UPDATE
National Toxicology Program
U.S. Department of Health and Human Services

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

What's Inside:

Committee Advises on Alternative Toxicological Methods

Article by Robin Mackar reprinted from *eFACTOR*, July 2010

Maximizing animal care and welfare, increasing awareness about alternative toxicological methods, vaccine potency testing, validation issues, and hearing updates on federal and international acceptance of alternative methods were just a few of the topics covered at the June 17-18 meeting of the Scientific Advisory Committee on Alternative Toxicological Methods Meeting (SACATM) held on the U.S. Environmental Protection Agency (EPA) campus in Research Triangle Park, N.C.

Warren Casey, Ph.D., the new deputy director for NICEATM, listened carefully to discussions. Casey presented on the validation of endocrine disruptor test methods the first afternoon of the meeting.

NIEHS/NTP Director Linda Birnbaum, Ph.D., provided a warm welcome to all, especially to the international partners in attendance, including Joachim Kreysa, Ph.D., of the European Centre for the Validation of Alternative Methods (ECVAM), Soon Young Han, Ph.D., director of the newly established Korean Center for the Validation of Alternative Methods (KoCVAM) and David Blakely, Ph.D., of Health Canada, who joined the meeting by teleconference. Birnbaum praised the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) for their progress, highlighting the endorsement or adoption of 33 new alternative methods. She also mentioned that she just forwarded two of the first "green technology" ICCVAM test method recommendations to federal agencies for their approval.

William Stokes, D.V.M., provided an update on activities. He drew attention to a new publication *The Biennial Progress Report 2008–2009: Interagency Coordinating Committee on the Validation of Alternative Methods* which describes ICCVAM activities, test method recommendations, and other progress made during the reporting period.

Stokes queried SACATM on the topics of outreach, industry participation, and how to address some regulatory responses to methods. The group had many ideas to share about how to create more awareness for study directors and Institutional Animal Care and Use Committees (IACUC) to make sure they consider alternative methods.

SACATM member Karen Brown, Ph.D., began the discussion by saying outreach efforts need to be expanded beyond the toxicology community to other groups, including industry. "Regulatory agencies and industry have to work together," said Brown. She suggested inviting industry representatives to workshops and presentations to hear about the savings in time, money, and labor that alternative testing methods can often provide.

Marion F. Ehrich, Ph.D., Sharon Meyer, Ph.D., Linda A. Toth, D.V.M., Ph.D., and others suggested more be done to reach out to laboratory animal veterinarians and personnel. They suggested placing articles in publications that lab technicians read such as Nature's "Lab Animal" as a way to increase awareness about alternative testing methods, as well as getting more concise, yet comprehensive articles into the peer-reviewed literature.

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Participants also offered ideas for expanding training grants and other NIH grant mechanisms. George Corcoran, Ph.D., suggested providing travel funds for IACUC members to attend more meetings and workshops.

Helen Diggs, D.V.M., proposed using new media to help create awareness and training for study directors and IACUC members. "I suggest developing Web-based or other training programs that people can access at their leisure," Diggs said. "These are very busy people who don't have time or money to travel to hear about the newest methods."

Members also offered ideas for encouraging industry to submit testing data to NICEATM and providing data for proprietary products from companies without revealing the product identity.

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

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CDC Taps Portier to Head Programs

Article by Larry Lazarus reprinted from *eFACTOR*, August 2010

Senior Advisor Chris Portier, Ph.D., left NIEHS July 29 to serve as director of two high-profile programs at the Centers for Disease Control and Prevention (CDC). Portier, a 32-year veteran of the Institute, assumes duties as director of the Agency for Toxic Substances and Disease Registry (ATSDR) and the National Center for Environmental Health (NCEH).

Portier said he was motivated to make the career change "due to my focus on direct public health issues during the last several years" and a commitment "to give back to my country and use what I have learned at the NIEHS to improve the health of the American public."

During his tenure at NIEHS, Portier served in several leadership roles. He was a principal investigator in the Environmental Systems Biology Group, former director of the Environmental Toxicology Program, and former associate director of the National Toxicology Program (NTP).

As senior advisor, Portier was a driving force in the NIEHS global health initiative. He helped design and fund a set of high-profile papers published in the journal *The Lancet* in November 2009 that identified important health benefits of interventions to alleviate global climate change (see story). He was the NIEHS lead on a white paper, *A Human Health Perspective on Climate Change*, published in April 2010 by a U.S. government-wide global health working group investigating the state-of-the-science on the human health consequences of climate change.

