Department Of Health And Human Services
National Institutes Of Health
National Institute of Environmental Health Sciences

Minutes of The National Advisory Environmental Health Sciences Council May 19, 2003

The National Advisory Environmental Health Sciences Council was convened for its one hundred ninth regular meeting on May 19, at 8:30 a.m., in Rodbell Auditorium, Building 101, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. The meeting was open to the public from 8:30 a.m. until 2:15 p.m. The meeting was closed for consideration of grant applications on May 19, 2:15 pm to 4:15 pm. Dr. Kenneth Olden presided as Chair on May 19, 2003.

Members Present:

Deborah Brooks
Joan Cranmer, Ph.D.
Dale Eastman
Deeohn Ferris, J.D.
Bernard Goldstein, M.D.
George Gray, Ph.D.
Frederick P. Guengerich, Ph.D.
Daniel W. Nebert, M.D.
Peggy Shepard
Frank Talamantes, Ph.D.
Peter Thorne, Ph.D h

Eric L. Stephens

Liaison Members Present:

Drue Barrett, Ph.D. - CDC
Elizabeth Ward, Ph.D. - ACS

Members of the Public Present:

Not Applicable

NIEHS Staff:

Kathy Ahlmark
Janice B. Allen, Ph.D.
Beth Anderson
Lisa Archer
Martha Barnes
Linda Bass, Ph.D.
Sharon Beard
David Brown
Gwen Collman, Ph.D.
Allen Dearry, Ph.D.
Dwight Dolby
Dorothy Duke
Sally Eckert-Tilotta, Ph.D.
Janet Guthrie
Kimberly Gray, Ph.D.
Jerry Heindel, Ph.D.
Mike Humble, Ph.D.
Ethel Jackson, D.D.S.
Laurie Johnson
Annette Kirshner, Ph.D.
Dennis Lang, Ph.D.
Cindy Lawler, Ph.D.
Charle League
Edith Lee
Francine Little
Elizabeth Maul, Ph.D.
Carolyn Mason
Patrick Mastin, Ph.D.
Michael McClure, Ph.D.

Liam O'Fallon
Ted Outwater
Michelle Owens
Joan Packenham, Ph.D.
Jerry Phelps
Warren Pope
Chris Portier, Ph.D.
Larry Reed
Les Reinlib, Ph.D.
Susan Ricci
Margarita Roque
Anne P. Sassaman, Ph.D.
Carol Shreffler, Ph.D.
Shobha Srinivasan, Ph.D.
William Suk, Ph.D., M.P.H.
Ray Tennant, Ph.D.
Claudia Thompson, Ph.D.
Fred Tyson, Ph.D.
Bennett Van Houten, Ph.D.
Charles Wells, Ph.D.
Brenda Weis, Ph.D.
Samuel Wilson, M.D.
Carolyn Winters
Leroy Worth, Ph.D.

Other Federal Staff:

Peggy Jones - FDA
Caroline Dean - FDA
Henry Startzman - FDA
Rass M. Shayiq, Ph.D. - CSR, NIH

I. CALL TO ORDER AND OPENING REMARKS

The one hundred ninth regular meeting of the National Advisory Environmental Health Sciences Council was called to order by Dr. Olden. Dr. Olden commented on the retreat and the importance for future directions of the Institute. He discussed how topics were solicited and how three topics were chosen. Dr. Olden also requested from the Council names of individuals for the advisory committees, individuals capable of both objectivity and quality science. Dr. Sassaman made note of Dr. Olden’s Honorary Doctorate that he received on May 18th from the University of Rochester.

II. REVIEW OF CONFIDENTIALITY AND CONFLICT OF INTEREST PROCEDURES - Dr. Kenneth Olden

Dr. Olden read the requirements of the Government in the Sunshine Act. All aspects of the meeting were open to the public except those concerned with review, discussion and evaluation of grant applications and related information. The Chairperson explained policies and procedures regarding confidentiality and avoidance of conflict of interest situations.

III. CONSIDERATION OF MINUTES OF February 10-11, 2003, MEETING

Council accepted the minutes without change.

FUTURE COUNCIL MEETING DATES

September 15-16, 2003 NIEHS
February 23-24, 2004 NIEHS
May 17-19, 2004 NIEHS with Leadership retreat

IV. REPORT OF THE DIRECTOR, NIEHS - Dr. Kenneth Olden

Dr. Olden began his report by commenting on the House and Senate Appropriation Hearings and the fact that they were uneventful. The committees are pleased with NIH and Dr. Zerhouni's leadership. There was some discussion of tripling the NIH budget. The tripling of the budget is not reflected in the President's budget, but will likely be favorably considered in Congress.

Dr. Olden noted recent hearings by Congressman Bohlert (Science Committee) on endocrine disruptors. There is pending legislation giving NIEHS major programs in this area which is supported by the World Wildlife Fund. Also included is a provision to create Women's Health Centers through NIEHS.

Dr. Olden noted a meeting with the Center for Disease Control and Dr. Jose Corderro, National Center for Birth Defects and Developmental Disabilities, attended also by Dr. Samuel Wilson and Dr. Allen Dearry, to discuss areas for collaboration. They found common interests in chronic diseases and will follow up on that. Two additional areas of possible collaboration with the National Center for Environmental Health (NCEH) are genomic approaches to exposure assessment - what does it mean to find chemicals in human populations-- and the built environment and obesity in children.

Dr. Olden has contacted Dr. Allen Speigel, Director, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), regarding NIEHS interests in obesity and is looking for collaboration. This area is clearly an interagency activity, with other institutes as well as the Department of Housing and Urban Development and Department of Transportation. Dr. Olden noted a recent meeting on the health effects of nanoparticles in New Orleans. Nanotechnology is beginning to flourish, but we need to know the potential health effects, especially toxicity. There needs to be research and Dr. Olden is interested in convening a meeting with the Food and Drug

Administration and the National Institute for Standards and Testing to discuss. There are new types of exposures and health issues appear to be related primarily to size.

Dr. Olden commented on two recent meetings sponsored by NIEHS:

- The Children's Health Conference in February at NIH was a big success. Former Assistant Secretary of Health, Philip Lee gave the keynote address. There was good press at this meeting.
- Another successful meeting to announce completion of Phase 1 of the Environmental Genome Project and held in conjunction with celebration of double helix also generated good press coverage and follow-up.

There have also been two Town Meetings:

- Syracuse with American Lung Association (ALA). There was good partnership, and interest sparked by the paper of Thurston, et al. related to lung cancer. There will be a second one in California co-sponsored with ALA.
- Miami, Dr. Donna Shalala, former DHHS Secretary, now president of University of Miami participated. There was also a "game show" the following day with high school students and local researchers in which Dr. Olden participated.

Dr. Olden had three papers of note:

1. The first showed that asthma rates in Harlem may be as high as 25%. The study involved actual examination of children. The investigator expects some follow up led by Senator Hilary Clinton. This study was not directly funded by NIEHS, but used NIEHS- funded infrastructure.
2. A paper in the New England Journal of Medicine (NEJM) looking at effects of low level 5 lead in blood concluded that blood lead levels of less than 10 micrograms/dl blood, were associated with decrements in IQ. The slope is not linear at higher blood lead levels.
3. A study of children and air pollution from nearby traffic and expressways found significant health effects.

Dr. Olden noted other NIEHS initiatives:

- NIH has decided to obtain the transcriptome of 50 organs of the mouse. NIEHS will contract with NIH to get this transcriptome and maybe others within the next two years. Three million dollars has been set aside for this.
- We will also invest in polymorphism effects related to haplotypes, working to get mouse haplotypes in several strains. These would be resources for the entire scientific community, giving NIEHS visibility for leadership.

