UPDATE
National Toxicology Program

JANUARY 2009

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

Linda S. Birnbaum to Lead NIEHS and NTP

On December 3, Dr. Raynard S. Kington, acting director of the National Institutes of Health, announced the appointment of Linda S. Birnbaum, Ph.D., D.A.B.T., A.T.S., as director of the NIEHS and NTP. Dr. Birnbaum is currently a senior advisor at the EPA, where she served for 16 years as director of the Experimental Toxicology Division. She will begin her appointment in mid January 2009.

Humane Society and Procter and Gamble Recognize NTP for Advancing Alternatives to Animal Testing

Article by Robin Mackar, reprinted from *eFACTOR*, January 2009

On December 18, the Humane Society of the United States (HSUS) and Procter and Gamble presented Ray Tice, Ph.D., of the National Toxicology Program (NTP) an award for the outstanding scientific contributions that he and others are making to advance viable alternatives to animal testing.

The North American Alternative Awards were presented at HSUS Washington office by the executive vice president of the HSUS, Andrew Rowan, Ph.D., and Len Sauers, Ph.D., vice president of product safety, regulatory and technical relations for Procter and Gamble. The awards recognize the efforts of the recipients to work toward the elimination of animal testing for consumer product safety while ensuring safe products for consumers and the environment.

Raymond Tice, Ph.D., chief of the NTP Biomolecular Screening Branch and deputy director of the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods, was joined by colleagues Christopher Austin, M.D., of the National Human Genome Research Institute (NHGRI) and Robert Kavlock, Ph.D., of the Environmental Protection Agency (EPA) to receive the award. The award includes a $25,000 grant to support the ongoing alternative methodologies efforts.

The three agencies signed a Memorandum of Understanding (MOU) in February 2008 to use the NIH Chemical Genomics Center's (NCGC) high-speed, automated screening robots to test suspected toxic compounds using cells and isolated molecular targets instead of laboratory animals. The award will be used to develop toxicity signatures that help determine how toxic a chemical might be and what type of toxicity it might cause.

"I am pleased that we are receiving recognition by our stakeholders for our efforts," said Tice. "The NTP and our sister agencies are working hard to implement the vision set out by the National Research Council's 2007 Report, Toxicity Testing in the 21st Century: A Vision and a Strategy."

NTP Unveils New Non-Cancer Study Criteria

Article by Eddy Ball, reprinted from *eFACTOR*, January 2009

The National Toxicology Program (NTP) is trying to bring the same rigorous standards it uses for classifying the outcomes of its cancer studies to many of its non-cancer studies, according to presentations made by speakers at the NTP Board of Scientific Counselors (BSC) meeting November 20 in Rodbell Auditorium. The Board voted to accept three working group reports addressing the establishment of new criteria for future NTP immunotoxicology, reproductive and developmental studies.

The three sets of criteria were presented by BSC Criteria Working Group (CWG) chairs Nancy Kerkvliet, Ph.D., who chaired the immunotoxicology group, and Edward Carney, Ph.D., who chaired the reproductive and developmental groups. The criteria are similar to those used with the agency's gold-standard cancer studies, which are based on five levels of evidence ranging from clear evidence to no evidence and inadequate study.

Toxicology Branch Acting Chief and reproduction and development discipline leader Paul Foster, Ph.D., opened the discussion by explaining why NTP decided to establish the strength of evidence criteria. "We've had a goal now for the best part of 18 months to employ the same rigorous standards used historically to review our carcinogenicity bioassays with the NTP non-cancer studies," he said. He also noted that the NTP did not undertake this effort in isolation and emphasized the critical role the working groups played in helping establish the criteria for the various studies. The working groups, made up of representatives from government, industry and academia, met in late summer 2008.

"We have a desire to have uniform [non-cancer] criteria for chemicals across studies and for studies across chemicals, much as we have for the cancer bioassays," Foster continued, "so that the Board, the NTP staff and the public can have some kind of consistency in the ways these findings are reported."