In a congratulatory note about Portier's new appointment, NIEHS/NTP Director Linda Birnbaum, Ph.D. said, "Over the years, I have often marveled at Chris' intellectual ability and what I would call brilliance when it comes to big picture visionary thinking. I know I can always count on Chris for new ideas and challenging discussion, which I believe has benefited our Institute in many ways."

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Upcoming Events

November 30 - December 1, 2010

NTP Board of Scientific Counselors

NIEHS
111 TW Alexander Drive
Research Triangle Park, NC

January 11-13, 2010

NTP Workshop: Role of Environmental Chemicals in the Development of Diabetes and Obesity

Raleigh Marriott Crabtree Valley
4500 Marriott Drive
Raleigh, NC

ICCVAM Workshop series on Best Practices for Regulatory Safety Testing:

January 19, 2011; Assessing the Potential for Chemically Induced Eye injuries

January 20, 2011; Assessing the Potential for Chemically Induced Allergic Contact Dermatitis

NIH Natcher Conference Center
Bethesda, MD

January 26, 2010

Peer Review of Draft
NTP Technical Reports

NIEHS
111 TW Alexander Drive
Research Triangle Park, NC

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

Portier leaves the NIEHS/NTP with the support and admiration of many colleagues and collaborators. In particular, they stressed his committed mentorship, dynamic leadership style, broad understanding and appreciation of basic and applied science, and dedication to using modeling to improve risk assessment.

John Prichard, Ph.D., NIEHS acting scientific director, noted that Portier "led the effort to develop a master plan – a roadmap – for the NTP [due to] his emphasis on mechanistic endpoints, and he set priorities which continue to have a strong impact." During Portier's tenure as NTP associate director from 2001-2006, the program put out its landmark document, *A National Toxicology Program for the 21st Century: A Roadmap for the Future*.

Current NTP Associate Director John Bucher, Ph.D., said Portier brought "quantitative rigor" to the NTP by "developing an NTP database and high throughput screening [which] led to the Tox-21 initiative working beyond our dreams." This sentiment was reiterated by Nigel Walker, Ph.D., NTP deputy program director for science, who remarked, "We are living in the vision he started."

Portier's mentor and former NTP Associate Director George Lucier, Ph.D., had this to say: "Dr. Portier combines a remarkable intellect with a keen understanding of how to translate science into sound public policy decision-making."

Both Lucier and Birnbaum see Portier's move as an opportunity to strengthen collaborations in addressing environmental causes of human disease. The NCEH/ATSDR is an important member of the NTP, which is an interagency program of the U.S. Department of Health and Human Services.

Explaining the mission of NCEH/ATSDR, Portier added, "The basic concept is anytime anyone needs help about toxic substances in their neighborhood, they call us. The NCEH looks at the people who live in an affected region." Portier said that the scientists and staff at NCEH/ATSDR also serve as boots on the ground as "the nation's first responders for health related issues."

This role involves not only major environmental disasters, such as waste dumps, toxic sites, hurricanes, and oil spills, but also "clusters of environmental health-related cancers," he added. Portier said the NCEH is also the "biomonitoring program for the U.S." and works hand-in-hand with organizations to conduct multidimensional evaluations that assess 450 known environmental chemicals in human blood and urine samples, to aid in understanding exposures in the United States.

(Larry Lazarus, Ph.D., is a principal investigator in the NIEHS Laboratory of Toxicology and Pharmacology)

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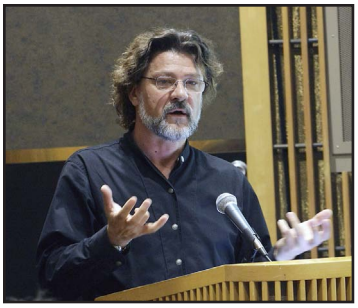
Evaluating the Science on Cell Phone Safety

Article by Mamta Behl reprinted from *eFACTOR*, October 2010



Leszczynski is actively involved in various task groups and steering committees associated with cell phone studies. He has also been invited by the World Health Organization and U.S. Food and Drug Administration to review research agendas related to mobile phone safety.
(Photo courtesy of Steve McCaw)

As part of its ongoing efforts to evaluate the toxicity and carcinogenicity of cell phone radiofrequency radiation, the National Toxicology Program (NTP) invited world-renowned researcher, Dariusz Leszczynski, Ph.D., D.Sc., to share his views on the status of current science regarding cell phone safety, as well as the need for more and better designed studies. Also speaking at the Aug. 30 seminar was NTP toxicologist Michael Wyde, Ph.D., project leader for cell phone radiation studies currently underway in a specially designed facility in Chicago.

