



UPDATE

National Toxicology Program

U.S. Department of Health and Human Services

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Health Sciences • NIH-HHS

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National Research Center honors Birnbaum



NIEHS/NTP Director [Linda Birnbaum](#) will receive the 2012 Health Policy Hero Award May 11 during the annual awards luncheon of the [National Research Center for Women & Families \(NRC\)](#), held at the historic Cosmos Club in Washington, D.C. Katharine Weymouth, publisher of The Washington Post, will serve as the emcee for the program, which is held each year on the Friday before Mothers Day.

Birnbaum joins a select group of influential women who have been so honored for their contributions to public health, including last year's winner, Margaret Hamburg, commissioner of the U.S. Food and Drug Administration; Catherine DeAngelis, former editor in chief of JAMA;

and two members of Congress, Rep. Rosa DeLauro and Sen. Chuck Grassley. The NRC is dedicated to improving the health and safety of adults and children, by using research to encourage more effective programs and policies. The organization achieves its mission by gathering, analyzing, critiquing, and explaining scientific and medical research. ●

(Ed Kang is a public affairs specialist in the Office of Communications and Public Liaison and a regular contributor to the Environmental Factor.)

Panel peer reviews and approves seven NTP technical reports

By Robin Mackar reprinted from *eFACTOR*, March 2012

A panel of external scientific experts convened by the National Toxicology Program (NTP) peer reviewed and approved the conclusions of [seven draft technical reports](#). The two-year studies outlined in the NTP technical reports covered a broad spectrum of substances ranging from a popular herbal supplement and industrial solvents to HIV treatment approaches. The meeting February 8-9 was webcast and included presentations and comments from the public.

After brief welcoming remarks by NIEHS/NTP Director Linda Birnbaum, Ph.D., and NTP Associate Director John Bucher, Ph.D., and accolades to NTP's Michelle Hooth, Ph.D., and David Malarkey, D.V.M., Ph.D., for overseeing the reports, the meeting began with a short presentation by Hooth outlining the panel's charge.



NTP's Hooth reminded the panel members of their charge at the onset of the NTP Technical Reports Peer Review meeting. (Photo courtesy of Steve McCaw)

Hooth discussed how NTP technical reports describe the methods, results, and NTP conclusions. She said NTP conclusions are presented as levels of evidence under the specific conditions of each study, and that both the clear and some-evidence conclusions indicate positive responses for carcinogenic activity in the rodent studies. It was the panel's role to determine whether the study's experimental design, conduct, and findings support the NTP's conclusions.

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Presentations on Ginkgo and DMPT

June Dunnick, Ph.D., of NTP kicked off the first studies to be reviewed, which were on N,N-Dimethyl-p-toluidine (DMPT), a high-production



Dunnick of NTP responded to comments made by panel members on the DMPT technical report.

chemical used in dental materials and bone cements. Dunnick presented data showing that both rats and mice developed tumors after being given DMPT for up to two years. The NTP also found anemia-like symptoms in rats and mice. All three primary reviewers, as well as the panel, concurred with the clear-evidence call made by the NTP.

The second report on *Ginkgo biloba* extract was presented by NTP's Cynthia Rider, Ph.D. Ginkgo is among the top five herbal supplements on the market, with an estimated 7.7 million Americans taking it in 2002. Ginkgo extract was given orally to mice and rats for up to 105 weeks. The NTP found an increase in liver cancer in male and female mice, and in cancer of the thyroid gland in male and female rats and male mice.

Written and oral comments from the American Herbal Products Association and from Intertek Cantox were presented to the panel. The comments largely focused on the *Ginkgo biloba* extract used in the NTP studies, claiming that it was not representative of ginkgo products in the U.S.

With citations in hand, Rider said, "The range of constituents in the ginkgo extract used in our NTP studies is within the ranges found in the U.S. market." After a brief discussion around this topic, Birnbaum commented on the importance of putting human-use doses into context for people, so they can better understand, appreciate, and use the NTP findings.

NTP pathologist Mark Hoenerhoff, D.V.M, Ph.D., also added to the discussion by presenting molecular work conducted by NTP on ginkgo, which begins to unravel the underlying mechanisms for the tumors in the rodent studies. "We really applaud this extra effort by the NTP. It is very helpful in interpreting the data," said Paul Howard, Ph.D., of the National Center for Toxicological Research (NCTR) at the U.S. Food and Drug Administration. Hoenerhoff's findings are being submitted to a peer-reviewed journal.

Remaining technical reports approved

After hearing the study overview and conclusions put forward by NTP study scientist Michael Wyde, Ph.D., on beta-picoline, a solvent used to make pharmaceuticals, resins, dyes, rubber accelerators, and insecticides, the panel voted to accept the NTP conclusions as written.