Foster and Toxicology Branch immunology discipline leader Dori Germolec, Ph.D., expressed their confidence that the new criteria will give NTP non-cancer studies the same rigor, consistency and authority that NTP cancer reports have enjoyed for decades. The criteria, they maintained, will increase the utility of the non-cancer studies for regulatory agencies by clarifying the official government opinion of the hazards posed by chemicals.

Following presentations by the chairs of the three CWGs, Board members engaged in a lively discussion before giving NTP the go-ahead to progress to the next phase of finalizing the criteria. NTP scientists agreed to address Board concerns about terminology that might lead to misinterpretation by those not as familiar with toxicology.

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Upcoming Events

February 24, 2009

NTP Board of Scientific Counselors Meeting

NIEHS, 111 TW Alexander Dr.
Research Triangle Park, NC

February 25, 2009

NTP Board of Scientific Counselors Technical Reports Review Subcommittee Meeting

NIEHS, 111 TW Alexander Dr.
Research Triangle Park, NC

June 25-26, 2009

Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) Meeting

Hilton Arlington
950 North Stafford Street
Arlington, VA

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

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In the course of the Board's discussion, Foster, Germolec and NTP Associate Director John Bucher, Ph.D., emphasized that the non-cancer studies will be clearly presented as hazard studies with multiple interrelated end points and not as risk assessments, which require exposure data along with consideration of hazards. The levels of evidence, they assured the Board, will be noted as applying under the conditions of the individual study in regard to specific sexes of specific species for the particular studies.

All CWGs proposed four levels of evidence: clear evidence, some evidence, equivocal evidence and no evidence. The reproductive and developmental toxicity CWG also included the category "inadequate study" for studies with qualitative or quantitative limitations that could not be interpreted for toxicity — a category that will also be incorporated into the final immunotoxicology criteria.

Timeline for Non-Cancer Criteria

According to Foster and Germolec, revisions to the levels of evidence statements for the three sets of criteria incorporating the Board's recommendations should be completed by the end of January 2009.

The proposed criteria are slated for formal presentation to attendees at the Society for Toxicology (SOT) 48th Annual Meeting and ToxExpo March 15-19 in Baltimore, MD by Foster and Germolec.

Germolec expects to begin applying the immunotoxicology criteria to studies for peer review by the end of 2009. Foster said that the first reproductive and developmental studies featuring the new criteria could appear as early as early as 2010.

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NTP Staff Honored

William Stokes Honored

Rear Admiral William Stokes, Director of NICEATM, received the James A. McCallam Award at the 114th meeting of the Association of Military Surgeons of the United States (AMSUS) in San Antonio, TX on November 12, 2008. RADM Stokes received the award in recognition of his outstanding accomplishments in the field of medicine and health. RADM Steven Galson, Acting Surgeon General of the United States, and Major General (ret) George Anderson, AMSUS Executive Director presented the award.

RADM Stokes was also honored with the Karl F. Meyer-James H. Steele Gold Head Cane Award by the American Veterinary Medical Association (AVMA). RADM Stokes received the award in recognition of significant career achievements that have advanced human health through the practice of veterinary epidemiology and public health. Dr. Gregory Hammer, the 2007-08 President of the AVMA, presented the award at the 145th AVMA Annual Convention in New Orleans on July 22, 2008.

Scott Auerbach Wins NC SOT Award

Dr. Scott Auerbach, a postdoctoral fellow in the Toxicology Branch, received second place in the President's Award for Research Competition at the October annual meeting of the NC Society of Toxicology (SOT) for the study titled "Prediction of hepatocarcinogenic potential in male rats using machine learning methods informed by genome-wide expression analysis." His mentor and collaborator on the research is Dr. Rick Irwin.

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Directors Annual Awards Ceremony

The NIEHS honored employees at the 2008 Director's Annual Honor Awards Ceremony on December 18. Several NTP staff received Merit Awards, which is the highest honor award an Institute Director can approve. It recognizes contributions in the areas of leadership, significant scientific research or administrative support, creativity, and notable competence or administrative management of the institute.