Leszczynski is a professor at Säteilyturvakeskus (STUK) and the Radiation and Nuclear Safety Authority in Helsinki, Finland. Leszczynski also held until recently the position of Guangbiao Professor at the Zhejiang University School of Medicine in Hangzhou, China. He is an adjunct professor of biochemistry at the University of Helsinki.

According to the International Telecommunication Union, an estimated 4.6 billion cell phones are in use worldwide, with some 270 million units operating in the U.S. alone. Concerns about the health effects from widespread exposure to radiofrequency radiation have led to a proliferation of studies but, according to Leszczynski, investigators haven't

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always been asking the right questions. "It is a common misconception that thousands of papers are published, but [in fact] most of them do not evaluate cell phone radiation," he said.

Leszczynski emphasized that studies have mostly focused on cancer while ignoring other important non-cancer endpoints such as general toxicity, stress response, blood brain barrier permeability, and effects on vital organs other than brain. In discussing limitations of epidemiological studies, which often utilize blood pressure, headaches, allergic responses, and electroencephalograms (EEGs) to study radiation effects, Leszczynski cautioned that results may be skewed by the experimental design and evaluation of inappropriate endpoints and non-objective responses of study subjects.

At this time, he maintained, "we do not know if humans respond to mobile phone radiation." In his opinion, more epidemiological studies like the Cohort Study of Mobile Communications (COSMOS), the largest European-based research effort on mobile phone safety, are needed to evaluate exposure to cell phone radiation in humans. He also stressed the need for studying molecular level responses to mobile phone radiation in humans, in order to determine whether the human body is indeed affected by this radiation.

"Currently we don't have any studies on children [and] we don't know if they are more sensitive," Leszczynski added. He concluded by emphasizing the need for more targeted research since "current literature does not provide sufficient evidence for safety standards in protecting mobile phone users." He identified the importance of developing more realistic dosimetry models and strongly advocated the use of alternative methods such as omics technologies to investigate genes and proteins which may potentially be altered as a result of exposure to cell phone radiation.

In the meantime, Leszczynski advises, "Use caution, especially for children with phones, and limit exposure to mobile phones when reasonable, feasible and possible."

Following Leszczynski's talk, Wyde presented an update on the current status of the NTP studies in rats and mice on the exposure to cell phone radiofrequency and radiation. Wyde's presentation reflected the careful design of the NTP study, which addresses the shortcomings that Leszczynski had pointed out in his review of animal studies. The studies will address several limitations in the literature and will make an important contribution to the better understanding of cell phone safety. Selection of radiofrequency in these studies is based on human exposure and lies in the non-thermal range. The studies are designed to assess carcinogenic and non-carcinogenic endpoints including general toxicity, blood-brain barrier permeability, and molecular alterations. They are expected to be completed by 2014.

(Mamta Behl, Ph.D., is a research fellow in the NTP Toxicology Branch)

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Nominations Requested for SOT Anniversary

Article by Ed Kang reprinted from *eFACTOR*, August 2010

NIEHS and NTP are helping the Society of Toxicology (SOT) mark its 50th anniversary next year with a special poster, banner, and Website commemorating "Benchmarks in Toxicology." Voting is currently underway at the special "Benchmarks" Web site, where visitors, whether they are SOT members or not, can nominate the most important people, events, and discoveries that have influenced the modern discipline of toxicology.

As in years past, NIEHS and NTP will figure prominently in the presentations, sessions and workshops at the SOT Annual Meeting, March 6-10, 2011 in Washington, DC. At the 50th anniversary celebration, NIEHS, NTP, and the NIEHS journal Environmental Health Perspectives (EHP) will proudly display the "Benchmarks" banner and toxicology timeline. The commemorative poster, banner, and Web site will be available during and after the 2011 SOT conference to individuals, schools, and other public or private institutions.