The reviewers also heard presentations on two dermal studies that were conducted by NTP. The panel approved of the studies and conclusions presented by NTP's Minerva Mercado-Feliciano, Ph.D., on pyrogallol, a byproduct of plant tannins used to make consumer products, such as some hair dyes and photography developers. After hearing the discussion on trimethylolpropane triacrylate from NTP's In Ok Surh, Ph.D., and a few

Upcoming Events

June 21-22, 2012

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

September 5-6, 2012

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

September 19-21, 2012

NICEATM-ICCVAM
International Workshop on
Alternative Methods for *Leptospira*
Vaccine Potency Testing: State of
the Science and the Way Forward
U.S. Department of Agriculture
Center for Veterinary Biologics
National Centers for Animal Health
Ames, Iowa, USA

November 28-29, 2012

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

December 11-12, 2012

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

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



Chair Stephen Roberts, Ph.D., center, of the University of Florida, kept the meeting and the discussion moving along nicely. He was flanked by NTP's Danica Andrews, left, the designated federal official and Malarkey. (Photo courtesy of Steve McCaw)

public comments, the panel recommended minor revisions to the NTP conclusions on this industrial compound. The NTP will consider the input from the panel in finalizing the technical reports.

On the second day of the meeting, the panel also approved the two final reports on AIDS therapeutics. After a brief introduction from Howard, Julian Leakey, Ph.D., also of NCTR, thoughtfully walked the panel through the details of the two reports on 3'-azido-3'-deoxythymidine (AZT) and its combined mixtures with lamivudine or nevirapine. AZT is widely used worldwide, as part of combination drug therapy, to stop mother-to-child transmission of HIV. However, the long-term or lifetime consequences of perinatal exposure to these therapies are unknown. The studies were conducted in genetically modified mice. ●

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

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Science showcases grantee and NIEHS/NTP tox efforts

By Eddy Ball reprinted from *eFACTOR*, April 2012



Prior to joining Johns Hopkins, Hartung was head of the European Center for the Validation of Alternative Methods of the European Commission from 2002 to 2008. (Photo courtesy of Johns Hopkins)

A feature in the March 2 issue of *Science*, "*LIFE SCIENCE TECHNOLOGIES: Animal-Free Toxicology: Sometimes, In Vitro is Better*," highlights NIEHS/NTP predictive toxicology efforts. The article discusses work by NIEHS [grantee](#) Thomas Hartung, M.D., Ph.D., toward mapping what he calls the toxome, the Tox21 consortium, and alternative testing.

Along with efforts well underway in Europe, writes Jeffrey Perkel in the *Science* [article](#), animal-free toxicology in the U.S. is demonstrating that, sometimes, *in vitro* is better than trying to subject tens of thousands of chemicals to time-consuming and expensive animal testing. The goal of these initiatives is to identify the biomolecular changes triggered by toxic exposure that lead ultimately to the pathological outcomes, or apical endpoints, measured in traditional animal studies.

Mapping perturbed pathways as a guide to high-throughput screening

[Hartung](#), a Doerenkamp-Zbinden professor and chair for evidence-based toxicology at the Johns Hopkins University Bloomberg School of Public Health, has embarked on a six-year NIEHS funding quest to map the biomolecular pathways perturbed in response to toxicity by endocrine disruption that will mark one important step forward toward the gargantuan task. "Mapping the entirety of these [toxicity] pathways (i.e., the Human Toxome)," Hartung wrote in his grant description, "will be a large-scale effort, perhaps on the order of the Human Genome Project."

Using gene expression microarrays and mass spectrometry-based metabolomics, Hartung's team is testing human breast cancer cell lines, exposed to some 53 endocrine-disrupting compounds, to chart gene expression and metabolomic changes in protein production.

Consortia to develop predictive toxicology and reduce animal testing

In addition to funding such researchers as Hartung, NIEHS/NTP is also a part of two forward-thinking interagency consortia working to expand use of *in vitro* testing and reduce reliance on animals. The newest, known as Tox21, is a partnership among NIEHS/NTP, the NIH Chemical Genomics Center (NCGC), the U.S. Environmental Protection Agency, and the U.S. Food and Drug Administration, to develop predictive toxicology using high-throughput screening.

Tox21 has set its sights on screening 10,000 plus compounds on an NCGC robotics platform. The information that high-throughput screening yields will help identify toxicity pathways that will be the basis for more targeted assays.

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The [NTP Interagency Center for the Evaluation of Alternative Toxicological Methods \(NICEATM\)](#) also administers a partnership known as the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). With representatives from [U.S. federal regulatory and research agencies](#) that require, use, generate, or disseminate toxicological information, ICCVAM is charged with promoting the validation and regulatory acceptance of new alternative test methods that may reduce, refine, and replace animal use for safety testing of various chemicals, medicines, and consumer products. To date, ICCVAM has contributed to the regulatory approval of more than 49 alternative methods. Replacing animals with *in vitro* testing to ensure consumer and worker safety isn't always possible. But when they can't replace animals, alternative methods strive to use many fewer animals and to make safety testing with animals less harmful, painful, and stressful.