Finally, Dr. Olden announced that Dr. Allen Dearry has relocated to Office of the Director, NIEHS to coordinate translation efforts. Some areas of development will be methods of communication and outreach, and integration across the Institute. He will also be charged with

improving the planning and evaluation process. Input and help from the Council will be needed for these efforts.

Council member Dr. Goldstein asked if there would be Environmental Protection Agency sessions on the elderly and the environment and if the NIEHS had a interest in this. Dr. Olden responded that the EPA, NIEHS, NIA will participate at some level and that this is under discussion at this time. There is a concern that EPA is switching emphasis from children to aging.

V. "Evaluation of the Scientific and Public Health Impact of NIEHS supported Research" - Dr. Ben Van Houten - See Attachment B

Dr. Van Houten has been compiling Public Health Impact Stories. He presented a handout with examples of the Public Health Impact Stories to the Council and members attending the meeting.

NIEHS-supported research covers a broad array of environmental health topics and forms a continuum from basic to applied translational research. Dr. Van Houten described the two tools to help evaluation of more than 1600 publications per year from the NIEHS portfolio.

Omniviz is a data mining tool that tranforms mounds of raw text into clusters of like topics, allowing the user a visual overview of the dataset. By using various vocabulary and synonym features, the data can be refined into topic and or time specific clusters for use in conducting publication and portfolio analyses. SPIRES , the Scientific Publication Information and Evaluation System, links information from the grants database (IMPACII) to the publications database (NLM), providing a means to track the publications that are produced as a direct result of a grant or grants. It also provides the ability to view publications by various other factors, including year or budget.

Dr. Shepard asked how this would measure public health impact. Dr. Gray pointed out that it is important to show that some agents may not be harmful and commented on determining what is and is not important. He pointed to the work of Dr. Clarkson on the effects of methyl mercury, which has been underway for nine years and shows no negative impact on children’s neurocognitive development at very low levels.

Ms. Brooks commented on the increased visibility in press if this type of evaluation is done in terms of the near term impacts on policy.

Dr. Goldstein pointed out that this is not an evaluation of public health impact, but is an evaluation of the science. He encouraged the inclusion of evaluation approaches in schools of public health to assist in a retrospective analysis of the role of NIEHS-supported science on public health.

VI. "Metabolic Profiling: Application to Toxicology and Risk Reduction, An International Conference" - Dr. Brenda Weis - See Attachment C

Dr. Brenda Weis discussed the objectives of the Metabolic Profiling Conference May 14-15, 2003 which were the following:

- Define the state of the science: technology, computational (bioinformatics) apprroaches, applications in health science
- Provide a forum for information exchange and partnering amongst academia, industry and government: approximately. 200 participants (and NIH webcast); 36% academia, 41% government, 23% private sector
- Define parameters for developing a research initiative in environmental health science.

Metabolic profiling involves measuring and interpreting complex, time-related metabolic changes in biofluids, cells or tissues that reflect the global physiological response to biological systems to genetic or environmental factors. Computational and bioinformatics tools are needed to maximize information recovery about biological mechanisms and pathways of toxicity and disease.

At the conference one of the speakers used the following to describe the new "omics": genomics represent "words," proteomoics, "grammar," and metabonomics, "the story."

Metabolic profiling holds the promise for improving health. Exposure assessment is done in dose, time, age and genetic polymorphism. Markers of toxicity and disease chart the status and progression from exposure. Models of human diseases will be developed and improve in the extrapolation from animals to humans. Metabonomics will help in the study of biological pathways and regulatory networks mediating response, developing clinical diagnostics and personalized risk assessment, treatment and prevention.

VII. Concept Clearance: Metabolic Profiling: Applications to Toxicology and Risk Reduction - Dr. Brenda Weis - See Attachment D

Dr. Brenda Weis continued with her concept clearance for Metabolic Profiling. The metabolic profiling perspective for the NIEHS started in 2000 with the DERT Science Retreat; 2002 NIEHS Congressional Testimony; and May this year with the International Conference. This initiative will be a multi-phased strategy focused on the development and application of metabolic profiling technologies, including data bases and bioinformatics tools, in basic and applied environmental health research.

There would be specific research foci in this initiative involving:

- Identification of dose, time-dependent markers of exposure, toxicity, disease status and progression in animals and humans;
- development and testing of animal models of human disease processes with emphasis on biological mechanisms, time course and genetic susceptibility;
- development and applications of bioinformatics, computational and modeling approaches to define metabolic and systems level responses to environmental stressors.

The initial phase of this initiative is projected to begin in 2004.

This concept clearance received unanimous approval from the Council.

VIII. Concept Clearance: The Role of Environmental Agents in Cardiovascular Disease - Dr. Pat Mastin - See Attachment E

Dr. Mastin began his presentation with some facts about cardiovascular disease (CVD). In 1999 over 950,000 people died of CVD. This number represents a death rate of 350 per 100,000 people and accounts for 40% of all deaths. The major risk factors for CVD include, race, age, lifestyle and family history. There have now been clear link between CVD and environmental agent exposure(s) but the mechanisms by which these agents cause or contribute to disease have not been well characterized.

There have been associations between CVD and inhaled particulate matter (PM) or other air pollutants, such as carbon monoxide and ozone. The current NIEHS portfolio, as reported by Dr. Mastin crosses many mechanisms but is rather limited.

This concept clearance received unanimous approval from the Council.

IX. Report of the Director, DERT - Dr. Anne P. Sassaman - See Attachment F

Dr. Sassaman began her report by introducing new staff and advising the Council that the reorganization of the Division is now final. The new deputy director, Dr. Dennis Lang will be getting involved in the Division's activities and taking on special assignments as he becomes familiar with its programs. She also highlighted some of the program developments and pointed out the new section in Environmental Health Perspectives where some of these are summarized each month.

She then reported to the Council on some of the administrative changes occurring at the NIH level, in particular the outsourcing of "non-inherently governmental" functions and consolidation of administrative functions. The FY2003 outsourcing involves functions associated with grant support staff, so there will likely be changes in procedures and operations within the Division.

A concept clearance for the FY2004 Small Business Innovation Research topics was presented with comments from Council reviewers. Council approved the concept with advice to staff on some of the topics. The document is included in the appendix.

Dr. Sassaman concluded her report with a presentation on the NIEHS's activities related to studies in and with Vietnam on the health and environmental effects of Agent Orange/dioxin. She briefed the Council on the history of these interactions, beginning in 1995, and focused on a Memorandum of Understanding signed at the conclusion of a jointly-sponsored international conference in Hanoi in March 2002. The Institute continues its efforts to implement the MOU and to encourage joint research and to build the research infrastructure in Vietnam. However, these efforts have been stalled due to lack of responsiveness on the part of the Vietnamese Government.

X. Report of the Director, DIR - Dr. Lutz Birnbaumer - See Attachment G

Dr. Birnbaumer began his report by saying that DIR was in good shape and had good strong programs. He updated the Council on recent hires and ongoing recruitments. Dr. Birnbaumer talked about some problems in renovating labs for new hires, which may necessitate a delay in some of the recruitments. NIEHS has also made a presentation to the NIH for funds to construct an additional module. We have been asked to prepare a business plan, and will do so in hopes of having this be a high priority for NIH budget planning.

CLOSED PORTION OF THE MEETING

This portion of the meeting was closed to the public in accordance with the determination that it was concerned with matters exempt from mandatory disclosure under Sections 552b(c)(4) and 552b(c)(6), Title 5, U.S. Code and Section 10(d) of the Federal Advisory Committee Act, as amended (5 U.S.C. Appendix 2).