"From Paracelsus' declaration that 'the dose makes the poison' to high-throughput assays, many people, discoveries and events have shaped the modern field of toxicology," said NIEHS/NTP Director Linda Birnbaum, Ph.D., who is a benchmark

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in her own right. Birnbaum is the first woman to lead the NTP and the first toxicologist to head an NIH institute. "We think this will be a great resource to highlight the formative benchmarks in our field, and we're excited to have the toxicology community help us shape the project by providing input," Birnbaum said.

According to SOT, the landmark 50th annual meeting expects to attract more than 7,000 scientists from industry, academia, and government, including confirmed plenary speakers Francis Collins, M.D., Ph.D., director of the National Institutes of Health, and Margaret Hamburg, M.D., commissioner of the U.S. Food and Drug Administration.

(Ed Kang is a public affairs specialist in the Office of Communications and Public Liaison)

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High-throughput screening of mitochondrial toxicity

Article by Mamta Behl reprinted from *eFACTOR*, September 2010

"RPTCs cultured under standard conditions in which they are stationary with glucose medium have basal oxygen consumption rates 100-fold lower than those for RPTCs cultured under optimized conditions in which they were shaken with lactate as media," explained Beeson.
(Photo courtesy of Steve McCaw)

The NTP Biomolecular Screening Branch (BSB) hosted a talk by Craig Beeson, Ph.D., July 30 on "High-Throughput Respirometric Assay for Mitochondrial Biogenesis and Toxicity" as part of the NTP High-Throughput Screening Initiative. Beeson described the results he has obtained using the Seahorse Bioscience XF analyzer for real-time measurement of mitochondrial function in adapted primary cultures of renal proximal tubular cells.

As an associate professor in the Department of Pharmaceutical and Biomedical Sciences and the director of the Metabolomics Core and Drug Design and Synthesis Core at the Medical University of South Carolina, Beeson focuses on the biochemical networks responsible for the regulation of energy metabolism and cellular proliferation and their potential applications in the areas of predictive toxicology and drug discovery.

Mitochondrial toxicity assays in the Tox21 partnership

"The BSB is evaluating mitochondrial toxicity as a potential toxicity pathway using *in vitro* studies as part of its high-throughput screening initiative," NTP molecular toxicologist and lecture host Scott Auerbach, Ph.D., said of the branch's decision to invite Beeson to speak at NIEHS.

Auerbach, above, moderated active discussion on potential upstream energy stress depletion signals which, in turn, may alter the ATP/AMP ratio in the mitochondria.
(Photo courtesy of Steve McCaw)

The NTP, NIH Chemical Genomics Center (NCGC), and U.S. Environmental Protection Agency (EPA), along with their most recent partner, the U.S. Food and Drug Administration (FDA), are striving to advance the state of toxicity testing in the 21st century through a consortium known as Tox21. The consortium is seeking to identify new mechanisms of chemical activity in cells, effectively prioritize the backlog of untested chemicals for more extensive evaluations, and develop better predictive models of human response to such toxicants as industrial and environmental chemicals and drugs.

Overcoming limitations in current mitochondrial cell-based assays

Although many drugs and chemicals are mitochondrial toxicants, according to Beeson, assessing mitochondrial function has posed a challenge for investigators, because there is no direct high-throughput assay for mitochondrial function. In addition, the current cell-based models of mitochondrial toxicity are inadequate, because immortalized cell lines have lost differentiated function and are highly glycolytic, with minimal aerobic metabolism and altered mitochondrial physiology.

Beeson said that although roughly half of the drugs with FDA black box warnings for hepatotoxicity or cardiotoxicity also have documented mitochondrial effects, the same level of attention has not been given for nephrotoxicity, despite the frequency of loss in renal function due to adverse drug effects and xenobiotic exposure.

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To address this issue, Beeson's group adapted primary renal proximal tubular cells (RPTCs) as a model to study mitochondrial loss following oxidative injury. "In contrast to immortalized cells, RPTCs are robust primary cells, which are completely differentiated and polarized with good brush border enzyme activity and sodium-dependent glucose transport," Beeson explained. "The RPTCs are somewhat unique among differentiated tissues in that they have some capacity for repair and regeneration," he added. Beeson described the use of multiple endpoints, such as basal and uncoupled respiration rates, and provided examples of compounds that induce increased uncoupled respiration, confirming that it is a biomarker of mitochondrial biogenesis.

Collaborative efforts for a promising future

Beeson provided examples of loss of respiratory capacity of the mitochondria in degenerative diseases such as retinitis pigmentosa and macular degeneration, as well as the development of novel molecular models, known as pharmacophores, that serve as computational templates for discovering drugs to use in their treatment. "Our strategy involves looking at protection of the uncoupled rate for new molecules in the prevention of these disorders," concluded Beeson.