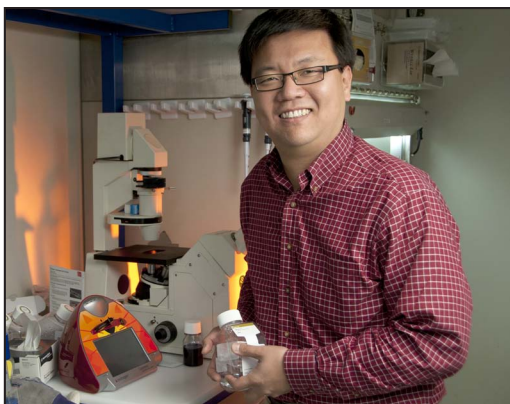
Everyone Perkel interviewed for his story conceded that the challenges are enormous, and that progress toward realizing predictive toxicology and reducing animal testing, as much as possible, will take several years. Still, the effort is receiving government support from such agencies as NIEHS/NTP and private sector involvement is growing. ●

Citation: [Perkel, JM](#). 2012. LIFE SCIENCE TECHNOLOGIES: Animal-Free Toxicology: Sometimes, *in Vitro* is Better. *Science* 335(6072):1122-1125.

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High-throughput screening finds surprising properties for antioxidants

By Larry Thompson reprinted from *eFACTOR*, April 2012



Myung's laboratory investigates genome instability, which is a characteristic of cancer and many genetic disorders, by examining the mechanisms of DNA repair and replication, as well as their roles in the production and suppression of gross chromosomal rearrangements. (Photo courtesy of NHGRI)



Tice is the lead coordinator for the NTP High Throughput Screening (HTS) Initiative and plays a key role in the efforts of the Tox21 community. (Photo courtesy of Steve McCaw)

Antioxidants have long been thought to have anti-aging properties, primarily by protecting a person's genetic material from damaging chemicals. The story, however, now appears to be much more complicated.

National Institutes of Health researchers from two institutes and one center have demonstrated that some anti-oxidants damage DNA and kill cells instead of protecting them. The [findings](#), published March 19 in the Proceedings of the National Academy of Sciences, also suggest that this surprising capability may be good for treating cancer, but may prove cautionary when using antioxidant-based medicines to treat other disorders, such as diabetes.

An unexpected discovery

"It's an unexpected discovery," said [Kyungjae Myung, Ph.D.](#), a senior researcher in the Genetics and Molecular Biology Branch of the National Human Genome Research Institute (NHGRI), and the senior author on the report.

"This report demonstrates the ability of the high throughput screening program to generate findings that may impact on human health," said co-author [Raymond Tice, Ph.D.](#), chief of the NIEHS/NTP Biomolecular Screening Branch. "In this case, the technology is helping to identify potential drug candidates for treating cancer."

Many people attempt to boost their levels of antioxidants by eating fruits and vegetables, nuts and grains, or by taking supplements. By adding antioxidants to the diet, many people hope to slow down the process that some believe contributes to the normal process of aging.

DNA repair

Myung did not set out to challenge this anti-aging strategy. His lab studies DNA repair, the enzyme systems within a cell that fix mistakes and other



damage accumulating routinely in DNA as cells live and divide. Researchers know that naturally occurring defects in DNA repair can lead to a number of disorders, including cancer.

Myung's group sought a new way to easily identify chemicals that damage DNA and then use those chemicals to study cellular repair mechanisms, a basic research question. Using a laboratory-grown cell line from human kidneys, the team developed a novel laboratory test that readily shows when a chemical exposure damages DNA.

With the test developed, Myung's team formed collaborations with two other NIH research groups. The first was with what is now the NIH National Center for Advancing Translational Sciences (NCATS). Over the last several years, a team lead by Christopher Austin, M.D., head of the NCATS labs, has developed high-throughput chemical screening systems using robotics. Austin agreed to use Myung's test to screen thousands of chemicals for their ability to damage DNA. But what chemicals should they test?

Tox21 – testing chemicals faster and more efficiently

In 2008, NCATS (then part of National Human Genome Research Institute (NHGRI)), NIEHS/NTP, and the U.S. Environmental Protection Agency (EPA) formed the Tox21 initiative to develop high-throughput screening tests that measure cellular harm caused by environmental chemicals. The Tox21 team created a library of some 2,000 compounds and agreed to test them against Myung's assay. The NHGRI researchers also added a commercially available chemical collection to the screening runs for a total of some 4,000 chemicals.

The screening runs produced surprises, identifying 22 antioxidants that damaged DNA. Three of the antioxidants – resveratrol, genistein, and baicalein – are currently used or being studied as an anti-aging intervention, as well as a treatment for several disorders, including heart disease, type 2 diabetes, osteopenia and osteoporosis, and chronic hepatitis.