Joseph Roycroft, Ph.D. and Molly Vallant *for significant and sustained contributions to the high quality of studies conducted for the National Toxicology Program.*

John Bucher, Ph.D., Paul Foster, Ph.D., Denise Lasko, Michael Shelby, Ph.D., Diane Spencer, M.S., Kristina Thayer, Ph.D., and Mary Wolfe, Ph.D., in a group award with Robin Mackar and Allen Dearry, Ph.D. *for exemplary service in facilitating and organizing the evaluation of the bisphenol A report*.

Paul Foster, Ph.D., John French, Ph.D., Robert Sills, Ph.D., Cynthia Smith, Ph.D., Raymond Tice, Ph.D., Nigel Walker, Ph.D., and Mary Wolfe, Ph.D., *for significant scientific and technical contributions to the implementation of the realignment of the National Toxicology Program*.

Dori Germolec, Ph.D., Grace Kissling, Ph.D., and Sharon Soward, received special recognition as recipients of the NIEHS Unsung Hero Award. This award recognizes individuals *for behind the scenes contributions that keep the NIEHS operating in harmony.*

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NTP at SOT

Plan to stop by booth #1673 and visit NTP at the Society of Toxicology (SOT) 48th Annual Meeting and ToxExpo March 15-19, 2009, in Baltimore, MD. NIEHS and its journal EHP will be adjacent to NTP in booth #1772.

NTP to Unveil New Non-Cancer Evaluation Criteria at SOT

The NTP is working to bring the same rigorous standards it uses for classifying the outcomes of its cancer studies to many of its non-cancer studies (see related story, page 2). On March 17, 2009, from 1:30-2:30 PM in Room 337, the NTP will sponsor a session to discuss the establishment of its new evaluation criteria for future NTP immunotoxicology, reproductive and developmental studies and how these criteria can be used to draw study conclusions. The NTP invites you to join them for the exhibitor-hosted session to hear about the new evaluation criteria.

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NTP Board of Scientific Counselors

The NTP Board of Scientific Counselors (BSC) will meet on February 24, 2009, at the NIEHS, 111 TW Alexander Drive, Research Triangle Park, NC. The primary agenda item is the public peer review by the BSC of draft substance profiles for five candidate substances (aristolochic acids, captafol *ortho*-nitrotoluene, riddelliine and styrene) under consideration for the 12th Report on Carcinogens.

This meeting was announced in the Federal Register on December 22 (73 FR 78365) and materials for the meeting, including the draft substance profiles, are posted on the NTP website (http://ntp.niehs.nih.gov/go/165) or can be obtained by contacting Dr. Barbara Shane (contact information below). This meeting is open to the public and public comment, both written and oral, is welcome on any draft profile.

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

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NTP Board of Scientific Counselors Technical Reports Review Subcommittee

The Subcommittee is scheduled to meet on February 25, 2009, at the NIEHS, 111 TW Alexander Drive, Research Triangle Park, NC to peer review the findings and conclusions from 6 draft NTP Technical Reports on studies conducted in conventional rats and mice.

The draft reports tentatively scheduled for review are:

TR 557	β-Myrcene	TR 560	Androstenedione
TR 558	3,3',4,4'-Tetrachloroazobenzene	TR 561	Tetralin
TR 559	2,3',4,4',5- Pentachlorobiphenyl (PCB 118)	TR 562	Goldenseal root powder

Information about the meeting was announced in the Federal Register on December 18 (73 FR 77026) and materials for the meeting are posted on the NTP website (http://ntp.niehs.nih.gov/go/15833) or can be obtained by contacting Dr. Barbara Shane (contact information below). Draft technical reports will be available by January 14, 2009. This meeting is open to the public, and public comment, both written and oral, is welcome on any draft report.

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

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Report on Carcinogens (RoC)

Cobalt-Tungsten Carbide Powders and Hard Metals

The public meeting of the RoC Expert Panel on Cobalt-Tungsten Carbide Powders and Hard Metals took place on December 9-10, 2008, at the Sheraton Chapel Hill Hotel, Chapel Hill, NC. The expert panel peer-reviewed the draft background document on cobalt-tungsten carbide powders and hard metals and made a recommendation on its listing status in the 12th RoC. The expert panel recommended that cobalt-tungsten carbide powders and hard metals be listed as *reasonably anticipated to be a human carcinogen* based on limited evidence of carcinogenicity in humans and supporting mechanistic data. The NTP plans to have the expert panel's recommendation on listing status and scientific justification (Expert Panel Report, Part B) posted on the RoC website (http://ntp.niehs.nih.gov/go/29682) and available for public comment by late January 2009. The final background document on cobalt tungsten carbide powders and hard metals should be posted on the RoC website in February.