There was a discussion of procedures and policies regarding voting and confidentiality of application materials, committee discussions and recommendations. Members absented themselves from the meeting during discussion of and voting on applications from their own institutions, or other applications in which there was a potential conflict of interest, real or apparent. Members were asked to sign a statement to this effect.

XIII. REVIEW OF APPLICATIONS

The Council considered 295 applications requesting $95,773,045 in total cost. The Council recommended 170 applications with the total cost of $58,201,645.

XIV. ADJOURNMENT OF THE NAEHS COUNCIL

The meeting was adjourned at 4:15 pm on May 19, 2003.

ATTACHMENTS:

To view these documents you will need to have Adobe Reader. You may download a copy at *Adobe Reader*.

A. Council Roster
B. Evaluation of the Scientific Health Impact of NIEHS Supported Research - Dr. Ben Van Houten; *Adobe Reader* Format
C. Metabolic Profiling - Dr. Brenda Weis; *Adobe Reader* Format
D. Concept Clearance - Dr. Brenda Weis; *Adobe Reader* Format
E. Concept Clearance - Dr. Pat Mastin; *Adobe Reader* Format
F. Report of Director, DERT - Dr. Sassaman; *Adobe Reader* Format
G. Report of Director, DIR - Dr. Birnbaum; *Adobe Reader* Format

Evaluation of the Scientific and Public Health Impact of NIEHS Supported Research

Ben Van Houten, Chief, Program Analysis Branch, Division of Extramural Research and Training, NIEHS.

NIEHS supported research covers a broad array of environmental health topics and forms a continuum from basic to applied translational research. A large segment of this research results in publications in peer-reviewed journals. The Program Analysis Branch has developed a tracking system that can now be used to systematically analyze the content, number, types, and scientific fields of journal articles produced by our portfolio of grants. This tracking system is being used to measure the scientific impact of the work supported by NIEHS. In addition, as part of a communication effort, each month PAB searches scientific literature databases and selects four of the 100-125 monthly NIEHS-supported publications to highlight in a feature called ***DERT Papers of the Month***. These publications are encapsulated into lay summaries describing the overall importance of this work and the potential public health impact. Over time NIEHS-supported publications lead to key advances in medicine, regulatory decision-making, and improved public health. Dr. Kenneth Olden, Director NIEHS, asked the Program Analysis Branch (PAB) to develop a series of short, one to two page descriptions of the impact NIEHS-sponsored research has had on public health. These ***Stories of Public Health Impact*** provide a historical perspective of NIEHS research and highlight how advances in basic research can lead to improved health care, new standards for environmental exposure, and a reduction in disease. PAB developed an example of such a write-up (Dramatic Decline in Lead Poisoning) and asked the Program Administrators in DERT for ideas of additional topics. Stories were developed by program staff and submitted to PAB. Drafts were edited a number of times and have all been reviewed by the investigator(s) whose work is being highlighted. Nine stories ranging from aflatoxin and liver cancer to particulate matter and cardiovascular disease have been completed; several more topics are under development.

Metabolic Profiling: Application to Toxicology and Risk Reduction
An International Conference

Dr. John Connelly
Director of Biomedical Sciences
Metabometrix Ltd.

The NIEHS hosted an international conference on *Metabolic Profiling:Application to Toxicology and Risk Reduction* on May 14-15, 2003. Meeting sponsors included the NIEHS and Office of Rare Disease of the NIH; Food and Drug Administration; Paradigm Genetics and Waters Corporation. The conference was designed to define the state of the science for the emerging technology of metabolic profiling, also called *metabonomics* or *metabolomics*, and its application in basic and applied health research. Three scientific sessions focused on the application of metabolic profiling to *toxicology*, *risk reduction*, and *computational and systems biology*. The conference was instrumental in identifying future directions for technology and research in metabolic profiling to significantly advance exposure assessment, predictive and mechanistic toxicology and clinical medicine.

The concept of metabolic profiling is not a new. It is essentially “biochemistry grown up” or what biochemistry would have been had methods now available been around in the 1930s when the components and dynamics of the Krebs cycle were being worked out. Previously, one at a time measurements, isolated tissue preparations and interspecies extrapolation often founded on hope, were successfully used to describe basal intermediary metabolism, but did not provide the flexibility and sensitivity to gain a whole-organism look at biological responses. A variety of approaches currently being used for metabolic profiling of biosamples (blood, urine, tissue) will be discussed. These approaches build on basic science technologies such nuclear magnetic resonance (NMR) spectroscopy, liquid and gas chromatography, tandem mass spectroscopy (MS/MS), and time-of-flight mass spectroscopy (MS-TOF).

Industry has played a leadership role in advancing metabolic profiling technologies as a means of streamlining and accelerating chemical toxicity testing and drug discovery and development. Thus, current applications focus largely on the identification and validation of predictive markers of liver, renal, cardiovascular and neurodegenerative toxicity and disease in animals and humans exposed to a variety of chemicals, drugs and nutritional agents. Currently with technology development, new computational, database and visualization tools are being developed to facilitate data mining, pattern recognition and information recovery from the vast amount of metabolic profiling data generated using high throughput approaches. Several example applications with relevance to environmental health science research will be discussed.

Metabonomics represents a new approach for environmental science research but realization of this potential will require new initiatives that focus on technology development and application to environmentally relevant exposures and disease. The conference provided a needed forum for information exchange and scientific partnering amongst academic, industry and government scientists, engineers, statisticians and information technologists. An emerging area of shared interest is

in systems biology: the integration of “omics” data in order to define and model dynamic, systems level responses to exogenous agents (chemicals, drugs, and nutritional factors). Systems biology approaches have been successfully used to model functional pathways and metabolic regulation in simple biological systems such as yeast. An important challenge in this post-genomic era will be the development of comparable model approaches for complex mammalian systems. This is critical for placing “omics” information in a real world disease context which links genetic composition and functional genomics with risk of prognosis for future disease.

Several collaborative efforts are underway to stimulate technology development, collaborative research, and communication about metabolic profiling programs on a global scale. These include the Consortium for Metabonomics in Toxicology (COMET), the European Nutrigenomics Organization (NuGO) and the NIEHS National Center for Toxicogenomics (NCT). Each of these forums will merge research and technology interests in metabolic profiling and systems biology and serve to advance the field in a positive direction in this post-genomic era.

NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES
Division of Extramural Research and Training
Center for Risk and Integrated Sciences

NATIONAL ADVISORY ENVIRONMENTAL HEALTH SCIENCES COUNCIL
May 19-20, 2003
Concept Clearance
for
Metabolic Profiling: Application to Toxicology and Risk Reduction

Introduction

Metabolites are the end products of cellular processes and their levels are reflective of the systems level response of biological systems. Metabolic profiling, also called *metabonomics* or *metabolomics*, is a high throughput approach to measuring and interpreting complex, time-related metabolic parameters in biosamples such as urine, blood, cells and tissues. This initiative proposes a new approach for environmental health research using metabolic profiling for identifying and validating predictive markers of environmental exposure, toxicity and disease and for understanding the time course of toxicological response in relation to genetic susceptibility, age and environmental exposures.

Background

There have been remarkable advances in the development of high throughput technologies for metabolic profiling. Technologies built on nuclear magnetic resonance (NMR) spectroscopy, mass spectroscopy (MS) and chemical separation have made it possible to produce biochemical fingerprints of metabolites from complex biological mixtures such as biofluids and tissue extracts. Changes in fingerprint profiles can be used to characterize the effects of toxic insult or genetic manipulation in *in vivo* systems. Metabolic spectra are information-rich and computational tools, pattern recognition methods, and expert modeling systems are developing concurrently with technology advancements to maximize information recovery (1,2).