Not only did the antioxidants damage the DNA, the researchers found, but, in dividing cells such as in tumors, the antioxidants could be lethal, killing the disease-causing cells. Despite their ability to damage DNA, the chemicals did not cause genetic mutations, making them particularly good candidates for improved cancer drugs.

Cool biology — new research avenues

"This is what's cool about biology," Austin said. "Just when we think we understand something, it turns out to be more complex than we thought. Not only did the NHGRI team produce a novel way to measure DNA damage, but their test has given us insights into the effects of chemical compounds that were not seen in more conventional strategies."

The discovery opens up several new lines of research. As a first step, the collaborators are dramatically expanding the number of compounds - more than 300,000 – to be screened with the new test. The Tox21 team also has decided to include the test in its standard screen for biological harm produced by environmental chemicals. ●

Citation: [Fox JT, Sakamuru S, Huang R, Teneva N, Simmons SO, Xia M, Tice RR, Austin CP, Myung K.](#) 2012. High-throughput genotoxicity assay identifies antioxidants as inducers of DNA damage response and cell death. *Proc Natl Acad Sci U S A*; doi:10.1073/pnas.1114278109 [Online 19 March 2012].

(Larry Thompson is the communications director for the National Human Genome Research Institute.)

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Sills president-elect of the Society of Toxicologic Pathology

By Eddy Ball reprinted from eFACTOR, April 2012



In conjunction with his interdisciplinary research activities in the NIEHS/NTP, Sills holds adjunct positions in pathology at the University of North Carolina at Chapel Hill School of Medicine and in the College of Veterinary Medicine at North Carolina State University. (Photo courtesy of Steve McCaw)

When the Society of Toxicologic Pathology (STP) gathers in June for its annual symposium, its new president-elect will be NIEHS/NTP pathologist [Robert Sills, D.V.M., Ph.D.](#), a diplomate of the American College of Veterinary Pathologists, and fellow of the International Academy of Toxicologic Pathology. In March, Sills who is chief of NIEHS and NTP pathology and the Cellular and Molecular Pathology Branch, was chosen as president-elect by the [STP](#) membership.

"We [at NIEHS and NTP] have been honored with my selection as president-elect of the Society of Toxicologic Pathology, which, personally, is very humbling," Sills said when he learned of the election results. The STP, which consists of 1,200 members in more than 20 countries, is a nonprofit association of pathologists and other scientists. Its principal aim is the advancement of pathology as it pertains to changes elicited by pharmacological, chemical, or environmental agents, and factors that modify these responses.

The STP's 31st [annual symposium](#), "Mechanisms of Toxicity," will take place June 24-28 in Boston. Featured events include a free pre-event NTP symposium June 23, as well as an awards ceremony and annual business meeting where Sills will be officially installed.

An active research and professional development agenda

Sills' research activities include the study of molecular mechanisms of carcinogenesis, neuropathology, and the inclusion of biologic-based interdisciplinary research in NIEHS/NTP studies.

Sills has served on the STP Executive Council and editorial board of the STP journal Toxicologic Pathology. He chaired STP annual meeting sessions on the human genome, implications for toxicologic pathology and carcinogenesis, and a session on cellular and molecular neurocarcinogenesis: toxicologic pathology of the nervous system. Also, he co-chaired a session of the STP annual meeting on cancer, the Scientific Program Planning Committee of the STP annual meeting on toxicologic neuropathology, and the International Life Sciences Institute seminar series on current issues in neuropathology.

Along with his affiliation with STP, Sills is very active in professional organizations as a member of the American College of Veterinary Pathologists, American Association for Cancer Research, and the American Veterinary Medical Association. He is the associate editor for the environmental pathobiology section of the journal Veterinary Pathology. Also, he has served on advisory committees, including the joint Food and Agriculture Organization of the United Nations/World Health Organization expert committee on food additives, International Agency for Research on Cancer, and U.S. Food and Drug Administration advisory committee. ●

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Enthusiasm for science reigns at SOT

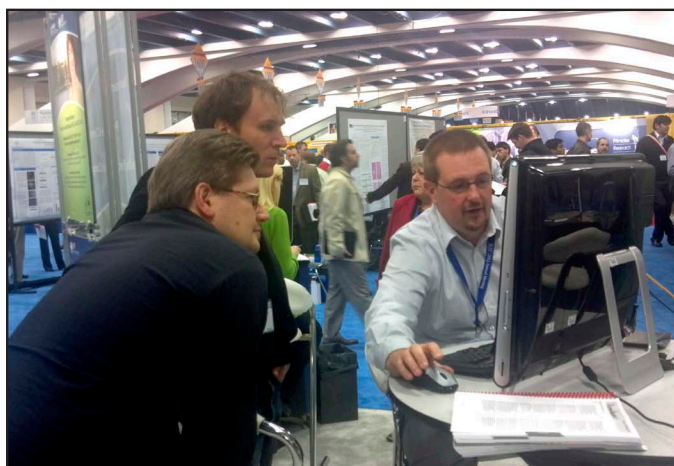
By Robin Mackar reprinted from *eFACTOR*, April 2012



NIEHS Deputy Director Rick Woychik, Ph.D., welcomed SOT President Jon Cook, Ph.D., to the NIEHS/NTP/EHP exhibit. (Photo courtesy of Hui Hu)



When not giving talks or meeting up with colleagues, Birnbaum could be found at the exhibit catching up on emails. (Photo courtesy of Hui Hu)



The database demos were quite popular. Auerbach was kept busy throughout the conference. (Photo courtesy of Denise Lasko)

The cool San Francisco weather and heavy rain didn't dampen the enthusiasm of the more than 7,000 scientists who attended the annual Society of Toxicology (SOT) Meeting March 11-15.

