research presented at the STP, Gao proposed a non-classical estrogen receptor binding mechanism for cadmium-induced proliferative changes in uterine fibroid cells. Gao and Dixon plan to conduct additional studies to support their hypothesis.

NIEHS is one of several sponsors of the STP Annual Symposium, which also features a special premeeting, full-day [NTP Symposium](#) each year, featuring a number of NTP scientists addressing issues of interpretation in pathology. ●



## NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

By Debbie McCarley and Cathy Sprankle reprinted from *eFACTOR*, September 2012

### Federal agencies accept ICCVAM-recommended testing methods



U.S. federal agencies, including NIEHS, have agreed with recommendations on test methods using human cells that can screen substances for their potential to interact with the estrogen receptor. Chemicals that interact with hormone receptors, known as endocrine disruptors or endocrine-active substances, may result in abnormal growth, development, or reproduction.

The [Interagency Coordinating Committee on the Validation of Alternative Methods \(ICCVAM\)](#) evaluated the scientific validity of the proposed methods, the BG1Luc estrogen receptor (ER) transactivation (TA) agonist and antagonist assays, and recommended how they could be used to identify substances that induce or inhibit human ER activity *in vitro*. Representatives of U.S. Environmental Protection Agency (EPA) responded that they regard the BG1Luc ER TA test methods as an alternative to similar test methods currently used in their [Endocrine Disruptor](#)

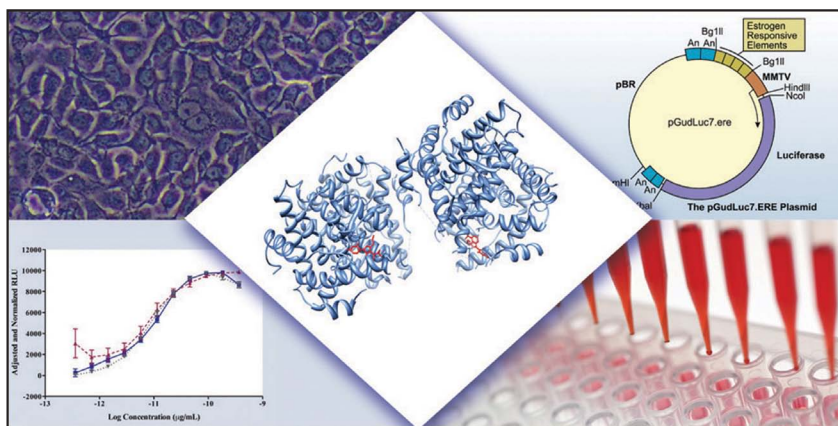
[Screening Program](#). Several agencies also indicated that they would communicate the ICCVAM recommendations to stakeholders and encourage their appropriate use.

In her [response](#) to the ICCVAM Committee, NIEHS/NTP Director Linda Birnbaum, Ph.D., noted the advantages offered by the BG1Luc ER TA test methods compared to other methods used to measure interaction with the estrogen receptor. "NIEHS and the NTP will ... promote and encourage the consideration and use of the BG1Luc ER TA for research and testing where determined appropriate," she wrote. "These alternative test methods should be routinely considered and used where appropriate, in order to avoid or minimize animal use."

Endocrine-active substances mimic or block the action of hormones, causing adverse health effects, by interfering with normal hormone function. Evidence suggests that environmental exposure to endocrine-active substances may cause reproductive and developmental problems in humans and wildlife. There is also concern that exposure to endocrine-active substances may increase cancer incidence in humans.

The Food Quality Protection Act of 1996 directed the EPA to screen pesticides and environmental contaminants for their potential to affect the endocrine systems of humans and wildlife. The EPA subsequently initiated an endocrine disruptor screening program, and began efforts to standardize and validate test methods to include in the program. At the request of EPA, ICCVAM and the [NTP Interagency Center for the Evaluation of Alternative Toxicological Methods \(NICEATM\)](#), which administers ICCVAM and provides scientific support for its activities, reviewed the validation status of *in vitro* test methods, and developed guidance for future validation studies. NICEATM then conducted validation studies of *in vitro* test methods that could identify potential endocrine disruptors without using animals.