Metabolic profiling has widespread application in basic and applied research because it is nonselective and requires little sample preparation or derivitization. Many of the technologies for metabolic profiling have been developed by industry for chemical toxicity screening, drug discovery and genetic linkage studies. Metabolic profiling of urine or blood has been used to identify dose- and time-dependent biochemical profiles, site of toxic action, mechanism of toxicity, onset, duration and dose-response relationships in animals exposed to a variety of kidney and liver toxins including hydrazine, cadmium, mercury, and acetaminophen (3-9). In these studies, metabolic profiles of response were related to toxicity as determined using histopathology and clinical chemistry. The technology is highly sensitive because characteristic profiles of response can be defined for individual strains of animals, polymorphic individuals, and individuals exposed to chemical doses that did not induce toxicity based on histopathology or clinical chemistry (2, 6, 10). This is a great improvement over histopathology as a means of toxicological evaluation because once the metabolic pattern recognition spectra have been generated based on histopathology and clinical chemistry future studies require only collection of

urine from the experimental animals. Thus, studies following the time course and progression of toxicological events can be pursued without the need for sacrificing animals at each time point (6).

In the clinical arena, metabolic profiling has proven to be a valuable tool for diagnosing the severity and pathological condition of cardiovascular, renal and neurodegenerative diseases in animals and humans (10-14). The technique has also been used to produce metabolic markers for disease progression and identify metabolic pathways that are perturbed in dystrophic tissue from the *mdx* mouse model of Duchenne muscular dystrophy (10). Thus, metabolic profiling may be of great biological significance in studying genetic differences, including those due to specific intervention such as genetic knock out models. The technology may also be useful for validating animal models of human disease processes and defining predictive profiles for the time course of toxicological response. The search for clinical biomarkers of target organ toxicity and progression of toxicity in humans is often hindered by variation in factors such as genetic composition, diet, age and idiosyncratic responses to drugs. Metabolic profiling offers a nonselective approach for determining target organ toxicity and classifying effects of multiple organ toxicities based on readily obtainable samples. The technique provides a framework for reducing risk through the development of targeted prevention strategies.

The NIEHS has significant interest in assessing the usefulness of metabolic profiling as a tool for basic environmental science research. Congressional testimony on behalf of the NIEHS highlighted the importance of this emerging technology for toxicogenomics and systems biology research. Metabolic profiling was also featured at the Division of Extramural Research and Training Science Retreat in 2000 as a tool for advancing basic research aimed at elucidating mechanisms of action and surrogate markers of environmental exposure and disease. An international conference hosted by the NIEHS on *Metabolic Profiling: Application to Toxicology and Risk Reduction* on May 14-15, 2003 defined the state of the science for metabolic profiling and highlighted recent studies where metabolic profiling has been used to identify predictive markers of exposure, toxicity and disease in experimental animals and humans. Several speakers emphasized the promise of metabolic profiling for personalizing the assessment of environmental exposure and disease risk and elucidating systems responses to environmental agents. Understanding and quantitating the effect of environmental agents on the metabolome provides fingerprints of past and current exposure to environmental agents and early markers of impending toxicity or disease. The definition of such markers in animals, and eventually in humans, will improve the definition of exposure, toxicity and disease and may help identify genetic polymorphisms that define more susceptible individuals or populations.

Research Goals and Scope

This is a multi-phased initiative focused on the development and application of metabolic profiling technologies in basic and applied environmental science research.

Specific research foci include:

?? Studies to identify dose- and time-dependent biomarkers (predictive profiles) of exposure, toxicity and disease status and progression in animal models, including genetic

knock out models, and in specific human populations for which archived biosamples are available;

?? Studies to develop and validate animal models of human disease processes with emphasis on biological mechanisms and pathways, time course and genetic susceptibility in animal models and humans;

?? Development and application of bioinformatics, computational and modeling approaches for analysis of metabolic profiling data and its integration into models of systems levels responses to environmental exposures.

The initial phase of the initiative, to begin in 2004, will focus on identifying and validating predictive markers of exposure, toxicity and disease in order to establish proof of concept for metabolic profiling as a technology to further the understanding of risk and disease processes resulting from environmental exposures. One approach for implementing this phase of the initiative will be enhancing capacity at academic institutions which have demonstrated capabilities in chemical separation and identification technologies, such as liquid chromatography, gas chromatography, MS and NMR, which are amenable to metabolic profiling. Enhancing capabilities within these research institutions builds on existing expertise and instrumentation applicable to metabolic profiling and fosters research that is complimentary to existing programs in toxicology, genetics, toxicogenomics and proteomics. This phase of the initiative may also be coordinated with intramural efforts of the National Toxicology Program to identify predictive profiles of toxicity and disease using archived biological samples and information on dose- and time-dependent pathology and disease for environmental chemicals and drugs. A critical aspect of the initial and future phases of this initiative is the development and mining of metabolic profiling databases using novel bioinformatics, computational and modeling approaches.

While the focus of this initiative is on identifying predictive markers in animal models, retrospective studies using archived human biosamples are also of interest. Ideally, predictive markers could serve as indicators of early response to exposure and toxicity that can lead to development of appropriate prevention and intervention strategies that can be tested in these models. Future initiatives will focus on the development and validation of computational models of systems level responses to environmental agents and on establishing predictive profiles for human diseases processes and genetic susceptibility in clinical populations.

Ultimately, this initiative will foster the introduction of novel scientific ideas, methods, model systems, tools and technologies that have the potential to substantially advance basic and applied research in metabolic profiling and integrated systems sciences. This research initiative takes advantage of state-of-the-art technologies that can used to develop predictive markers of exposure, toxicity and disease in established animal models and to better understand mechanisms of toxicity and disease. Under this initiative, productive collaborations between academic institutions and private sector groups are encouraged as a means of promoting technology transfer and capacity building within academic institutions.

This initiative is timely as the new technologies of metabolic profiling complement existing and potential future NIEHS initiatives in genetic polymorphisms, toxicogenomics, proteomics and systems biology. Utilizing metabolic profiling techniques may, for the first time, offer real-time

evaluation of exposures and provide predictive markers for the time course and progression from exposure to disease. The data generated by this program may also be important for deciphering gene-environment interactions in disease and understanding the role of polymorphisms in susceptibility to disease.

