UPDATE
National Toxicology Program

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Headquartered at the
National Institute of Environmental
Health Sciences NIH-DHHS

What's Inside:

Dr. Stokes Wins Award

Dr. William S. Stokes received the Charles River Prize for distinguished contributions to the field of laboratory animal medicine and science during the 143rd American Veterinary Medical Association (AVMA) Annual Convention held in Honolulu, July 15-19, 2006. The Charles River Laboratories Foundation, an international authority on the care and use of laboratory animals for biomedical research and testing, sponsors the award.

Dr. Stokes is director of the NTP's Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). During his career with the National Institutes of Health, he established procedures to validate and gain regulatory acceptance of new safety testing methods that would reduce, refine, and replace animal use. As co-chair of the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) from 1994-2001, he led the review and adoption of several new methods that significantly reduced the numbers of animals and the pain and distress involved in regulatory testing. Dr. Stokes commented that this award acknowledges significant progress resulting from the sustained efforts and teamwork of the many dedicated scientists on the ICCVAM and at NICEATM, and the contributions of many scientists and stakeholders in industry, academia, animal protection groups, and the international community.

Dr. Stokes served as a council member for the Institute for Laboratory Animal Research of the National Research Council from 1998-2004. On October 1, 2006, Dr. Stokes was promoted to Assistant Surgeon General and Rear Admiral in the Commissioned Corps of the U.S. Public Health Service. In 2003, the U.S. Surgeon General appointed him Chief Veterinary Officer for the U.S. Public Health Service.

Special Issue of Toxicologic Pathology

A special issue of the journal Toxicologic Pathology (Vol. 34, Issue 5) was published October 6th, highlighting the normal structure and pathology of the lymphoid system. Featured in this issue is a full-color CD-ROM of lymphoid organ histopathology.

"The papers have used large numbers of photomicrographs to provide a practical atlas for toxicologic pathologists, toxicologists, and immunologists," says Robert Maronpot, guest editor of the special issue and Chief of Laboratory of the Experimental Pathology at the National Institute of Environmental Health Sciences.

The special issue follows up on guidelines developed by the Society of Toxicologic Pathology (STP) in 2005 to address new FDA rules requiring immunotoxicity testing on all new investigational drugs or medicinal products. It fulfills a need among pathologists and toxicologists for a guide to gross and microscopic examinations of lymphoid tissues as necessary and pivotal first steps in the assessment of new drugs for immunotoxic potential. *(Continued on page 2)*

Upcoming Events

October 17-20, 2006
NIH Research Festival, Natcher Conference Center, NIH in Bethesda, MD
http://researchfestival.nih.gov/default.htm

November 13-14, 2006
Alternative Methods to Replace the Mouse LD50 Assay for Botulinum Toxin Potency Testing Workshop, Crowne Plaza Hotel, Silver Spring, MD

November 30, 2006
Scientific Advisory Committee on Alternative Toxicological Methods (SACATM),
NIEHS, 111 TW Alexander Drive
Research Triangle Park, NC 27709

December 1, 2006
NTP Board of Scientific Counselors Meeting,
NIEHS, 111 TW Alexander Drive
Research Triangle Park, NC 27709

January 24-26, 2007
CERHR Expert Panel Meeting on Hydroxyurea,
Radisson Hotel Old Town,
Alexandria, VA

May 16-17, 2007
Technical Reports Review Subcommittee,
NIEHS, 111 TW Alexander Drive,
Research Triangle Park, NC 27709

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Special Issue of Toxicologic Pathology

Dealing with normal structure, function, and histology as well as pathology of each major lymphoid system tissue, there are also papers in this issue on enhanced histopathology for evaluating lymphoid tissues and a paper dealing with immunohistochemistry of lymphoid organs.

"The special issue serves as a mechanism to provide photomicrographs to illustrate the 2005 STP Best Practice Guidelines for Routine Evaluation of the Immune System," says Maronpot.