The subject of one of these validation studies was the BG1Luc ER TA agonist and antagonist assays, also known as the LUMI-CELL® ER test method. Xenobiotic Detection Systems Inc. (XDS) developed the LUMI-CELL® ER test method with the support of an NIEHS Small Business Innovation Research grant. NICEATM coordinated an international validation study of the BG1Luc ER TA agonist and antagonist assays at laboratories in Europe, the United States, and Japan.



This collage of pictures includes a drawing representing estrogen receptor molecules bound to 17beta-estradiol surrounded by, clockwise from top left, cells used for the BG1Luc ER TA test method; drawing of a DNA plasmid molecule contained in the cells used for the BG1Luc ER TA test method; pipettor and assay plate representing lab automation; and an example of data from a positive control substance in the BG1Luc ER TA agonist assay. (Photo courtesy of NICEATM-ICCVAM)



The ICCVAM evaluation of the BG1Luc ER TA test methods was reviewed by an independent scientific peer review panel that met in March 2011. Appearing here with the panel are NICEATM Director William Stokes, D.V.M., seated third from right, and NICEATM Deputy Director Warren Casey, Ph.D., seated third from left. (Photo courtesy of NICEATM)

The ICCVAM recommendations on the BG1Luc ER TA test methods were based on data from the NICEATM-sponsored independent validation studies. In developing the recommendations, ICCVAM considered comments from its [scientific advisory committee](#), an [independent scientific peer review panel](#), and members of the public.

NICEATM also nominated the BG1Luc ER TA test methods for evaluation in [Tox21](#). The assays have now been adapted to a high-throughput format using 1536-well plates by the [National Center for Advancing Translational Sciences](#) and have been used to screen all compounds in the Tox21 10K chemical library.

Details on the ICCVAM evaluation of the BG1Luc ER TA test methods, including a recommended protocol, more information on the ICCVAM recommendations, and the agency responses, can be found on the [NICEATM-ICCVAM website](#). ●

*(Debbie McCarley is a special assistant to Stokes. Cathy Sprankle is a communications specialist with ILS, Inc., support contractor for NICEATM)*

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## NICEATM-ICCVAM and International Partners Convene Workshop on Alternative Methods for *Leptospira* Vaccine Potency Testing

Over 80 international scientific experts from the United States, Europe, and Asia attended the “International Workshop on Alternative Methods for *Leptospira* Vaccine Potency Testing: State of the Science and the Way Forward” on September 19-21. Scientists representing industry, government, and academia met to review available methods and approaches for *Leptospira* vaccine potency testing as well as recent advances in science and technology. Participants also developed a strategy to achieve global acceptance and implementation of scientifically valid alternative methods.

Leptospirosis is a zoonotic disease caused by bacteria of the genus *Leptospira*. An estimated 500,000 human cases of leptospirosis occur worldwide each year with a fatality rate of up to 25% in some regions. Designated as a Neglected Tropical Disease by the U.S. National Institutes of Health and a Neglected Zoonotic Disease by the World Health Organization, leptospirosis is a global public health priority.

In the United States and other countries, *Leptospira* vaccines are used in cattle, swine, and dogs to protect them from disease and to reduce the risk of animal-to-human transmission. Manufacturers test the potency of vaccine lots prior to their release to ensure their effectiveness. However, methods currently used to test the potency of *Leptospira* vaccines



Above are the participants of the NICEATM-ICCVAM International Workshop for *Leptospira* Vaccine Potency Testing, U.S. Department of Agriculture Center for Veterinary Biologics, Ames, IA, September 19-21, 2012.



use large numbers of laboratory animals that experience significant pain and distress, accounting for over one third of the animals reported to the U.S. Department of Agriculture (USDA) in this category. Vaccine testing overall is estimated to use about twice as many animals as toxicity testing.

An international workshop organized in 2010 by NICEATM, ICCVAM, and their international partners identified *Leptospira* vaccines as a high priority for future research, development, and validation of alternative test methods. Last month's workshop was convened to consider methods and approaches for *Leptospira* vaccine testing that could provide improved accuracy, efficiency, and worker safety and that are more humane and use fewer or no animals.