References

1. Holmes, E and Antti, H. 2002. Chemometric contributions to the evolution of metabonomics: mathematical solutions to characterizing and interpreting complex biological nmr spectra. *Analyst*, Dec 127(12): 1549-57.
2. Gavanagh CL, Holmes, E, Lenz E, et al. 2000. An NMR-based netabonomics approach to investigate the biochemical consequences of genetic strain differences: application to the c57blj10J and alpk:apfcd mouse. *FEBS Letters* (2002) 484: 169-174.
3. Nicholson, JK, Lindon, JC and Holmes, E. 1999. Metabonomics: understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological nmr spectroscopic data. *Xenobiotica* 11: 1181-1189.
4. Robertson, DG, Reily, MD, Sigler, RE, et al. 2000. Metabonomics: evaluation of nuclear magnetic resonance (nmr) and pattern recognition technology for rapid in vivo screening of liver and kidney toxicants. *Toxicol. Sci.* 57: 326-337.
5. Holmes, E, Nicholls, AW, Lindon, JC, et al. 2000. Chemometric models for toxicity classification based on nmr spectra of biofluids. *Chem. Res. Toxicol.* 13: 471-478.
6. Holmes E, Nicholson, JK and Tranter, G. 2001. Metabonomic characterization of genetic variations in toxicological and metabolic responses using probabilistic neural networks, *Chem. Res. Toxicol.* 14:182-191.
7. Griffin, JL, Walker, LA, Shore, RF, et al., 2001. Metabolic profiling of chronic cadmium exposure in the rat. *Chem. Res. Toxicol.* 14(10): 1428-1434.
8. Bundy, JG, Lenz, EM, Bailey, NJ, et al. 2002. Metabonomic assessment of toxicity of 4-fluoroaniline, 3,5-difluoroaniline and 2-fluoro-4-methylaniline to the earthworm eisenia veneta (rosa): identification of new endogenous biomarkers. *Environ. Toxicol. Chem.* Sep 21(9): 1966-72.
9. Coen, M, Lenz, EM, Nicholson, JK, et al. 2003. An integrated metabonomics investigation of acetaminophen toxicity in the mouse using nmr spectroscopy. *Chem. Res. Toxicol.* 16: 295-303.
10. Griffin, JL, Williams, HJ, Sang, E, et al. 2001. Metabolic profiling of genetic disorders: a multitissue 1h nuclear magnetic resonance spectroscopic and pattern recognition study into dystrophic tissue. *Anal. Biochem.* 293: 16-21.
11. Stockcor, JP and Holmes, E. 2002. Metabonomic applications in toxicity screening and disease diagnosis. *Curr. Top. Med. Chem.* Jan 2(1): 35-51.
12. Brindle, JT, Antti, H, Holmes, E, et al. 2002. Rapid and noninvasive diagnosis of the presence and severity of coronary heart disease using 1h-nmr-based metabonomics. Nature Medicine 8(12): 1439-1444.
13. Brindle, JT, Nicholson, JK, Schofield, PM, et al. 2003. Application of chemometrics to 1h nmr spectroscopic data to investigate a relationship between human serum metabolic profiles and hypertension. *Analyst*, Jan 128(1): 32-6.
14. Mitchell, S, Holmes, E and Carmichael, P. 2002. Metabonic and medicine: the biochemical oracle. *Biologist* (London) 49(5): 217-21.

NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES
Division of Extramural Research and Training
Office of Program Development
Organs and Systems Toxicology Branch

NATIONAL ADVISORY ENVIRONMENTAL HEALTH SCIENCES COUNCIL
May 19, 2003

CONCEPT CLEARANCE
For
The Role of Environmental Agents in Cardiovascular Disease

Introduction

Cardiovascular disease is the primary cause of mortality in the industrialized world. According to the American Heart Association, over 950,000 people died of cardiovascular disease (CVD) in the United States in 1999. This represents a death rate of 350 per 100,000 people and accounts for 40% of all deaths. The major "traditional" risk factors for CVD include, race, age, lifestyle (such as smoking, physical inactivity, serum lipids, and diet) and family history. In addition to these "traditional" risk factors, clear links between CVD and environmental agent exposure(s) have now been established, but the mechanisms by which these agents cause or contribute to disease have not been well characterized.

Associations between CVD and inhaled particulate matter (PM) or other air pollutants, such as carbon monoxide and ozone, have been noted. The association between exposure to ambient particulate matter and CVD has been somewhat surprising but is nonetheless robust. Increases of 0.5 - 2.0% in premature CVD deaths per 10 mg/m^3 of PM have been noted. Epidemiologic studies have also shown associations between exposure to PM and CVD morbidity (e.g., hospital admissions for CVD), as well as between PM and pathophysiologic changes that are associated with CVD, such as changes in heart rate variability (HRV) and blood parameters. PM exposure has also been shown, in experimental animal studies, to increase the severity of atherosclerosis. Polycyclic aromatic hydrocarbons (PAHs), such as benzo(a)pyrene, are components of outdoor air pollution, primarily from combustion of fossil fuels, as well as of indoor air, primarily from environmental tobacco smoke (ETS). PAHs have been shown to alter the redox environment in vascular walls, activating signaling pathways that can lead to proliferation of vascular smooth muscle cells. Similarly, cigarette smoke causes mitochondrial damage, which is associated with increased atherosclerosis. Certain aldehydes on the EPA=s list of "Air Toxics" can present workplace as well as environmental exposure risks. Aldehydes, both exogenous and endogenous, can induce proliferation of vascular smooth muscle cells, one of the hallmarks of atherosclerosis. The mechanisms by which PM toxicity induces the CVD-related pathophysiological changes are not adequately known or understood. It has been suggested they might involve anomalous stimulation of the autonomic nervous

system, production of inflammatory mediators, generation of reactive oxygen species in the lung, and/or direct toxic effects on cardiovascular tissues.

Airway exposures to agents that are associated with CVD are not the only vector of exposure of consequence. It is known that exposure to arsenic (As) through occupational exposures or via drinking water is associated with increased CV morbidity, including a condition known as "blackfoot," a peripheral vascular disease. Arsenic has been shown to cause oxidative stress in the vascular tissues of occupationally exposed workers. This is a condition associated with the development of atherosclerosis. Given that environmental arsenic exposure is a reality in the US as well as in other countries, the contribution of arsenic to CVD prevalence needs further study. In addition, *in vitro* experiments with arsenic hold the promise of further elucidating CVD-related cellular dysfunctions in endothelial and vascular smooth muscle cells.

There are, therefore, many avenues of research that have pointed to an important role for environmental agents in cardiovascular disease. However, a more focused effort to better understand this role in the adult is needed.

On August 6 and 7, 2002, a workshop entitled, "The Role of Environmental Agents in Cardiovascular Disease" was held in Durham, N.C. The workshop was sponsored by the NIEHS, EPA, NHLBI, the American Heart Association Council on Epidemiology and Prevention and Expert Panel on Population and Prevention Science, and St. Jude Medical, Inc. The participants identified numerous questions and research issues, which included:

- What diseases of the cardiovascular system are associated with exposure to environmental agents?
- What other factors might interact with environmental exposure to increase the risk of CVD?
- By what mechanisms do environmental agents cause or contribute to CVD?
- What factors make individuals more susceptible to the effects of environmental agents?

NIEHS maintains an active program in environmentally related cardiovascular disease. Many of the studies describing the association between PM and CVD were funded by prior activities of the NIEHS, as were the studies on the effects of PAHs and ETS on vascular smooth muscle cells. NIEHS previously released two Program Announcements related to CVD linkages resulting from prenatal exposures: "Environmentally Induced Cardiovascular Malformations" (PA-02-093) and "The Fetal Basis of Adult Disease: Role of the Environment" (PAR-02-105). However, given the importance of CVD as a public health concern, the growing evidence that exposure of adolescents and adults to various ambient environmental agents plays a role in subsequent CVD prevalence, and the recommendations from the above-mentioned workshop, it appears timely and highly warranted to focus additional research efforts on specifically identifying environmental cardiovascular toxicants and elucidating their toxicity mechanisms. Recent, rapid advances in the fields of cellular biology (e.g., cell signaling and signal transduction) and

molecular biology technology, including genomics and proteomics, now greatly increase the likelihood of significant advancements in the field. Multidisciplinary approaches to complex questions like these are generally more successful, some environmental health science and cardiovascular researchers have successfully bridged the gap between multiple disciplines to develop innovative approaches to the study of environmentally induced CVD. Therefore, enhanced multidisciplinary research collaboration is now seen as vital to the success of contemporary research efforts.

Research Goals and Scope

The purpose of this initiative is to support innovative, multidisciplinary research to identify environmental agents that cause or exacerbate cardiovascular disease, to elucidate mechanisms of cardiovascular toxicity by these agents, and to identify susceptibility factors, such as genetics and pre-existing disease. The major focus of the initiative will be the identification and evaluation of the mechanisms of environmentally induced adult CVD. The initiative is also designed to expand the number of researchers working in the area of environmentally related cardiovascular disease, by encouraging cardiovascular researchers to apply the newest tools and models to the problem of environmentally related CVD. Collaborative, multidisciplinary approaches are seen as the best way to address gaps in knowledge in this area. Cardiovascular researchers have developed models and technologies that could prove invaluable in the study of the cardiovascular effects of environmental agents. Therefore, collaborations between environmental health science researchers and cardiovascular researchers or cardiologists would be required for the R01 grant applications responsive to this initiative.