The emphasis is primarily on rodents, although a small number of canine and non-human primate photomicrographs have been added when available. Contributing authors are from government, industry, and research organizations. ●

NTP Board of Scientific Counselors Meeting

The NTP Board of Scientific Counselors will meet on December 1, 2006, at the NIEHS, 111 TW Alexander Drive, Research Triangle Park, NC. Tentatively, on the agenda, are 1) a report on a retreat to review the recommendations on four workshops relating to the NTP Roadmap, 2) testing of chemicals in high throughput screening assays and in *Caenorhabditis elegans*, 3) concept reviews on the host susceptibility program and magnetic resonance imaging for pathological evaluations, 4) the NIEHS Exposure Biology Program, 5) reports from meetings of the NTP Board's Technical Reports Review Subcommittee and 6) substances nominated for the Center for the Evaluation of Risks to Human Reproduction evaluations. Topics may be added or modified as the agenda is finalized. Details about this meeting will be announced in the *Federal Register* and posted on the NTP web site http://ntp.niehs.nih.gov (see Advisory Committees and Board) or can be obtained by contacting the executive secretary, Dr. Barbara Shane. This meeting is open to the public and public comment, both written and oral, is welcome on any agenda topic. ●

Contact Information: Dr. Barbara Shane, Executive Secretary, NTP Liaison and Scientific Review Office, NIH/NIEHS, P.O. Box 12233, MD A3-01, Research Triangle Park, NC 27709; T: (919) 541-4253; FAX: (919) 541-0295; shane@niehs.nih.gov

Recent Peer Review of Draft NTP Technical Reports

The Technical Reports Review Subcommittee of the NTP Board of Scientific Counselors met on June 12 and August 28, 2006, at the NIEHS to peer review the findings and conclusions from draft NTP technical reports using conventional rodent models or genetically modified models. The subcommittee made the recommendations recorded below regarding the findings and conclusions of these reports. These recommendations will be reported to the NTP Board of Scientific Counselors at its meeting on December 1, 2006. Additional details about the meeting are available on the NTP website at http://ntp.niehs.nih.gov, see Advisory Committees and Board. *(Continued on page 3)*

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Recent Peer Review of Draft NTP Technical Reports

June 12, 2006 meeting

Genistein

Multigenerational Study (TR 539)

- The Subcommittee accepted unanimously (10 yes, 0 no) the summary of the findings for this continuous breeding study as written. Offspring from the F1 and F3 generations exposed *in utero* and during lactation to genistein were either exposed to genistein or a control diet until 2 years of age (TR 545).

Bioassay (TR 545)

- The Subcommittee accepted unanimously (10 yes, 0 no) the conclusions as written, *no evidence* of carcinogenic activity in F1 male Sprague-Dawley rats and *some evidence* of carcinogenic activity in F1 female Sprague-Dawley rats exposed to genistein from conception to 2 years of age and *no evidence* of carcinogenic activity in F1 male Sprague-Dawley rats and *equivocal evidence* of carcinogenic activity in F1 female Sprague-Dawley rats exposed to genistein from conception to 20 weeks of age followed by control feed to 2 years of age.
- The Subcommittee accepted unanimously (10 yes, 0 no) the conclusions, *no evidence* of carcinogenic activity in F3 male Sprague-Dawley rats and *equivocal evidence* of carcinogenic activity in F3 female Sprague-Dawley rats exposed to genistein from conception through weaning at postnatal day 21 followed by control diet to 2 years of age. The Subcommittee stated that the effects of genistein on estrous cycling and the incidences of common, hormonally related spontaneous neoplasms of female Sprague-Dawley rats are consistent with an estrogenic mechanism of toxicity.

α-Methylstyrene (TR 543)

- The Subcommittee accepted unanimously (9 yes, 0 no) the conclusions as written, *some evidence* of carcinogenic activity of α-methylstyrene in male F344/N rats, *no evidence* of carcinogenic activity in female F344/N rats, *equivocal evidence* of carcinogenic activity in male B6C3F1 mice, and *clear evidence* of carcinogenic activity in female B6C3F1 mice. The Subcommittee stated that the kidney toxicity in male rats exhibited some features of α-2u-globulin nephropathy.