NICEATM, ICCVAM, and partner organizations in the International Cooperation on Alternative Test Methods organized last month's workshop, which was hosted by the USDA Center for Veterinary Biologics at the National Centers for Animal Health in Ames, Iowa. Cosponsors of the workshop included the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), the International Alliance for Biological Standardization, and the Animal Health Institute.

A summary of the conclusions and recommendations from the workshop is [available on the NICEATM–ICCVAM website](#). A report from the workshop, which will include manuscripts contributed by many of the speakers, will be published in 2013 as a special issue of *Biologicals*. ●

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## Upcoming workshop on innovative safety tests for pertussis vaccines

By Debbie McCarley and Cathy Sprankle reprinted from *eFACTOR*, October 2012

NIEHS and U.S. Food and Drug Administration (FDA) scientists will join other scientific experts from around the world this fall to consider improved methods and approaches for safety testing of vaccines that protect against pertussis. The “[International Workshop on Alternatives to the Murine Histamine Sensitization Test \(HIST\) for Acellular Pertussis Vaccines: State of the Science and the Path Forward](#)” flyer will take place on November 28-29 at the William H. Natcher Conference Center on the NIH campus in Bethesda, MD.

The [NTP Interagency Center for the Evaluation of Alternative Toxicological Methods \(NICEATM\)](#), is organizing the workshop in collaboration with the [Interagency Coordinating Committee on the Validation of Alternative Methods \(ICCVAM\)](#) and partner organizations in the [International Cooperation on Alternative Test Methods](#). The organizing committee for the workshop includes NIEHS scientists and scientists from the FDA, international vaccine manufacturers, and international research and regulatory agencies.



[play linked multimedia clip](#)

Watch as President of the National Foundation of Infectious Diseases William Schaffner, M.D., discusses why pertussis vaccine development and safety testing need to continue (01:11).

[Download Media Player](#)

### **Pertussis is an important public health concern**

Pertussis, also known as whooping cough, is a highly contagious bacterial disease that was once a major cause of childhood mortality. While widespread vaccination has substantially decreased the incidence of pertussis, recent outbreaks have led public health officials to recommend renewed and expanded vaccination efforts. For example, a sharp increase in the number of pertussis cases in early 2012, in central North Carolina, prompted the state health department to offer free pertussis vaccinations.

Regulatory authorities require testing of each production lot of a vaccine to ensure safety, potency, and purity. The murine HIST is a key safety test performed on pertussis vaccines to ensure that residual pertussis toxin has been effectively inactivated, to avoid adverse effects caused by the toxin. However, such testing requires large numbers of mice, some of which experience significant unrelieved pain and distress. An international [workshop](#) in 2010 identified the HIST as a priority for future research, development, and validation of alternative test methods that could reduce, refine, or replace animal use for pertussis vaccine safety testing.





“There is significant international interest in supporting innovation in this area,” notes Rear Adm. William Stokes, D.V.M., director of NICEATM, which administers and provides scientific support for ICCVAM. “In addition to animal welfare concerns, the HIST is time-consuming and technically challenging. Both vaccine manufacturers and regulators are actively working to develop and validate improved alternatives that can achieve acceptance and use.”

The ICCVAM Authorization Act of 2000 charges ICCVAM with coordinating the interagency evaluation of new testing methods that can replace or reduce the use of animals, and refine animal use to enhance animal well-being and lessen or avoid pain and distress. Vaccine testing uses significantly more animals, and results in more animals experiencing pain and distress than toxicity testing. Therefore, promoting improved alternative test methods for vaccine potency and safety testing is one of the highest priorities in [ICCVAM's five-year plan](#).

### About the workshop

The upcoming workshop will provide a forum for participants to review protocols and available data from an ongoing international study of *in vitro* alternatives to the HIST. Participants will also review recent advances and innovations in science and technology that may provide greater accuracy, precision, and efficiency, and that are more humane and use fewer or no animals. Finally, the workshop will address the path to achieve validation, global acceptance, and implementation of new alternative methods.