The lively discussion enabled researchers at the NTP to identify several areas of mutual interest where collaborations might be possible. "One area of immediate interest is in applying Dr. Beeson's mitochondrial toxiphore descriptor model to the Tox21 10K-compound library that will be tested for mitochondrial toxicity in high-throughput mode at the NIH Chemical Genomics Center," said BSB Chief Ray Tice, Ph.D.

Beeson was the latest in a series of speakers Tice has invited to NIEHS whose research has direct bearing on the high-throughput screening initiative. "Given our interest in identifying mitochondrial toxicants, we are very pleased that Dr. Beeson found the time in his busy schedule to present his research findings and meet with key NTP staff," Tice said.

Citation: Beeson CC, Beeson GC, Schnellmann RG. 2010. A high-throughput respirometric assay for mitochondrial biogenesis and toxicity. *Anal Biochem* 404(1):75-81. Epub ahead of print.

(Mamta Behl, Ph.D., is a research fellow in the NTP Toxicology Branch)

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Board of Scientific Counselors

The BSC meeting will be held on November 30 – December 1, 2010 at the NIEHS. Preliminary agenda topics include report of the NIEHS/NTP Director, report of the NTP Associate Director, a contract concept: NTP Sperm Count and Vaginal Cytology, review of the Biomolecular Screening Branch, and the draft concepts for carrying out testing/research on nominations to the NTP including drinking water disinfection by-products, bisphenol A, and N-butylbenzenesulfonamide, and an update on study plans for phosphate ester flame retardants.

The preliminary agenda, roster of BSC members, background materials, public comments, and any additional information, when available, will be posted on the BSC meeting website (http://ntp.niehs.nih.gov/go/165).

Contact Information: Dr. Lori White, Designated Federal Officer, NTP Office of Liaison, Policy and Review, NIH/NIEHS, P.O. Box 12233, K2-03, Research Triangle Park, NC 27709; T: (919) 541-9834; FAX: (919) 541-0295; whiteld@niehs.nih.gov

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

OECD Adopts International Test Guidelines and Guidance Documents Based on ICCVAM Recommendations

Test method evaluations and recommendations by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and an international validation study directed by NICEATM formed the basis for four international safety testing guidelines and guidances recently adopted by the Organisation for Economic Co-operation and Development (OECD).

ICCVAM is an interagency committee administered by NICEATM that was established to promote the development, validation and harmonization of test methods. NICEATM worked with ICCVAM working groups to draft OECD test guidelines for an updated version of the murine local lymph node assay (LLNA) and two non-radioactive versions of the LLNA. They also prepared a draft guidance document on using *in vitro* cytotoxicity assays to estimate starting doses for acute oral systemic toxicity tests. OECD adopted the guidance document on July 20 and the test guidelines on July 22. The documents incorporate recommendations made to Federal agencies by ICCVAM based on its evaluation of the test methods for specific regulatory testing applications. Implementation of the ICCVAM recommendations are expected to further reduce, refine, and replace the use of animals in required safety testing of chemicals and products.

Updated Test Guideline for the LLNA

The LLNA measures cell proliferation in lymph nodes to detect substances with the potential to cause chemically induced allergic contact dermatitis. U.S. Federal agencies originally accepted the LLNA in 1999 for safety testing, based on ICCVAM recommendations. The LLNA has many advantages compared to guinea pig methods, including using fewer animals, eliminating potential discomfort, providing dose-response information, and requiring less time to conduct.

The recently adopted OECD Test Guideline (TG) 429, *Skin Sensitization: Local Lymph Node Assay*, is an update of the version of TG 429 adopted in 2002. The updated method reduces animal use by 20% and provides improved reproducibility and accuracy compared to the original LLNA protocol. A reduced LLNA procedure included in the updated TG 429 allows additional animal use savings of 40% compared to the multi-dose LLNA. The updated TG 429 also provides a standardized approach and improved guidance for establishing the highest test dose, as well as performance standards that can be used to expedite the validation of modified versions of the LLNA. These revisions of the LLNA are based on recommendations forwarded to U.S. Federal agencies by ICCVAM in September 2009.