Methylene Blue Trihydrate (TR 540)

- The Subcommittee accepted unanimously (10 yes, 0 no) the conclusions as written, *some evidence* of carcinogenic activity of methylene blue trihydrate in male F344/N rats and B6C3F1 mice, *no evidence* of carcinogenic activity in female F344/N rats, and *equivocal evidence* of carcinogenic activity in female B6C3F1 mice.

August 28, 2006 Meeting

Allyl bromide (GMM 7)

- The Subcommittee accepted (7 yes, 0 no) the conclusions as written, *no evidence* of carcinogenic activity of allyl bromide in male and female p53 haploinsufficient mice.

Benzene (GMM 8)

- The Subcommittee accepted (7 yes, 0 no) the conclusions as written, *clear evidence* of carcinogenic activity of benzene in male $p16^{Ink4a}/p19^{Arf}$ haploinsufficient mice and *no evidence* of carcinogenic activity of benzene in $p16^{Ink4a}/p19^{Arf}$ haploinsufficient female mice.

Dicyclohexylcarbodiimide (GMM 9)

- The Subcommittee accepted (7 yes, 0 no) the conclusions as written, *no evidence* of carcinogenic activity of dicyclohexylcarbodiimide in female p53 haploinsufficient mice.

Glycidol (GMM 13)

- The Subcommittee accepted (7 yes, 0 no) the conclusions as written, *clear evidence* of carcinogenic activity of glycidol in male haploinsufficient $p16^{Ink4a}/p19^{Arf}$ mice and *some evidence* of carcinogenic activity of glycidol in female haploinsufficient $p16^{Ink4a}/p19^{Arf}$ mice.

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Recent Peer Review of Draft NTP Technical Reports

Phenolphthalein (GMM 12)

- The Subcommittee accepted (7 yes, 0 no) the conclusions as written, *no evidence* of carcinogenic activity of phenolphthalein in haploinsufficient $p16^{Ink4a}/p19^{Arf}$ male or female mice. The Subcommittee also recommended that because this is a new model, there is uncertainty whether the study possessed sufficient sensitivity to detect a carcinogenic effect. ●

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The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) Meeting

The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) will meet on November 30, 2006, at the NIEHS, 111 TW Alexander Drive, Research Triangle Park, NC to discuss issues related to toxicological methods that reduce, refine, or replace the use of animals in testing. Details about this meeting, as available, will be announced in the *Federal Register* and posted on the NTP web site http://ntp.niehs.nih.gov (select Advisory Board and Committees). This meeting is open to the public and public comment, both written and oral, is welcome. ●

Contact Information: Dr. Kristina Thayer, NIH/NIEHS, P.O. Box 12233, MD A3-01, Research Triangle Park, NC 27709; T: (919) 541-5021; FAX: (919) 541-0295; thayer@niehs.nih.gov

Center for the Evaluation of Risks to Human Reproduction (CERHR)

Genistein and Soy Formula Evaluations

CERHR held an expert panel meeting on genistein and soy formula on March 15-17, 2006, in Alexandria, VA. An independent panel of 14 scientists evaluated information on human exposure, reproductive toxicity, and developmental toxicity of genistein and soy formula.

The final expert panel reports are now available on the CERHR web site http://cerhr.niehs.nih.gov and in hardcopy or on CD from CERHR (contact information below). Public comments received on these reports are posted on the CERHR web site and will be included in the NTP-CERHR monographs on these substances. The NTP considers all public comments during preparation of the NTP Brief, a document that articulates the NTP's opinion on the reproductive and/or development hazard for humans. The draft NTP Briefs on genistein and soy formula are in preparation.

Hydroxyurea and Bisphenol A Expert Panel Meetings Planned

Expert panel meetings on hydroxyurea and bisphenol A are planned for January 24-26, 2007 and March 2007, respectively. Draft expert panel reports, information about submitting public comments, and details about the meetings will be announced later this year. ●

Contact Information: Dr. Michael D. Shelby, Director CERHR, NIH/NIEHS, P.O. Box 12233, MD EC-32, Research Triangle Park, NC 27709, T: (919) 541-3455; FAX: (919) 316-4511; shelby@niehs.nih.gov