Registration information and a workshop program are available on the NICEATM-ICCVAM [website](#). NICEATM and ICCVAM also invite the submission of abstracts for scientific posters to be displayed during this workshop. Abstracts should be submitted by October 12.

Stokes and Warren Casey, Ph.D., deputy director of NICEATM, are serving on the organizing committee for the workshop. The committee also includes Richard McFarland, Ph.D., M.D., co-chair of the ICCVAM Interagency Biologics Working Group, Juan Arciniega, D.Sc., and Lev Sirota, Ph.D., all from the FDA Center for Biologics Evaluation and Research. ●

*(Debbie McCarley is a special assistant to Stokes. Cathy Sprankle is a communications specialist with ILS, Inc., support contractor for NICEATM.)*

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## ICCVAM Makes Recommendations to Federal Agencies on Using Fewer Animals to Identify Chemical Eye Hazards

ICCVAM has forwarded recommendations to U.S. Federal agencies that will provide for identifying chemical eye hazards with fewer animals. When it is determined necessary to use animals for eye hazard testing, the recommendations provide procedures that use 50% to 83% fewer animals than some current testing procedures. The recommendations also harmonize the number of animals used for eye safety testing across U.S. regulatory agencies and international test guidelines.

ICCVAM also recommends that *in vitro* test methods should always be considered before using animals for eye safety testing, and these should be used where determined appropriate. When it is necessary to use animals for eye safety testing, ICCVAM recommends that medications and humane endpoints should always be used to avoid or minimize pain and distress.

Each year, an estimated 2 million eye injuries occur in the United States. Of these, more than 40,000 cause permanent visual impairment. Chemicals and compounds are the third most common cause of eye injuries, with many eye injuries in consumers associated with the use of household cleaning products. To warn consumers and workers of the potential for chemicals and products to cause eye injuries, eye safety testing is performed to determine if substances may cause temporary or permanent eye damage. Test results are then used for hazard classification of chemicals and products using appropriate national and/or international hazard classification systems.

Eye safety testing procedures vary among U.S. agencies. Current testing procedures specified in the U.S. Code of Federal Regulations (16 CFR 1500.42) require 6 animals per test and may require up to three sequential tests for each substance, thereby requiring 6, 12, or 18 animals to reach a hazard decision.



Based on previous initiatives in the United States to reduce the number of animals used for eye safety testing, some U.S. and international test guidelines for eye irritation/corrosion testing have been modified, with a maximum of 3 animals typically used. However, current testing procedures (16 CFR 1500.42) do not provide criteria to classify results from 3-animal tests. Therefore, NICEATM and ICCVAM conducted an analysis (reported in Haseman et al., Regul Toxicol Pharmacol 61: 98-104, 2011) to determine classification criteria based on results from a 3-animal test that would maintain eye hazard classification equivalent to current testing procedures (16 CFR 1500.42). The ICCVAM recommendations are based on this analysis.

ICCVAM concluded that using a classification criterion of one or more positive animals in a three-animal test to identify chemicals and products that are eye hazards will maintain hazard classification equivalent to that provided by current testing procedures (16 CFR 1500.42). ICCVAM recommends that alternative *in vitro* test methods should always be considered and used where appropriate for eye safety testing. While currently approved *in vitro* test methods can identify some eye hazards, they are not sufficiently validated and accepted to completely replace all animal testing. When eye safety testing using animals is necessary, testing should be conducted using the minimum number of animals in the most humane manner possible consistent with testing objectives. Consistent with ICCVAM's duty to foster interagency and international harmonization (42 U.S.C. 285I-3), this recommendation harmonizes the number of animals used for eye safety testing across U.S. regulatory agencies and international test guidelines.

The ICCVAM recommendations are detailed in the "ICCVAM Test Method Evaluation Report: Identifying Chemical Eye Hazards with Fewer Animals" (NIH Publication No. 12-7930), available on the [NICEATM-ICCVAM website](#). ICCVAM recommendations have been transmitted to U.S. Federal agencies, which have 180 days to respond to the recommendations. Transmittal letters are posted on the [NICEATM-ICCVAM website](#). Agency responses will be posted on this page as they are received.