The initiative will encourage basic (*in vitro and in vivo*) research, as well as controlled human exposure studies and small clinical studies (but not large-scale population-based or epidemiological studies) using state-of-the-art technologies, such as proteomics/genomics/systems biology and the use of transgenic and gene-targeted mutant animal models. As it is clear that certain populations and individuals are more susceptible to the effects of certain environmental agents, studies using animal and /or human exposure models to consider the role of such factors as genetic predisposition, race, age, gender (including the influence of hormones), pre-existing disease (e.g., lung disease, or diabetes), diet, socioeconomic status, and obesity will be encouraged.

Proposals will be expected to directly address the role of environmental agents in specific cardiovascular diseases or disease processes, such as atherosclerosis, cardiac hypertrophy, heart failure, sudden cardiac death, stroke, arrhythmias, hypertension, and cardiomyopathy. Examples of environmental agents of interest include air pollutants (such as particulate matter or gases like carbon monoxide or ozone), metals, aldehydes, PAHs, and xenoestrogens (e.g., bisphenol). If the agent is a complex mixture, such as PM, efforts to characterize the agent and identify the important components will be encouraged. Examples of potential mechanisms might include changes in cell signaling (including apoptosis) and signal transduction, inflammatory processes (e.g., inflammatory

mediators, cytokines, adhesion molecules), changes in gene expression, oxidative stress (e.g., the roles of reactive oxygen and nitrogen species, enzymes such as NAD(P)H oxidase and the cyclooxygenases, and antioxidants), electrophysiological changes (such as heart rate variability), and changes in blood components. Proposals would be encouraged be to study organs and tissues from all levels of the cardiovascular system, including cardiac (e.g., myocardium and the conducting system) and vascular tissue, including the large vessels, coronary arteries, peripheral vasculature, microcirculation, various vascular beds (e.g., kidney glomeruli), and blood (e.g., leukocytes and plasma proteins and lipids). Within these tissues, cellular functions of specific interest would include endothelial cell functions, ion channel function and contractility in cardiomyocytes and vascular smooth muscle cells (VSMCs), and leukocyte function as it relates to CVD (e.g., macrophages in atherosclerotic plaques).

It is anticipated that this initiative will involve collaboration with the NHLBI and/or the Environmental Protection Agency.

FEATURED ACTIVITIES of DERT
May 2003

MEETINGS

Symposium on Children's Environmental Health: Identifying and Preventing Environmental Risks
February 24-26, 2003
Natcher Conference Center, NIH Campus, Bethesda, Maryland

For three days, scientists, community members, members of advocacy groups and representatives of the media came together to discuss what is known about environmental health threats to children, how well we are translating important scientific findings to the public and how we can share our information more effectively with the press and media. The symposium, which included 250 attendees and over 40 speakers, was divided into five key topic areas: respiratory disease and air quality, neurological impairments, childhood cancer, birth defects and endocrine disruption. There were also special sessions on obesity and nutrition, built environment, autism, fetal origins of adult disease and international perspectives.

In each session, lead speakers from laboratories, organizations that care deeply about the health of children, and from policy arenas summarized the state of the science, shared success stories from outreach and translational programs that work, and defined the gaps to fill in order to reduce morbidity and mortality among children exposed to hazardous substances.

The work of the NIEHS supported Children's Environmental Health and Disease Prevention Research Program was highlighted throughout the three days in scientific sessions and with poster presentations. Meeting highlights were published in *the April issue of Environmental Health Perspectives*. A more comprehensive meeting report is being written for future publication.

Meeting Highlights

- Dr. Phillip Lee, former Assistant Secretary of DHSS was the keynote speaker. He spoke about children's vulnerabilities to toxic substances and the impact neurodevelopmental diseases, such as ADHD, have on children and their families in terms of school performance, drop out rate, future drug abuse and risk of suicide. A number of environmental agents could contribute to this disease, and are not well studied. Dr. Lee also talked about manganese, which is an essential element that is added in high levels to infant formula and could be dangerous to infants.

- Other speakers discussed lead and mercury as classic neurotoxicants and how much our understanding about the threats to childhood development have come from understanding the mechanism of these metals.

- The systemic effects of air pollution were discussed, noting that it is not just detrimental to children's respiratory health, but new data indicate the components of air pollution may contribute to pregnancy loss, reduced birth weight, cancer, sudden infant death syndrome and cardiovascular disease in adulthood.

- Risk factors for childhood cancers were discussed and the process of developing new risk assessment cancer guidelines considering adult and childhood risks was also discussed.

- Representatives from organizations such as the Learning Disabilities Association, the Birth Defects Research for Children, Children's Environmental Health Coalition, etc., put forth the public

perspective and discussed actions that these groups are taking to raise awareness for these issues. Attendees joined in the discussion during break out sessions.

- A three-pronged approach to children's environmental health was articulated by Dr. Kenneth Olden, Director of NIEHS: Identify the risk factors, reduce exposure, and translate this information into public health policy and the practice of medicine.

Environmental Factors in Autoimmune Disease
February 4-5, 2003
Durham Marriott at the Civic Center, Durham, North Carolina

Autoimmune diseases are chronic, potentially life-threatening conditions. There are more than 80 recognized autoimmune diseases, which include systemic lupus erythematosus, glomerulonephritis, multiple sclerosis, autoimmune thyroiditis, rheumatoid arthritis, and myositis. Although some of the conditions afflict only small numbers of individuals, as a group autoimmune diseases represent an important public health concern. The common characteristics of these diseases are immune responses directed against normal tissue or cellular components, which are normally protected from immune attack and the resultant inflammatory response. Although genetic susceptibility and exposure to infectious agents have been identified as possible contributors to autoimmune disease and have been extensively studied, these factors cannot account for most cases. This suggests the likelihood of exposure to environmental agents as an etiologic factor, and research has linked environmental agents with autoimmunity. Human studies have shown an association between exposure to vinyl chloride, silica, and organic compounds. Likewise, experimental studies have shown numerous immunologic changes, related to autoimmunity, induced by exposure to metals, polycyclic aromatic hydrocarbons, and mycotoxins. Possible mechanisms for environmentally induced autoimmunity include molecular mimicry, alteration of lymphocyte signaling, and interference in the development of tolerance to "self" antigens. Despite the accumulation of these research data, there are still gaps in knowledge, including how to link results from human and animal studies.

The goals of the workshop were to get input from the environmental health science and autoimmune research communities on the most appropriate and productive directions for research in the area of environmentally related autoimmune disease. The format of the workshop, which included six breakout sessions, was designed to enhance interactions among research scientists that will lead to identification of gaps in knowledge, appropriate questions for future research, innovative uses of existing technology and ideas for new technologies (including animal models), and types of collaborations needed to address these issues.

Meeting Highlights

The workshop was attended by over 100 participants, including basic scientists, epidemiologists, clinicians, and disease advocates. It was in part a grantees' meeting, to allow some of the awardees from the 1999 RFA, "Environment / Infection / Gene Interactions in Autoimmune Disease," to present findings from these studies. There was also a session consisting of six breakout groups focused on the following topics: Gene-Environment Interactions, Altered Antigens, Immune Modulations, Signal Transduction, Translational Research: Systemic Autoimmune Disease, and Translational Research: Organic-Specific Diseases. The two primary outcomes of the meeting were:

- While experimental animal data are strong, many more human studies, epidemiologic and clinical, are needed to link environmental exposure to autoimmune disease.