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

Workshop Planned for November

NICEATM will hold the public workshop Alternative Methods to Replace the Mouse LD_{50} *Assay for Botulinum Toxin Potency Testing* at the Crowne Plaza Hotel, Silver Spring, MD on November 13 and 14, 2006 (*Federal Register* 71FR47505-6; August 17, 2006). Scientists from leading governmental and academic institutions, national and international regulatory agencies, industry, and the animal protection community will review the current state-of-the-science for alternative methods and identify high priority research, development, and validation studies. Individuals who wish to attend the workshop are strongly encouraged to register with NICEATM by October 30, 2006. Registration information, agenda, and additional information will be available on the workshop web site. Available at http://iccvam.niehs.nih.gov/meetings/schedule.htm.

Botulinum toxin, the most poisonous substance known, causes paralysis by acting on the nervous system. Botulism has been a public health hazard for centuries and is emerging as a significant bioterrorism threat. Recently, the toxin has been used to treat many serious and painful medical conditions. Currently, the most frequently used method for detecting or assessing the potency of botulinum toxin is a test called the mouse LD_{50} assay. This assay involves dosing mice with dilutions of the toxin and identifying the dilution at which 50% of the mice die. The LD_{50} assay has been in use for many years and is currently accepted as the method-of-choice by all U.S. and European regulatory agencies. However, recent advances are affording opportunities for alternative methods that may be faster, more accurate, and potentially refine (cause less pain and distress), replace, or reduce the use of mice for testing botulinum toxin.

Revised List of Reference Substances for Validation of ER and AR Binding and Transcriptional Activation Assays

NICEATM announces the availability of an addendum to the report, "Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Evaluation of *In Vitro* Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays" (*NIH Publication* 03-4503). The addendum describes the rationale for the revisions to the original list of recommended reference substances for validation of *in vitro* estrogen receptor (ER) and androgen receptor (AR) binding and transcriptional activation (TA) assays.

The original list of ICCVAM recommended reference substances was published in 2003 (*Federal Register* 68FR33171-33172; June 3, 2003 available at http://iccvam.niehs.nih.gov/docs/docs.htm#endocrine). NICEATM recently reviewed the commercial availability and cost for the 78 recommended substances. A minimum of 44 substances are recommended for AR binding and TA assays and 53 substances for ER binding and TA assays. This review indicated that three substances (anastrazole, CGS 18320B, fadrozole) are not commercially available, one substance has restricted commercial availability (ICI 182,780) and six others (actinomycin D, hydroxyflutamide, 4-hydroxytamoxifen, methyltrienolone, 12-O-tetradecanoylphorbol-13-acetate, zearalenone) are expensive. ICCVAM has now replaced the four substances that are not commercially available or have restricted availability with ones having similar ER and AR activity profiles (4-hydroxyandrostenedione, chrysin, dicofol, raloxifene HCl). Suitable replacements (19-nortestosterone and resveratrol) were also identified for metyltrienolone and zearalenone, respectively, two of the expensive substances. NICEATM wanted to replace four other highly priced substances (actinomycin D, hydroxyflutamide, 4-hydroxytamoxifen, 12-O-tetradecanoylphorbol-13-acetate), but was unable to identify suitable replacements because of their unique activity profiles and/or chemical/physical properties. ●

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Report on Carcinogens (RoC)

The NTP recently announced its proposed review process for nominations to the Twelfth Edition of the Report on Carcinogens (12th RoC *Federal Register* 71FR47507; August 17, 2006). The NTP is currently reviewing the public comments received on the proposed process and will consider them as the program works to finalize the process. The final 12th RoC review process will be announced in a future *Federal Register* notice and through NTP publications.

Two important new elements in the proposed RoC review process are (1) the public peer review of draft background documents by ad hoc scientific expert panels and (2) the peer review of draft substance profiles by the NTP Board of Scientific Counselors. In addition, the NTP will also, on a trial basis, prepare a response to public comments for the 12th RoC. The proposed RoC review process is described in detail on the NTP web site http://ntp.niehs.nih.gov (select Report on Carcinogens).