### **ICCVAM Seeks Comments and Data on Nominated Methods**

ICCVAM recently requested public comments, nominations of experts, and data submissions on nominated test methods for three safety testing applications.

#### ***In Vitro* Test Method to Assess Allergic Contact Dermatitis Hazard Potential**

The electrophilic allergen screening assay (EASA) is an *in vitro* test method that may be useful for identification of substances with the potential to produce allergic contact dermatitis (ACD). NICEATM recently requested public comments on the EASA, which was nominated for validation studies to evaluate its usefulness and limitations as a screening assay to identify potential sensitizers. NICEATM also requested data generated using *in vivo* and *in vitro* test methods for assessing ACD hazard potential. Data will be used to develop integrated testing and decision strategies that will also consider incorporation of the EASA following adequate validation studies.

Sensitizers are substances with the potential to cause ACD, and skin sensitization is the process by which a sensitizer induces the development of ACD. The initial molecular event in the pathway leading to skin sensitization involves binding of the potential sensitizer to proteins in the skin. The EASA identifies a potential sensitizer by measuring binding of a test substance to chemical probes that contain structures commonly found in skin proteins.

The nomination of the EASA for validation studies was considered at the recent meeting of the ICCVAM advisory committee; the advisory committee agreed with ICCVAM's draft high priority for the proposed studies.

For more information about the NICEATM-ICCVAM evaluation of the EASA, please visit the [NICEATM-ICCVAM website](#).

#### ***Up-and-Down Procedure for Acute Dermal Systemic Toxicity Testing***

NICEATM and ICCVAM are planning to convene an independent scientific peer review panel to assess the validation status of an up-and-down procedure (UDP) for acute dermal systemic toxicity testing. NICEATM requested nominations of scientific experts to be considered for the panel and data for substances tested in *in vivo* acute dermal and oral systemic toxicity tests.

Poisoning by dermal exposure (absorption through the skin), while not as common as poisoning by ingestion, accounted for over 172,000 poisonings in the U.S. in 2010. Alternative test methods for acute dermal systemic toxicity testing are an ICCVAM priority because such testing is required by multiple U.S. regulatory agencies, can involve large numbers of

animals, and can result in significant pain and distress to test animals. NICEATM is developing a UDP procedure for acute dermal systemic toxicity testing, which is one of the four most commonly conducted product safety tests worldwide. If accepted, this procedure could reduce the number of animals required for this testing compared to current guidelines.

For more information about the NICEATM–ICCVAM evaluation of the dermal UDP, please visit the [NICEATM–ICCVAM website](#).

### **In Vitro Test Methods to Assess Eye Injury Hazard Potential**

NICEATM and ICCVAM are planning to convene an independent scientific peer review panel to assess the validation status of *in vitro* tests and integrated non-animal testing strategies proposed for identifying eye injury hazard potential of chemicals and products. NICEATM has requested nominations of scientific experts who can be considered for the panel. NICEATM has also requested data from substances tested in *in vitro* tests for identifying eye injury hazard potential. Of particular interest are data generated in the short time exposure (STE) and isolated rabbit eye (IRE) tests and data from approaches using two or more *in vitro* tests. However, NICEATM also requests data from other *in vitro* tests, as well as corresponding *in vivo* data from any ethical human or animal studies or accidental human exposures.

Past ICCVAM evaluations of *in vitro* eye safety test methods have supported the national and international acceptance of several methods that can be used to obtain eye hazard classification data on chemicals and products without using animals. NICEATM and ICCVAM are currently evaluating additional *in vitro* test methods for their potential usefulness for this purpose. The IRE test is an organotypic test method that evaluates the eye injury potential of a test substance by measuring corneal opacity, corneal swelling, epithelial integrity, and fluorescein staining. The STE test measures the viability of cultured cells from an established rabbit corneal epithelial cell line following test substance exposure.

For more information about the ongoing NICEATM and ICCVAM evaluations of eye safety test methods, please visit the [NICEATM–ICCVAM website](#).