- Greater efforts are needed to establish collaborations between epidemiologists and clinicians, on the one hand, and basic scientists on the other.

Hopefully, the workshop can generate interest in this field among epidemiologists and encourage the types of collaborations in which data from basic research and human studies can inform further research in the other discipline.

The products of this meeting will include a workshop report to serve as a framework for future program planning and a publication of the workshop highlights.

Human Health Effects of Phthalate Exposure Workshop
March 26-27, 2003
Radisson Hotel, Research Triangle Park, North Carolina

For something so ubiquitous in the environment of most Americans, phthalates are poorly understood. The compounds are used in everything from time-release capsules and children's toys to plastic tubing and pesticides**.**

Recently, researchers learned that exposures to phthalates are much more common in humans than first thought. An analysis of the latest National Health and Nutrition Examination Survey (NHANES) data on body burden by researchers at the Center for Disease Control and Prevention revealed that a majority of Americans are exposed and some at significantly high levels.

In fact, levels of phthalates are generally highest in children and women of reproductive age. These exposure levels create the potential for developmental effects in the fetus and children. For this reason, understanding the health effects of phthalate exposure in humans may be important for protecting populations at risk. Researchers, however, would be just as interested to confidently know that phthalates are harmless to humans.

Compared with many other chemicals in widespread use, the research gaps for phthalates are relatively extensive.

Dr. Kimberly Gray, NIEHS/SPHB, and Dr. Russ Hauser, Harvard School of Public Health, organized and convened the workshop, which was sponsored by the office of the Director, NIEHS, and co-sponsored by Harvard's Environmental Health Center. The multidisciplinary workshop was designed to describe the current state of knowledge on the health effects of phthalates, to identify gaps and deficiencies in that knowledge base, and to identify future directions for exposure assessment, toxicologic and epidemiologic studies.

The conference assembled some of the nation's leading phthalate researchers across disciplines to address many of the issues and focus the future research agenda regarding phthalates.

Meeting Highlights & Recommendations

The workshop was divided in to three sessions: Toxicology & Mechanisms, Exposure Assessment and Epidemiology. Learning objectives and discussion points were prepared for each presentation, and these were used as talking points for the one-hour open discussion that followed each session. The following is an abridged summary list of questions, comments and future recommendations discussed at the workshop.

- Rodent endpoints need to be better harnessed for human studies. It is unclear what animal models indicate for humans. In particular, researchers should look at what cholesterol data might indicate. Short-term problems need to be identified that could be studied now.

- Gaps exist in all models of endpoints. Researchers need endpoints that can be extrapolated to humans. Species differences between reproductive effects and liver effects make extrapolation very difficult. More data on developmental differences in species is desirable.

- Human markers need to be identified if indicated. For example, placenta blood and amniotic fluid should be studied. Different biomarkers for males and females may be required. A better understanding of how male and female effects compare on a mechanistic and molecular level will help. Gene changes that offer hope for potential molecular markers need to be identified.

- Better methods should be developed to look more carefully at the varying effects of different phthalates. Which exposure routes are most important for each of the phthalates needs to be defined.

- Researchers would like to see all sources of potential phthalate exposure identified. This includes the range and concentration of phthalates in assorted products.

- Diet is still thought to be the largest source of phthalate exposure but it has hardly been studied due to experimental difficulties in accurately making such assessments. However, this may be a key area to explore, especially in terms of specific routes of exposure and contamination of food sources.

- Participants hope a better sharing of questionnaire approaches will result from the workshop. Questions linger about how to best sample urine and best store samples for stability. Researchers might investigate the stability of urinary phthalate metabolite levels in individuals over time.

- Leading researchers would like to see expanded use of NHANES data. On a population basis, differences in phthalate metabolism will likely be a big factor. Populations with the highest exposures should be targeted for study. Though phthalates are not persistent in the body, life time exposures may be important for human effects and should be considered. Susceptible subpopulations such as neonates should also be targeted for study. Genetic polymorphisms likely to be of interest should also be identified.

NIEHS Worker Education & Training Program (WETP)

4th National Trainers Exchange - Training for Change: Changing Our Training
March 26-28, 2003
Rosen Centre Hotel, Orlando, Florida

"Worker Training" is a constantly changing field, particularly in the area of occupational safety and health where scientific research, regulatory and legislative initiatives, and innovations in educational methodology are applied on an almost daily basis. With funding from the NIEHS WETP, 18 different awardee consortia, representing over 80 individual organizations, have developed one of the most highly skilled networks of trainers in this country. As these expert trainers are in the forefront of this dynamic profession, staff organized a two-day conference entitled the National Trainers Exchange, for them to showcase new translational methods and techniques and to share and evaluate their current practices. The NIEHS WETP believes that this Trainers Exchange is important in advancing the profession of occupational safety and health training; therefore, the proceedings from this conference were carefully documented and will be published in the near future by NIEHS National Clearinghouse for Worker Safety and Health Training on their website at http://www.wetp.org.

The 4th National Trainers Exchange was the largest held by the NIEHS WETP with over 250 health and safety trainers participating in 40 interactive workshops and plenary sessions. This year, based on extensive input from trainers and consortia, these sessions were concentrated in the areas of Advanced Training Technologies (e-learning), Instructor Development, Life Skills and Literacy, Weapons of Mass Destruction (WMD) and Emergency Response. Each workshop/plenary was organized according to adult

learning principles and included, as appropriate, participatory activities, hands-on demonstrations of particular training techniques, skill building exercises, or facilitated discussions of technical issues.

Of special note were those workshops in the Life Skills, Instructor Development and the WMD/ Emergency Response tracks. The Life Skills sessions conducted by Xavier University and the Laborers-AGC Education & Training Fund on "Cultural Awareness and Competence" allowed many trainers to grapple with sensitive issues of cultural stereotyping including race, age, abilities, and language and how it impedes training within our diverse workforce. Each session provided attendees, especially instructors, with a series of tools that can be used in re-shaping their own classroom and teaching environments.

The WMD sessions on "Critical Incident Stress Management" (CISM) and "What You Need to Know About Bioterrorism Diseases" also received very positive evaluations. The CISM model by the International Association of Firefighters shared the key elements for effective stress management before, during and after critical incidents. This CISM approach was instrumental in addressing the concerns of firefighters at the 255 firehouses in New York City after September 11. The Community College Consortium conducted the bioterrorism session, which included presentations, facilitated discussion and covered biological agents, exposure routes, emergency response requirements, personal protection needs, long and short-term health impacts and fatality rates.

The application of scientific knowledge is fundamental to the field of occupational and safety and health. The National Trainers Exchange is one excellent example of this application. The NIEHS WETP expects to conduct another exchange in the spring of 2005.

Healthy Environments for Children: The Promotion of Collaborative Research
February 3-5, 2003
Pattaya, Thailand

This meeting, which was chaired by Dr. Suk, was a follow-up to the "International Conference on Environmental Threats to the Health of Children: Hazards and Vulnerability," held in Thailand in March, 2002. Exposure to deleterious chemicals pose significant health effects worldwide but especially to maternal and child populations. This meeting addressed new scientific data and research results on children's vulnerability, discussed how to improve the current health conditions of children, and promoted the protection of children's environmental health. Scientific and methodological approaches to understanding mechanisms by which chemical substances pose a risk to human health and the environment were identified for international action. Products from this meeting will identify and resolve key issues with regards to mechanistic research, exposure assessment, risk assessment, risk management, and health effects.