The RoC is a congressionally mandated document published by the Secretary of Health and Human Services that identifies agents, substances, mixtures, or exposure circumstances (collectively referred to as "substances") that may pose a carcinogenic hazard to human health. The Secretary has delegated responsibility for preparing the draft report to the NTP. Substances are listed in the RoC as either *known or reasonably anticipated* to be a human carcinogen. Development of the RoC is based upon a review of nominations (for new substances that are under consideration for listing or for reclassification of the listing status for a substance already listed) and a multi-step, scientific review process with opportunity for public comment. ●

Contact Information: Dr. C. W. Jameson, Report on Carcinogens, NIH/NIEHS, P.O. Box 12233, MD EC-14, Research Triangle Park, NC 27709; T: (919) 541-4096, FAX: (919) 541-0144; jameson@niehs.nih.gov.

NTP Testing Program

Request for Study Nominations

With a broad mandate to provide toxicological characterizations for chemicals and other agents of public health concern, the NTP accepts nominations for new toxicological studies at any time. Labor unions, academic scientists, federal and state agencies, industry, and the general public are welcome to make nominations for specific substances or for general issues related to potential human health hazards of occupational or environmental exposures. As available, a rationale for study should accompany the nomination along with background information describing sources of exposure and possible adverse health effects or concerns associated with exposure, the chemical name and the Chemical Abstract Service (CAS) registry number. Details about the nomination review and selection process are available on the NTP web site http://ntp.niehs.nih.gov select Nominations to the Testing Program under the heading Testing Information) or by contacting the NTP Office of Chemical Nomination and Selection *(contact information at the end of this article)*.

Current areas of focus in the NTP's testing program include potential hazards associated with nanoscale materials, perfluorinated compounds, herbal dietary supplements, photoactive chemicals, brominated flame retardants, certain complex occupational exposures, dioxin-like compounds, contaminants of finished drinking water, and endocrine-disrupting substances, and methods for assessing potential cardiac toxicity.

All nominations undergo several levels of review before being selected by the NTP for study. These steps of review help to ensure that the NTP's testing program addresses toxicological concerns pertinent to all areas of public health and helps maintain balance among the types of substances and issues evaluated. Studies are initiated on selected nominations as time and resources permit.

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To subscribe to the listserv and receive the NTP Update as well as other NTP news and announcements electronically, register online at http://ntp.niehs.nih.gov or send e-mail to ntpmail-request@list.niehs.nih.gov with the word "subscribe" as the body of the message or contact the NTP Liaison and Scientific Review Office. Additional information about the NTP along with announcements of meetings, publications, study results and its centers is available on the Internet at http://ntp.niehs.nih.gov.

The NTP web site 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 web site at http://ntp.niehs.nih.gov (see Resources).

Contact Information: NTP Liaison and Scientific Review Office, NIEHS, P.O. Box 12233, MD A3-01, Research Triangle Park, NC 27709; T: (919) 541-0530; FAX: (919) 541-0295; liaison@starbase.niehs.nih.gov

Recent NTP Publications

NTP Technical Reports:

TOX 48: *Toxicity Studies of Allyl Acetate, Allyl Alcohol, and Acrolein*

TOX 74: *Atmospheric Characterization, Particle Size, Chemical Composition, and Workplace Exposure Assessment of Cellulose Insulation*

TR 522: *Toxicology and Carcinogenesis Studies of Transplacental AZT*

TR 530: *Toxicology and Carcinogenesis Studies of a Binary Mixture of PCB 126 and PCB 153*

Available at:
http://ntp.niehs.nih.gov/go/reports

NICEATM/ICCVAM:

Addendum: *Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Evaluation of In Vitro Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays* (NIH Publication 03-4503)

Available at:
http://iccvam.niehs.nih.gov/methods/endocrine.htm

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NTP Testing Program

A list of study nominations currently in review, along with supporting documents and pubic comments, can be accessed through the NTP web site at http://ntp.niehs.nih.gov/go/nom. Information on nominations reviewed in previous years can also be accessed through this page.

Contact Information: Dr. Scott A. Masten, Director, Office of Chemical Nomination and Selection, NIH/NIEHS, P. O. Box 12233, MD A3-07, Research Triangle Park, NC 27709; T: (919) 541-5710; FAX: (919) 541-3647; masten@niehs.nih.gov