### **International Partners Meet at NTP**



Attendees at the September ICATM coordination meeting included: (back row, left to right) Dr. Patric Amcoff, Operational Manager, EURL/ECVAM; Dr. Warren Casey, Deputy Director, NICEATM; (middle row, left to right) Dr. Ki Hwan Choi, Vice Director, KoCVAM; Dr. Hajime Kojima, JaCVAM; Dr. Joanna Matheson, U.S. Consumer Product Safety Commission, Vice-chair, ICCVAM; Dr. William Stokes, Director, NICEATM; (front row, left to right) Dr. Jodie Kulpa Eddy, U.S. Dept. of Agriculture, Chair, ICCVAM; Dr. Chea Hyung Lim, KoCVAM; Dr. Tim Singer, Director, Environmental Health Science and Research Bureau, Health Canada.

NICEATM hosted a coordination meeting of the International Cooperation on Alternative Test Methods (ICATM) on September 4. ICATM, which includes member organizations from the United States, the European Union, Japan, Canada, and South Korea, was established in 2009 to provide a forum for cooperation and harmonization of international efforts to reduce the number of animals required for chemical safety testing.

Topics discussed at the meeting included progress reports on ongoing validation studies. ICATM member organizations each have representatives on the management teams of ongoing validation studies of new test methods with the potential to reduce, refine, or replace animal use. NICEATM and ICCVAM have representatives on the study management teams for ongoing EURL ECVAM studies of *in vitro* methods to identify potential eye irritants, to better characterize metabolism of chemicals, and to identify potential skin sensitizers. NICEATM and ICCVAM also have representatives on the study management team of an evaluation of an *in vitro* method to identify potential skin sensitizers being conducted by the Japanese Center for the Validation of Alternative Methods (JaCVAM). In turn, the ICATM partner organizations have representatives on the management team of an ongoing NICEATM–ICCVAM evaluation of an *in vitro* test method to identify substances with the potential to interact with the estrogen receptor.

ICATM holds coordination meetings to coincide with scientific conferences and other events that representatives of the member organizations normally attend. The September coordination meeting was scheduled to coincide with the annual meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), which took place on September 5 and 6. The visiting ICATM representatives provided updates on their organizations' activities to SACATM.



## Stokes Presents at Japanese Workshop on Adverse Outcome Pathways

NICEATM had additional opportunities to develop relationships and harmonize approaches to alternative methods with international collaborators when NICEATM Director William Stokes, DVM, attended September meetings and workshops in Japan.

One of these events was a workshop on "Adverse Outcome Pathways for Skin Sensitization," which was held in Kyoto on September 13. Adverse outcome pathways, or AOPs, describe chains of biological events that begin with the interaction of a chemical with a biological molecule or structure and result in adverse outcomes such as allergic contact dermatitis, or skin sensitization. This workshop focused on the adverse outcome pathway that results in skin sensitization and how test method developers around the world are using what is known about this pathway to develop *in vitro* test methods to identify potential skin-sensitizing substances. Stokes spoke on NICEATM development of an AOP-based integrated testing and decision strategy for skin sensitization and ICCVAM-recommended test methods that incorporate AOP critical events.

The workshop coincided with a meeting of the study management team for a JaCVAM-led study of an *in vitro* method to identify potential skin sensitizers. Stokes is a member of the validation management team. Attendees at the validation management team meeting received updates on current progress of the study and planned the next phase of the study. ●

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**Contact Information:** Dr. William S. Stokes, D.V.M., Director, NICEATM, NIH/NIEHS, P.O. Box 12233, MD K2-16, Research Triangle Park, NC 27709; T: (919) 541-2384; FAX: (919) 541-0947; niceatm@niehs.nih.gov

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**Contact Information:** NTP Office of Liaison, Policy and Review, NIEHS, P.O. Box 12233, MD K2-03, Research Triangle Park, NC 27709; T: (919) 541-0530; FAX: (919) 541-0295; [CDM@niehs.nih.gov](mailto:CDM@niehs.nih.gov)

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