Contact Information: Dr. Ruth M. Lunn, Report on Carcinogens Office, NIH/NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709; T: (919) 316-4637; FAX: (919) 541-0144; lunn@niehs.nih.gov

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

Updated Evaluations of Genistein and Soy Formula Planned

In 2006, CERHR initiated the evaluations of genistein and soy formula. An expert panel meeting was held in March 2006, and the final expert panel reports for both substances were released in May 2006. Draft NTP briefs, which provided the NTP's conclusions regarding the potential for genistein or soy formula to adversely affect reproduction and/or development in exposed humans, were released for public comment and peer review in November 2006. CERHR has not finalized these evaluations, finalized the briefs, or issued the NTP-CERHR monographs on these substances. Since 2006, a substantial number of new studies related to human exposure or reproductive or developmental toxicity have been published and CERHR has determined that updated evaluations of genistein and soy formula are needed. Plans are underway to convene a second expert panel to consider this new literature and to review and update the conclusions drawn by the previous panel.

A Federal Register notice (http://ntp.niehs.nih.gov/files/73_FR_192_Updt_Evals_508.pdf) was published on October 2, 2008, requesting public comment on the proposed review as well as nominations of expert panel members. As plans for these evaluations are finalized, they will be announced in the Federal Register and this newsletter.

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

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Educational Opportunities in Pathology

Rodent Pathology Course

NTP staff from the Cellular and Molecular Pathology Branch including Drs. Angela King-Herbert, David Malarkey, Susan Elmore, and Ron Herbert recently served on the organizing committee for the Fourth RTP Rodent Pathology Course on Immunopathology held September 14-16, 2008. Dr. Susan Elmore spoke on "Structure and Function of the Immune System" and Ms. Julie Foley, also from CMPB, presented timely information on "FAQs regarding the Collection and Fixation of Tissue for Immune System Regulation." The course series, initially conceived by Drs. Robert Maronpot and Jeff Everitt, began in 2002 and is designed to provide useful information on current issues and techniques in rodent pathology to research and diagnostic pathologists, pathologists-in-training, and interested members of the research community. Past courses have covered reproductive pathology, neurological pathology, and cardiopulmonary pathology. To learn more about this continuing education series go to http://continuingeducation.ncsu.edu/rodentpath/.

NTP Satellite Symposium

The 10th Annual NTP Satellite Symposium will be held on June 20, 2009, in conjunction with the Society of Toxicologic Pathology (STP) 28th Annual Meeting in Washington, DC. This all-day pathology symposium is designed to provide continuing education to the toxicological pathology community using an interactive approach to diagnostic

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histopathology. This year's topic is tumor pathology, which complements the STP annual meeting's theme on cancer. Case studies will be presented and 100 audience members will have an opportunity to vote anonymously on the diagnosis for each case using wireless keypads; the fun begins when the voting results are displayed to everyone for general discussion. Dr. Susan Elmore from the NTP Cellular and Molecular Pathology Branch (elmore@niehs.nih.gov) is the symposium chair. It is a free event, but due to space limitations registration is required. To learn more and register for the symposium, go to http://www.toxpath.org/AM2009/gen.asp.

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

ICCVAM Issues Recommendations on *In Vitro* Pyrogenicity Test Methods

NICEATM announces availability of the (1) *ICCVAM Test Method Evaluation Report: Validation Status of Five* In Vitro *Test Methods Proposed for Assessing Potential Pyrogenicity of Pharmaceuticals and Other Products* (NIH Publication Number 08-6392) and (2) *Final Background Review Document: Validation Status of Five* In Vitro *Test Methods Proposed for Assessing Potential Pyrogenicity of Pharmaceuticals and Other Products* (NIH Publication Number 08-6391). The evaluation report provides recommendations from the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) on the usefulness and limitations of five *in vitro* pyrogen test methods for detecting Gram-negative bacterial endotoxin in human parenteral pharmaceuticals. The recommendations are based on ICCVAM's comprehensive review of the scientific validity of the methods that included an independent scientific peer review by an international expert panel. The report also provides recommended test method protocols for the five methods and recommendations for future studies that might broaden the usefulness of the test methods.