"The energy level at SOT was very high," said NIEHS/NTP Director Linda Birnbaum, Ph.D. "Not only does the annual meeting provide a chance to hear and see the newest findings in the world of toxicology, but also offers many opportunities to catch up with colleagues and friends and, sometimes, you can even find time to make some new acquaintances." As a past president of SOT, Birnbaum is well known and a popular draw at the meeting.

A few highlights

NIEHS/NTP staff led more than 110 science-related activities at the meeting, including talks and posters highlighting new findings, symposia, keynote lectures, demos of databases, exhibitor-hosted sessions, continuing education classes, and meetings with Superfund directors and grantees, as well as shared information about funding opportunities and recruited postdocs.

One of the busiest spots at the meeting was the combined NIEHS/NTP and Environmental Health Perspectives (EHP) exhibit. In addition to it being the hub for information about all NIEHS/NTP programs and activities, the exhibit served as a place for attendees to get hands-on experience with NTP databases. Scott Auerbach, Ph.D., of the NTP Biomolecular Screening Branch, formally introduced the DrugMatrix[®] and ToxFX[®] toxicogenomic database and analysis tools, at an exhibitor-hosted session on Monday that was very well attended.

Auerbach also gave demos at the booth. Laura Hall, Asif Rashid, and Hui Gong, from the NTP Program Operations Branch, presented a poster and walked many attendees through some of the upgrades and new information on the Chemical Effects in Biological Systems (CEBS) database. "We were all very pleased at the interest shown in our databases," Hall said. As a first-timer at the meeting, she also admitted being a little overwhelmed by the breadth of the meetings' offerings.

Another hot spot at SOT was the NIEHS Division of Extramural Research and Training (DERT) research funding and resource room, where Carol Shreffler, Ph.D., Annette Kirshner, Ph.D., and other DERT staff discussed federal research funding opportunities. According to Kirshner, "The grant writing brown bag session was a big hit with first-time grant writers."



The numerous continuing education sessions chaired by NIEHS/NTP staff were also very well attended, ranging from sessions on harmonized guidance for risk assessment and techniques for assessing chemical mixtures to nanotechnology.

Birnbaum continued to be a huge draw at all the sessions she participated in, especially the annual "Meet the Directors" special symposium. She provided a budget update and spent much of her time talking about the [strategic planning](#) process taking place at NIEHS. Birnbaum shared the stage with leaders from other agencies, including former NIEHS/NTP leader Chris Portier, Ph.D., who now heads the Agency for Toxic Substances and Disease Registry.

SOT leaders among us

NIEHS/NTP is also proud to have two elected [SOT leaders](#) representing the Institute. Dori Germolec, Ph.D., immunology discipline leader for the NTP, and Michael Waalkes, Ph.D., chief of the NTP Laboratories Branch, serve as SOT councilors. Both commented about how pleased they were with the meeting.

"NIEHS was very well represented at the SOT, with wonderful presentations from the director on down to numerous postdoctoral fellows," Waalkes said with a sense of pride. "Toxicology continues to shine at the Institute." ●

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

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Staffers and trainees gain toxicology certification

By Eddy Ball reprinted from *eFACTOR*, February 2012

Two NIEHS/NTP scientists and two NTP postdoctoral fellows recently took an important step along toxicology's professional track, by satisfying requirements for Diplomate of the American Board of Toxicology (DABT) certification. DABT certification often offers an advantage in the job market and career advancement, and has been associated with higher levels of compensation.

The [American Board of Toxicology](#) was established in 1979 to advance standards in the field of toxicology and confer recognition upon those members of the profession who, measured against such standards, demonstrate competence. Certification requirements include a combination of education and experience and a three-part examination.

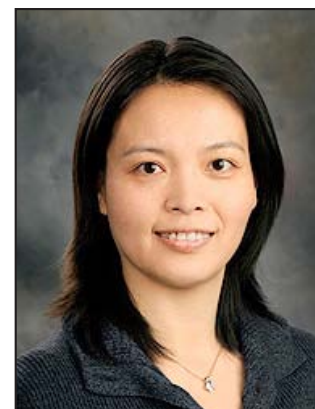
Several toxicologists at NIEHS/NTP have qualified for the coveted DABT, among them NIEHS/NTP Director Linda Birnbaum, Ph.D., who is the first toxicologist to head the Institute. Birnbaum offered the new diplomates her sincere congratulations. "Welcome to the club!" she wrote. "This marks a very important milestone in your careers."