Test Guidelines for Nonradioactive LLNA Methods

Two new test guidelines have protocols for nonradioactive versions of the traditional LLNA. The LLNA: DA, described in TG 442A, *Skin Sensitization: Local Lymph Node Assay: DA*, measures adenosine triphosphate content, while the LLNA: BrdU-ELISA, described in TG 442B, *Skin Sensitization: Local Lymph Node Assay: BrdU-ELISA*, measures bromodeoxyuridine (BrdU) incorporation to quantitate lymphocyte proliferation. The availability of OECD test guidelines for these nonradioactive LLNA methods will broaden their use and the practical issues associated with using radioactivity, which prohibits testing in the traditional LLNA by some laboratories, can be avoided. ICCVAM forwarded its recommendations on the use of these methods to U.S. Federal agencies in June 2010. Similar to the LLNA, the LLNA: DA and LLNA: BrdU-ELISA provide an advantage over guinea pig tests by reducing and refining animal use. These methods will also eliminate the environmental hazard associated with use and disposal of radioactive materials used in the traditional LLNA.

These three test guidelines can be found on the OECD website at:
http://puck.sourceoecd.org/vl=67652263/cl=12/nw=1/rpsv/cw/vhosts/oecdjournal s/1607310x/v1n4/contp1-1.htm

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ICCVAM members, NICEATM staff, and contract support staff were among the participants, pictured here, at an OECD expert meeting on the murine local lymph node assay in October 2009. This international meeting was a key event in the development of the updated OECD Test Guideline 429 for the LLNA and the recently adopted test guidelines for the nonradioactive LLNA methods.

Guidance Document on Using Cytotoxicity Tests to Estimate Starting Doses for Acute Oral Systemic Toxicity Tests

The OECD Guidance Document No. 129, *Guidance Document on Using Cytotoxicity Tests to Estimate Starting Doses for Acute Oral Systemic Toxicity Tests*, is based on recommendations forwarded to U.S. Federal agencies by ICCVAM in March 2008. The guidance document provides protocols for *in vitro* neutral red uptake assays and describes the use of the assays in estimating starting doses for acute oral systemic toxicity tests. When determined to be appropriate and used to estimate starting doses, these assays can reduce animal use by as much as 50% for each study. ICCVAM recommended these test methods for routine consideration before using animals for acute toxicity studies, a recommendation that was accepted by U.S. regulatory and public health agencies.

The guidance document can be found on the OECD website at: http://www.oecd.org/document/30/0,3343,en_2649_34377_1916638_1_1_1_1,00.html

ICCVAM Recommendations Will Reduce or Eliminate Pain and Distress During Eye Safety Testing

ICCVAM has issued final recommendations on the use of alternative methods and strategies for performing eye safety testing. Dr. Linda Birnbaum, Director of NIEHS and NTP, forwarded the recommendations to U.S. Federal agencies in September 2010 on behalf of the Secretary of Health and Human Services.

ICCVAM recommends the routine use of anesthetics, analgesics, and humane endpoints whenever animals must be used for eye safety testing. ICCVAM also provides recommendations for several *in vitro* test methods and a testing strategy proposed for identifying eye safety hazards without animals. Adoption and implementation of these ICCVAM recommendations will contribute to more humane and reduced animal use for required product safety testing, while continuing to protect public health.

To protect workers and consumers, regulatory agencies require testing to determine if chemicals and products may cause temporary or permanent eye injuries such as blindness. Each year, approximately 2 million eye injuries occur in the United States, of which more than 40,000 result in permanent visual impairment. Data on consumer product-related eye injuries in children indicates that over 15% of such injuries are related to household cleaning chemicals and other chemical products.

ICCVAM recommends that pain management procedures should always be used whenever it is necessary to use rabbits for eye safety testing required by Federal regulatory agencies. The ICCVAM evaluation report includes a recommended test method protocol that describes how to use topical anesthetics (similar to those used in human eye surgeries) and systemic analgesics prior to and after test substance administration in order to avoid or minimize animal pain and distress. The report also identifies specific clinical signs and lesions that can be used as humane endpoints to allow the investigator to end a study early in order to reduce or eliminate pain and distress.