Endotoxin is a component of the Gram-negative bacterial cell membrane that can induce fever (i.e., is pyrogenic). Parenteral pharmaceuticals, fluids for injection, medical devices, and human biological products must be properly and accurately evaluated for the presence of endotoxin contamination prior to their release for clinical use.

ICCVAM recommends that the test methods should be considered for use on a case-by-case basis to detect Gram-negative endotoxin in human parenteral drugs, subject to product-specific validation to demonstrate equivalence to the rabbit pyrogen test, in accordance with applicable U.S. Food and Drug Administration regulations. When determined to be valid for specific products and used in this manner, these methods can reduce the number of animals needed for pyrogenicity testing. ICCVAM also recommends that these and other *in vitro* alternative test methods should be considered prior to *in vivo* pyrogenicity testing, and should be used where determined appropriate for a specific testing situation. However, ICCVAM concludes that none of these test methods can be considered as a complete replacement for the rabbit pyrogen test for all testing situations for detecting Gram-negative endotoxin.

The final background review document provides the data and analyses that support the current validation status of these five *in vitro* test methods. Availability of the test method evaluation report and background review document was announced on November 24, 2008, in the Federal Register (73 FR 71003) and are available on the NICEATM/ICCVAM web site (http://iccvam.niehs.nih.gov/methods/pyrogen/pyrogen.htm). The European Centre

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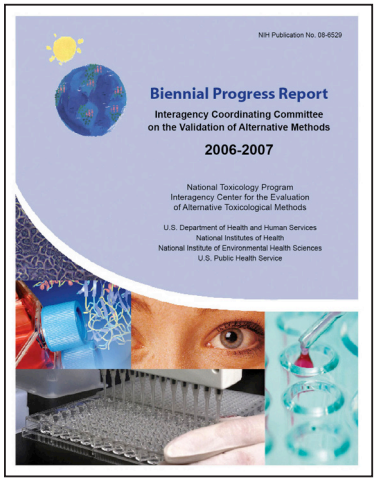
for the Validation of Alternative Methods submitted the five *in vitro* pyrogen test methods to ICCVAM for formal evaluation of their scientific validity for regulatory testing purposes.

The ICCVAM recommendations have been forwarded to U.S. Federal agencies for consideration of regulatory acceptance according to their specific statutory requirements. The deadline for the agencies' responses is April 22, 2009; the responses will be posted on the NICEATM-ICCVAM website.

2006-2007 ICCVAM Biennial Report



The Biennial Progress Report for the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) for 2006-2007 was released on October 23, 2008 (73 FR 63150). The biennial report describes major activities over the past two years and reiterates ongoing efforts by NICEATM and ICCVAM to promote the development, validation, and regulatory acceptance of new test methods that will reduce, refine, and replace the use of animals in testing while maintaining and promoting scientific quality and the protection of people, animals, and the environment. Selected highlights include:

- ICCVAM recommended to Federal agencies the first non-animal, ocular, safety-testing methods that can identify substances causing severe eye damage, such as blindness.
- ICCVAM determined that two non-animal, cell-based assays could reduce animal use for testing that is required to determine if chemicals and products can cause acute poisoning, the most common product safety test performed.
- ICCVAM completed evaluation of five non-animal test methods for assessing the fever inducing, or pyrogenicity, potential of injectable pharmaceuticals and other products.
- NICEATM initiated an international validation study of a cell-based test method to determine if it can identify substances that may disrupt normal hormonal function.
- NICEATM and ICCVAM strengthened collaborations with European and Japanese counterparts in the areas of validation studies and review activities.