New holders of the DABT are:

- Danielle Carlin, Ph.D., a health scientist administrator with the NIEHS Superfund Research Program, who joined the NIEHS Division of Extramural Research and Training in 2010, after completing a postdoctoral fellowship at the U.S. Environmental Protection Agency
- [Xiaoqing Chang, Ph.D.](#), a visiting fellow in the NTP Biomolecular Screening Branch headed by Raymond Tice, Ph.D., mentored by Michael DeVito, Ph.D., leader of the Experimental Toxicology Group in the NTP Toxicology Branch



Carlin, above, and Rider co-chaired a September 2011 workshop in Chapel Hill, N.C., on "Advancing Research on Mixtures." (Photo courtesy of Steve McCaw)



In 2011, Chang earned a Society of Toxicology [Nanotoxicology Specialty Section](#) Outstanding Postdoc Award and an NIEHS Fellows Award for Research Excellence for her work with micro- and nano-sized fluorescent polystyrene spheres. (Photo courtesy of Steve McCaw)



- [Minerva Mercado-Feliciano, Ph.D.](#), an Intramural Research Training Award fellow in the NTP Toxicology Branch headed by Paul Foster, Ph.D.
- [Cynthia Rider, Ph.D.](#), a toxicologist in the NTP General Toxicology Group who joined NIEHS/NTP in 2010, after completing postdoctoral work at Duke University and the U.S. Environmental Protection Agency

Diplomates hold initial DABT certification for 5 years and must demonstrate that they actively practice toxicology, engage in continuing education, and maintain expert knowledge in their field prior to receiving recertification. ●

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Mercado-Feliciano has designed and monitored several toxicology studies on the herbal supplement black cohosh, or *Actaea racemosa*, and is actively presenting study results at scientific meetings and preparing them for publication in peer-reviewed journals. (Photo courtesy of Minerva Mercado-Feliciano)



Rider currently serves as secretary and treasurer of the Mixtures Specialty Section of the Society of Toxicology. (Photo courtesy of Steve McCaw)

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

Federal Agencies Respond to ICCVAM Recommendations on Use of the Murine Local Lymph Node Assay for Potency Categorization



An interagency committee administered by NICEATM recommended to Federal agencies that the murine local lymph node assay, or LLNA, may be used to categorize the potency of chemicals causing allergic contact dermatitis (ACD) in humans. Specifically, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recommended that the LLNA may be used to categorize some substances as strong sensitizers, thus identifying those substances considered to have a significant potential for causing skin hypersensitivity resulting in ACD. U.S. Federal agencies have responded to the ICCVAM recommendations. Regulatory agencies, including FDA, EPA, CPSC, and OSHA, have indicated that they will take actions in response to the ICCVAM recommendations to encourage use of the LLNA for this purpose where appropriate.

Skin diseases are the most common type of occupational illness in the United States, according to the U.S. Bureau of Labor Statistics. Many skin disease cases arise from repeated exposures to skin-sensitizing substances, which can lead to ACD, an immunologically mediated hypersensitivity reaction. Studies have shown that ACD has a significant adverse impact on quality of life in affected individuals.

Since recommended by ICCVAM in 1999, the LLNA has been accepted worldwide as a valid alternative to traditionally accepted guinea pig test methods for assessing ACD hazard potential for most testing applications. The LLNA uses fewer animals than traditional guinea pig test methods, requires less time to perform, provides dose-response information, and, in most cases, eliminates pain and distress in the test animal.

The new ICCVAM recommendation provides guidance on how to use the LLNA to categorize some chemicals and products as strong skin sensitizers. However, since only half of the known strong human skin sensitizers can be identified as such in the LLNA (52% or 14 out of 27), all remaining substances require additional testing or information to determine that they are not strong skin sensitizers.



The ICCVAM evaluation is detailed in a report entitled *ICCVAM Test Method Evaluation Report: Usefulness and Limitations of the Murine Local Lymph Node Assay for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans* (NIH Publication No. 11-7709). In June 2011, ICCVAM forwarded recommendations to Federal agencies and made these recommendations available to the public (76 FR 18639). In accordance with the ICCVAM Authorization Act of 2000 (42 U.S.C. 285I-3), agencies have notified ICCVAM in writing of their findings, and ICCVAM has made these responses available to the public.

NIEHS/NTP Director Linda Birnbaum, PhD, responded to ICCVAM stating that NIEHS agreed with the recommendations. Noting the animal welfare advantages of the LLNA, she indicated that NIEHS and the NTP would ensure that the LLNA test method protocol is routinely considered whenever studies are proposed to assess ACD hazard potential and to categorize the potency of substances identified as having the potential to cause ACD in humans.