ICCVAM also recommends that the Cytosensor microphysiometer (CM) test method can be used as a screening test to identify some types of water-soluble substances that may cause permanent or severe eye injuries. A limited range of specific test chemicals and substances (i.e., water soluble substances and mixtures) that are positive in the CM test method can be classified as having the potential to cause severe or permanent eye injuries without additional testing using animals. The CM test method can also be used for a few types of substances (water-soluble surfactants and surfactant-containing formulations such as some cosmetics and personal care products) to determine that chemicals and products do not cause eye injuries that are severe enough to require eye hazard labeling. If accepted by Federal agencies, the CM test method will be the first *in vitro* test method available in the United States for this purpose. However, a chemical that produces a response in the CM test method between these two extremes would require additional testing (*in vitro* and/or *in vivo*) to establish a definitive classification. The CM test method is not considered appropriate for the identification of mild or moderate ocular irritants.

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ICCVAM evaluated four other *in vitro* test methods for their validity for identifying substances with the potential to cause nonsevere and reversible ocular injuries and substances that do not require ocular hazard labeling. ICCVAM concluded that the predictivity of these methods must be improved before they can be used in regulatory safety testing to classify such substances.

ICCVAM also evaluated and developed recommendations on use of an *in vitro* ocular safety testing strategy proposed for characterizing eye injury hazards for products used as antimicrobials regulated by the U.S. Environmental Protection Agency (EPA). While some of the methods appear promising, ICCVAM concluded that there are currently insufficient data with which to adequately demonstrate that the proposed strategy can classify test substances to the appropriate EPA ocular hazard category, and recommended that further studies are needed.

Finally, ICCVAM recommended that a proposed low volume eye test (LVET) should not be used for future regulatory testing due to poor predictivity when compared to the current standard rabbit eye test. However, ICCVAM concluded that data from past studies that used the LVET could be used in a weight-of-evidence approach to classify ocular hazards.

ICCVAM carried out the technical evaluation of the proposed methods and strategies in conjunction with NICEATM. Four recently published ICCVAM test method evaluation reports include the ICCVAM recommendations, ICCVAM-recommended protocols, final background review documents, independent peer review panel report, and the data used for the ICCVAM evaluations. Links to these and other ICCVAM recommendations on alternative methods to identify potential eye safety hazards can be found on the ICCVAM website at: http://iccvam.niehs.nih.gov/methods/ocutox/ocutox.htm.

NICEATM Convenes International Workshop on Vaccine Safety Testing

Nearly 200 scientists from 13 countries gathered last month at the "International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions." Workshop participants reviewed the current state of the science and recommended future research, development, and validation needed to advance alternative methods that can reduce, refine (decrease or eliminate pain and distress), and replace the use of animals for human and veterinary vaccine post-licensing potency and safety testing. The workshop, which took place on September 14-16 at NIH in Bethesda, Maryland, was organized by NICEATM and ICCVAM in partnership with the European Centre for the Validation of Alternative Methods, Japanese Center for the Validation of Alternative Methods, and Health Canada and co-sponsored by the Society of Toxicology.

Workshop participants identified knowledge and data gaps that need to be addressed to develop methods that can further reduce, refine, and replace the use of animals in vaccine testing. Participants also identified and prioritized research, development, and validation activities needed to address these knowledge and data gaps including the application of new science and technology to develop improved methods. They agreed that vaccines, which use the largest number of animals and are associated with the greatest pain and distress, should be given the highest priority for development and validation of alternative test methods. Participants also emphasized the need to find ways to avoid or minimize testing with live viruses and bacteria that are hazardous to workers. Ways to promote the increased use of accepted methods was also discussed. Implementation of the workshop recommendations is expected to advance the availability of alternative methods for vaccine potency and safety testing while ensuring continued protection of human and animal health.

Representatives from the U.S. Food and Drug Administration (FDA) and U.S. Department of Agriculture (USDA) joined scientists from Health Canada, the United Kingdom, Japan, and the World Health Organization to discuss their regulatory processes for evaluating human and veterinary vaccines. Although there are differences in these processes, each requires potency testing to ensure that each lot of a vaccine maintains the antigenic characteristics that make it effective, and safety testing to prevent the release of vaccine lots that might cause serious adverse health effects. Vaccine testing in the United States accounts for the largest number of animals that experience unrelieved pain and distress in research facility reports to the USDA, and is one of the four highest priority activities in the NICEATM-ICCVAM 2008-2012 Five-Year Plan.