Since its establishment in 1997, ICCVAM has contributed to the approval or endorsement of 18 alternative safety-testing methods by Federal regulatory agencies. These test methods have significantly reduced animal use and improved animal welfare. ICCVAM has also identified critical research, development, and validation efforts needed to further advance numerous other alternative methods.

The biennial report is available on the NICEATM-ICCVAM website (http://iccvam.niehs.nih.gov/).

Update on the Murine Local Lymph Node Assay (LLNA)

NICEATM has received and is reviewing additional data and information on three non-radioactive LLNA test methods and formulation data from over 70 traditional LLNA studies. These data will be used in an updated evaluation of the usefulness and limitations of the LLNA test methods. In addition, previous analyses performed to evaluate the use of the LLNA for predicting skin sensitization potency in humans have been updated to include new human reference data. An independent peer review panel is scheduled to meet in public session on April 28-29, 2009, to consider draft ICCVAM test method recommendations on the updated LLNA test method analyses.

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

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NIEHS Scientists Shine at Research Festival

Article by Eddy Ball, reprinted from *eFACTOR*, November 2008

NIEHS and National Toxicology Program (NTP) scientists were among the thousands of people attending the 21st annual NIH Research Festival October 14-17 at the Masur Auditorium and Natcher Conference Center on the NIH campus in Bethesda, MD. The event included a plenary session on obesity, 18 concurrent symposia sessions, poster sessions with more than 500 entries, a Fellows Award for Research Excellence (FARE) program and award ceremony, a symposium and career fair for postdocs, and special exhibits spread over the three days.

Representing NIEHS on the program were three senior investigators presenting at a symposia on "Genetic Susceptibility — The Link between Environmental Exposure and Human Disease," chaired by NTP Acting Chief and Staff Scientist in the Host Susceptibility Branch Jef French, Ph.D. Seven NIEHS postdoctoral fellows also made the trek to Bethesda, six of them to participate in the poster competition (see text box). An additional postdoctoral fellow did not attend the festival although his research was judged as part of the poster presentation.

In addition to serving as chair for the symposia, French made a presentation on analyzing DNA strand break repair and susceptibility to tumor suppressor gene loss associated with loss of heterozygosity in response to human carcinogen exposure. He was joined by NIEHS Senior Investigator and Chief of the Laboratory of Respiratory Biology Steve Kleeberger, Ph.D., and NTP Toxicology Branch Staff Scientist June Dunnick, Ph.D., who also spoke at the session.

In his presentation, Kleeberger described his work in identifying the transcription factor NRF2 as a critical determinant of susceptibility to hyperoxic lung injury. Dunnick's presentation explored how environmental factors may contribute to cardiac disease and how the NIEHS plans to use mouse models to identify highly penetrant allelic variants of genes that modify or influence cardiotoxicity in order to determine orthologous human genes. Two National Cancer Institute investigators, Kent Hunter, Ph.D. and Karlyne Reilly, Ph.D., also presented at the symposium.

One of the young scientists from NIEHS, Visiting Fellow Wataru Nakai, Ph.D., of the Chromosome Stability Group headed by Principal Investigator Mike Resnick, Ph.D., won a FARE Award for his research. His submission, "Transition of a Double-Strand Break to a Chromosome Break is Efficiently Prevented by RMX, Exonuclease I and MCD1," was part of the Genetics/Genomics Poster Session on October 15.

NIEHS Postdoctoral Fellow Jennifer Adair served as a member of the FARE Committee for the Research Festival.

NIEHS Postdocs at the NIH Research Festival

The annual FARE competition, now in its 14th year, selects the top twenty-five percent of abstracts from fifty different study sections to receive a $1,000 travel award. In addition to Visiting Fellow Wataru Nakai, Ph.D., six other NIEHS fellows presented their work in the competition:

Scott Auerbach, Ph.D., in the Cancer session

Yin Li, Ph.D. in the Cell Biology session

Yang Cao, Ph.D., in the Epidemiology session

Xueqian (Shirley) Wang, Ph.D., in the Imaging session

Jianxin Shen, Ph.D., in the Neurobiology and Behavior session

Saurabh Chatterjee, Ph.D., in the Oxidative Stress session (in absentia)

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NTP Publications July-September 2008

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