The NIEHS and other Federal agency responses to the ICCVAM recommendations and more information about the ICCVAM evaluation of the LLNA for potency categorization can be found on the [NICEATM-ICCVAM website](#). The ICCVAM Test Method Evaluation Report is also available on the [NICEATM-ICCVAM website](#).

NICEATM and ICCVAM are also currently evaluating several *in vitro* and *in chemico* methods for their potential to further reduce and eventually replace the use of animals for ACD safety testing.

ICCVAM Recommends Non-animal *In Vitro* Method to Identify Potential Endocrine-active Substances

ICCVAM recently recommended to Federal agencies a non-animal test method that can be used as a screening test to identify substances with *in vitro* estrogen receptor agonist and antagonist activity. This test method, the BG1Luc estrogen receptor (ER) transactivation (TA) test method (also known as the LUMI-CELL[®] ER test method) uses cultured human cells to identify substances that can induce or inhibit human ER activity *in vitro*.

Xenobiotic Detection Systems, Inc. (XDS, Durham, NC, USA) developed the LUMI-CELL[®] ER test method with support from an NIEHS Small Business Innovation Research Grant and nominated the method to ICCVAM for an interlaboratory validation study. ICCVAM and its advisory committee, the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) recommended the study as a high priority. NICEATM subsequently coordinated an international validation study with counterparts in Japan (the Japanese Center for the Validation of Alternative Methods; JaCVAM) and Europe (the European Centre for the Validation of Alternative Methods; ECVAM).

ICCVAM's Interagency Endocrine Disruptor Working Group (EDWG), composed of scientists from ICCVAM member agencies, JaCVAM, and ECVAM, worked with NICEATM to carry out relevant evaluation activities following completion of the international validation study. Members of the EDWG from NIEHS include Warren Casey, PhD, Jerry Heindel, PhD, Bill Stokes, DVM, and Julius Thigpen, PhD.

A background review document, test method performance standards, and ICCVAM test method recommendations were reviewed by an independent international peer review panel. ICCVAM considered the Panel report and comments from the public, the EDWG, and SACATM in preparing the final test method recommendations.

ICCVAM recommends that the accuracy and reliability of the BG1Luc ER TA test method support its use as a screening test to identify substances that can induce or inhibit human ER activity *in vitro*. ICCVAM concludes that the accuracy of this assay is at least equivalent to the only ER TA test method currently listed in a U.S. regulatory test guideline, the Environmental Protection Agency's "OPPTS 890.1300: Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa- 9903))". The BG1Luc ER TA test method was found to offer several advantages over the existing ER TA method, including (1) validation for use over a wider concentration range of test substances, (2) potential to detect a wider range of ER-active substances, (3) ability to identify both substances that induce and inhibit the estrogen receptor, and (4) availability of the cell line used for the test from more than one source.

The ICCVAM evaluation is detailed in a report entitled *ICCVAM Test Method Evaluation Report: The LUMI-CELL[®] ER (BG1Luc ER TA) Test Method: An In Vitro Assay for Identifying Human Estrogen Receptor Agonist and Antagonist Activity of Chemicals* (NIH Publication No. 11-7850), available on the [NICEATM-ICCVAM website](#). The report also provides (1) performance standards that can be used to evaluate functionally and mechanistically similar test methods, (2) recommended test method protocols, (3) a final background review document describing the current validation status of this test method, and (4) recommendations for future studies.



The ICCVAM report and recommendations have been transmitted to Federal agencies for their review and response to ICCVAM in accordance with the provisions of the ICCVAM Authorization Act of 2000, which requires agencies to review the recommendations and respond to ICCVAM within 180 days.

Casey, who is Deputy Director of NICEATM, received a 2011 NIH Individual Merit Award in recognition of his excellent performance in leading the international validation and interagency evaluation of new testing methods to support the Federal government's endocrine disruptor chemical screening program, including the evaluation of the BG1Luc ER TA test method.

The BG1Luc ER TA test method was adapted to a high-throughput format using 1536-well plates by the NIH Center for Advancing Translational Sciences (NCATS; formerly the NIH Chemical Genomics Center). Preliminary results are promising, and it is expected that this method will be incorporated into the Tox21 screening paradigm in 2012.

More information about the ICCVAM evaluation of the use of the BG1Luc ER TA test method for identification of potential endocrine-active substances can be found on the [NICEATM-ICCVAM website](#).

Hold the Date: NICEATM and ICCVAM International Workshop on Alternative Methods for *Leptospira* Vaccine Potency Testing, September 19–21, 2012

NICEATM and ICCVAM will convene an "International Workshop on Alternative Methods for *Leptospira* Vaccine Potency Testing: State of the Science and Planning the Way Forward" on September 19–21, 2012. The workshop will be hosted by the U.S. Department of Agriculture (USDA) Center for Veterinary Biologics at the National Centers for Animal Health in Ames, Iowa. NICEATM and ICCVAM are organizing the workshop in collaboration with partner organizations in the International Cooperation on Alternative Test Methods.