The workshop provided a unique opportunity for stakeholders from the human and veterinary vaccine sectors to interact and gain important insights on similarities and differences in how potency and safety testing is currently conducted in each sector. Invited participants included scientists from the FDA, Centers for Disease Control and Prevention,

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Dr. Jodie Kulpa-Eddy, USDA, and Dr. William Stokes, NIEHS/NTP, lead discussions with the veterinary breakout group at the International Workshop on Alternative Methods in Vaccine Potency and Safety Testing.

USDA, Department of Defense, National Institute of Allergy and Infectious Diseases, as well as representatives from the governments of Japan, Canada, the United Kingdom, the Netherlands, and the European Union. National and multinational corporations and research institutions were also represented.

Presentations from the workshop will soon be available on the NICEATM-ICCVAM website. Complete proceedings of the workshop, including manuscripts from speakers and breakout group sessions, will be published next year as a dedicated issue of *Procedia in Vaccinology*. An article summarizing the workshop discussions and conclusions will also be published in the journal *Biologicals*. The workshop's conclusions and recommendations will be provided to ICCVAM for prioritization of future research, development, and validation activities for alternative test methods that reduce, refine, and replace the use of animals in vaccine potency and safety testing.

Two ICCVAM Workshops on Best Practices for Regulatory Safety Testing to be Held in January 2011

NICEATM and ICCVAM will host the first of a series of workshops on "Best Practices for Regulatory Safety Testing" in January 2011. These one-day workshops will provide information on the use of available alternative testing methods to evaluate the hazard potential of chemicals and products that minimize animal use and avoid animal pain and distress.

- A workshop on Assessing the Potential for Chemically Induced Eye Injuries will be presented January 19, 2011.
- A workshop on Assessing the Potential for Chemically Induced Allergic Contact Dermatitis will be presented January 20, 2011.

These workshops will bring together scientific experts representing relevant stakeholder organizations to discuss available alternative test methods and strategies for ocular and allergic contact dermatitis (ACD) safety and hazard assessments. Participants will become familiar with the strengths and weaknesses of available alternative methods, the types of data they provide, and the use of these data in hazard, safety, and risk assessments. The workshops will provide a practical understanding of the theory and application of available methods that can be used to evaluate the hazard potential of chemicals and products while minimizing animal use and avoiding pain and distress.

Speakers at the workshops will discuss U.S. requirements for the consideration of available alternatives, current regulatory requirements for safety testing, and the acceptance status of alternative methods. Detailed presentations will provide practical instruction on application of the test methods including standard protocols and data interpretation. Workshop participants will also apply knowledge gained from the program using case studies in breakout group discussion sessions.

Topics discussed at these workshops will be of particular interest to toxicologists and study directors involved in conducting tests for ocular safety and ACD hazards, members of Institutional Animal Care and Use Committees responsible for reviewing study protocols prior to testing, and regulators that would review data generated by such tests. Those interested may attend one or both workshops.

The workshops will be open to the public with no registration or daily session fees charged; attendance is limited only by the space available. They will be held at the National Institutes of Health in Bethesda, Maryland. More information on the workshops, a draft agenda, and a link to an online registration form are available on the NICEATM–ICCVAM website at: http://iccvam.niehs.nih.gov/meetings/Implement-2011/ImplmtnWksp.htm.

ICCVAM Chair Dr. Marilyn Wind Retires

Dr. Marilyn Wind, Chair of ICCVAM, retired in July from her post as Deputy Associate Executive Director in the Directorate for Health Sciences at the Consumer Product Safety Commission (CPSC). She had worked at CPSC for 30 years. Dr. Wind received a Ph.D. in pharmacology from New York University School of Medicine and did postdoctoral work in teratology at the National Institute of Dental Research and Craniofacial Research.

She served as the principal representative from the CPSC to ICCVAM since ICCVAM's inception and was elected Chair of ICCVAM in January of 2007. Dr. Wind also served as Chair of the ICCVAM Acute

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Toxicity Working Group and Co-chair of the Endocrine Disruptor Working Group. She was also a member of the ICCVAM Five-Year Plan Development Subcommittee, the Five-Year Plan Implementation Subcommittee, and three additional ICCVAM working groups.

In addition to her service to ICCVAM, Dr. Wind represented CPSC on a number of NTP groups including the NTP Executive Committee, the Center for the Evaluation of Risks to Human Reproduction Core Committee, and served as Chair of the NTP Interagency Committee for Chemical Evaluation and Coordination.

Please join NICEATM in thanking Dr. Wind for her dedicated service to NICEATM and NTP, and wishing her the best in her retirement.

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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).

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

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