Leptospirosis is an emerging and widespread bacterial zoonotic disease caused by spirochetes of the genus *Leptospira*. More than 500,000 human cases of *leptospirosis* are reported worldwide each year with a fatality rate of up to 25% in some regions. Designated as a Neglected Tropical Disease by the NIH and the WHO, *leptospirosis* is a global research and public health priority. *Leptospirosis* also affects many animal species including livestock, pets, and wildlife. In the United States, vaccines are used to protect cattle, swine, and dogs. Vaccines for people are available in some other countries, and NIH is supporting the development of new human vaccines.

Manufacturers perform potency testing prior to release of each production lot of *Leptospira* vaccine to ensure that it will be effective. However, current methods for this testing require large numbers of laboratory animals. Many of the animals experience significant unrelieved and distress without the benefit of pain-relieving drugs, accounting for over one-third of the animals reported to the USDA in this pain category. While *in vitro* potency assays are now approved by the USDA and available for four *Leptospira* serovars, these new assays have not yet been widely implemented. Accordingly, NICEATM, ICCVAM, and their international partners recently identified *Leptospira* vaccines as one of its top three vaccine priorities for development, validation, and implementation of improved test method alternatives.

This workshop, the second in a series of specialized vaccine workshops organized by NICEATM and ICCVAM, will review recent advances and innovations in science and technology that can be translated to methods that are more humane, use fewer or no animals, and provide improved accuracy, efficiency, and worker safety. The workshop will also address global acceptance and implementation of scientifically valid alternative methods.

Registration information, a workshop program, and other information will soon be 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 August 13, 2012. If you have questions about the workshop or would like more information, please contact NICEATM at niceatm@niehs.nih.gov.

NICEATM and ICCVAM Presentations at the 51st Annual Meeting of the Society of Toxicology

NICEATM and ICCVAM presented seven scientific posters describing recent accomplishments at the 51st Annual Meeting of the Society of Toxicology, which took place on March 11–15, 2012 in San Francisco, CA, USA. NICEATM-ICCVAM presentations are available on the [NICEATM-ICCVAM website](#).



Two posters focused on the ICCVAM evaluation of the BG1Luc ER TA test method. This test method uses human cells to identify substances with *in vitro* estrogen agonist and antagonist activity (see article above). One poster summarized the ICCVAM recommendations on the BG1Luc ER TA test method, and the second described performance standards that have been developed for the test method.

Three posters described NICEATM evaluations of methods to identify substances with the potential to cause allergic contact dermatitis (ACD). One poster described an updated evaluation of the reduced LLNA, and another presented an evaluation of the use of two nonradiolabeled LLNA methods for potency categorization of substances causing ACD in humans. A third poster described a NICEATM analysis comparing use of the direct peptide reactivity assay with a three-test battery for *in vitro* identification of potential skin sensitizers, and proposes an integrated testing strategy that can significantly reduce animal testing.

A sixth NICEATM–ICCVAM poster presented the results of a NICEATM analysis to determine if acute oral systemic toxicity data can be used to estimate and avoid acute dermal systemic toxicity testing. The final poster presented a summary of conclusions and recommendations from the October 2011 International Workshop on Alternative Methods for Human and Veterinary Rabies Vaccine Testing.

Former ICCVAM Advisory Committee Member Receives SOT Award



Donald Fox, PhD, of the University of Houston College of Optometry has received a Career Achievement Award from the SOT Ocular Toxicity Specialty Section. The award recognizes lifetime achievement or contribution of a particularly influential body of work to the field of ocular toxicology.

Fox served on the Scientific Advisory Committee for Alternative Toxicological Methods (SACATM) from June 2005 to June 2009. SACATM, which is one of the formal advisory committees to the NTP, advises the NIEHS Director, ICCVAM, and NICEATM on ICCVAM functions and activities.

In addition to his service on SACATM, Fox participated on the NICEATM-ICCVAM Expert Panel convened in January 2005 to evaluate the validation status of *in vitro* test methods for identifying ocular corrosives and severe irritants. In that capacity, he reviewed and commented on ICCVAM recommendations on *in vitro* test methods that were subsequently adopted by U.S. agencies and internationally through the Organisation for Economic Co-operation and Development.

Fox is Professor of Vision Sciences, Biology & Biochemistry, Pharmacology, and Health & Human Performance at the University of Houston College of Optometry. His research interests are in the areas of mammalian retinal cell biology/ biochemistry, molecular biology, and neurotoxicology. Some of his recent research has focused on the effects of prenatal lead exposure on retinal development ([see article in Environmental Health Perspectives](#)). ●

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The NTP website offers electronic files of the Report on Carcinogens and the library of NTP Technical Reports and NTP Toxicity Reports. The PDF files of these reports are available free-of-charge through the NTP website at <http://ntp.niehs.nih.gov> (see Resources).

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