The meeting, though largely regional in scope, focusing on children's environmental health issues most relevant to the South-East Asia and Western Pacific regions, provided a forum for interactions among environmental health scientists whose activities focus on research, public health, education, and environmental exposures as related to children's health. It also provided an opportunity to work directly with academic institutions, government organizations, and industry in the area on specific children's environmental health issues.

Besides international organizations including WHO and the United Nations Environment Program, and South-East Asia organizations and industries based in China, Vietnam, Japan, Singapore, and Thailand, there was co-sponsorship by NIEHS, U.S. EPA, U.S. Trade and Development Agency, and USAID. In conjunction with this meeting in Thailand, Dr. Suk traveled to Hanoi, Vietnam, to assist in the planning of a conference to be held next year that will focus on exposure monitoring and remediation technologies as detailed in the Memorandum of Understanding (MOU) between Vietnam and the U.S on scientific issues surrounding Agent Orange/Dioxin.

DERT PAPERS OF NOTE

Rod-Shaped Eye Cells Die When Exposed to Lead
Donald A. Fox, University of Houston College of Optometry and
Mark H. Ellisman, University of California, San Diego
R01ES12482 and P42ES10337

Background: Some health effects of lead, cognitive and behavioral impairments, high blood pressure, and kidney disorders, are well known and documented. However, the effects lead has on rod-shaped photoreceptor cells, or rods, of the retina are not well or frequently studied. Rods assist in seeing in dim light. The other type of retinal cells, cones, are responsible for color and spatial vision. Cones are used primarily in daylight and for activities such as reading. A person can lose up to 20% of their rods and not experience any functional loss of vision. However, for people who need to see clearly at night or for people who are losing their rods due to disease or injury, finding a way to prevent the rods from dying could be critical.

The results of a previous research project conducted by this research team demonstrated that 7-10 year-old children whose mothers had elevated levels of lead in their blood during the first trimester of pregnancy developed retinal abnormalities. It isn't clear whether the children's rods are dying, but there are unique abnormalities in the children. These researchers continued their studies by examining the retinas of lead-exposed mice.

Advance: The animal study demonstrated that the rods died from lead-induced apoptosis. Lead triggers an increase in calcium entering the mitochondria, which in turn induces the production of Bax, a "death factor" protein. Bax then causes the release of cytochrome C, which initiates DNA damage and subsequent cell death. Electron micrographs confirmed that more gates or contact sites, thought to be associated with cytochrome C release, were open in cells of the eyes from the lead-exposed mice. Other studies found that an excess of an anti-death protein called Bcl-xL completely blocked the death of the rod cells and maintained normal mitochondrial function in the rods throughout adulthood.

Implication: Over expression of Bcl-xL prevented the effects of Bax and reduced the formation of contact sites preventing the release of cytochrome C. For people losing rods because of retinitis pigmentosa, diabetes, or traumatic injury, finding a way to increase the concentration of Bcl-xL or a similar factor in the eye could prevent cell death.

Citation: He L, Perkins GA, Poblenz AT, Harris JB, Hung M, Ellisman MH, Fox DA. Bcl-xL overexpression blocks bax-mediated mitochondrial contact site formation and apoptosis in rod photoreceptors of lead-exposed mice. *Proc Natl Acad Sci USA*. 2003 Feb 4;100(3):1022-7.

Breakthrough in Understanding Disease-Causing DNA Instability
Richard Sinden, Ph.D.
Texas A&M University
R01ES05508

Background: Genes in normal individuals contain short lengths of trinucleotide repeats in which a combination of nucleotides, the building blocks of DNA, are repeated a number of times, usually less than 30. Research has identified 18 human genetic diseases associated with expansion of the number of these repeats, sometimes numbering in the thousands. Fragile X syndrome, myotonic dystrophy, and Huntington's disease are few of these devastating diseases, which become increasingly severe and have earlier onsets in successive generations, a process known as anticipation. Scientists have theorized that if the cause of the repeat expansion can be discovered, there is hope in preventing them from occurring.

Advance: Researchers at Texas A&M University recently discovered that a repeat associated with spinocerebellar ataxia type 10 (SCA 10) is unlike any repeat identified to date. The repeat is made up of 10 nucleotides in the sequence $(ATTCT)_n \cdot (TAAGA)_n$. Experiments demonstrated that the repeat unpairs and acts as a false site of DNA replication.

Implication: While it remains to be seen if repeats associated with other expansion-related diseases support incorrect DNA replication initiation, this finding gives researchers a new target on which to focus. It also sheds light on the mechanism of repeat expansion and may lead to further discoveries on how to prevent and repair these genetic defects.

Citation: Potaman VN, Bissler JJ, Hashem VI, Oussatcheva EA, Lu L, Shlyakhtenko LS, Lyubchenko YL, Matsuura T, Ashizawa T, Leffak M, Benham CJ, Sinden RR. Unpaired Structures in SCA10 (ATTCT)(n).(AGAAT)(n) Repeats. *J Mol Biol*. 2003 Feb 28;326(4):1095-111.

Calcium Supplements Lower Blood Lead in Nursing Mothers

Howard Hu, M.D, Sc.D.
Harvard School of Public Health
R01ES07821, P42ES05947, P30ES00002

Background: Exposure to lead from a variety of sources has been known for centuries to cause adverse health effects. Children are especially vulnerable to learning and behavioral deficits resulting from lead exposure. Pregnancy and breast-feeding are known to cause a marked turnover of lead stored in bones, which account for 95% of lead found in adults. Therefore, lactation places women and their breast-fed infants at an increased risk of lead exposure. Dietary calcium supplements have been shown to reduce fetal lead exposure; however no reports in the literature exist of testing this hypothesis with a properly conducted clinical trial. To address this issue, these investigators conducted a double-blind randomized clinical trial to determine if taking 1,200 mg of calcium each day lowered blood lead levels in lactating women.

Advance: Calcium supplementation produced a small reduction in blood lead levels. The effect was more apparent for women with higher bone lead levels and who were more compliant with taking the supplements. Women with high bone lead levels experienced a 16% decline in blood lead levels.

Implications: This trial demonstrates that calcium supplementation may be effective in decreasing blood lead levels in lactating women. Because dietary lead absorption and bone lead mobilization are likely to be similar during pregnancy and lactation, calcium supplementation is likely to reduce lead exposure to the fetus as well. This kind of intervention is not intended to be a substitute for public health efforts to reduce environmental lead exposure from all sources; however, it may constitute an important secondary prevention effort, because dietary lead exposure is difficult to eradicate and lead exposure from long-lived bone stores is likely to persist for decades.

Citation: Hernandez-Avila M, Gonzalez-Cossio T, Hernandez-Avila JE, Romieu I, Peterson KE, Aro A, Palazuelos E, Hu H. Dietary calcium supplements to lower blood lead levels in lactating women: a randomized placebo-controlled trial. *Epidemiology*. 2003 Mar;14(2):206-12.

Component of Plastic Linked to Chromosome Damage in Mice

Patricia Hunt, Ph.D.
Department of Genetics, Case Western Reserve University
R21ES11172

Background: Bisphenol A (BPA) is a widely used component for the production of polycarbonate plastics used in food and beverage packaging and dental sealants. Though it is a man-made compound, BPA has hormone-like properties that mimic the effects of naturally produced estrogens. Recently, the accidental use of a harsh detergent used to clean animal cages led to the release of small amounts of BPA. Mice housed in the cages were exposed to the compound resulting in meiotic disturbances in the oocytes from the mice. This finding was later replicated in a controlled experiment.

Advance: Researchers in the Department of Genetics at Case Western Reserve University noticed the abnormalities and went looking for an answer. When the detergent was determined to be the cause, the researchers dosed mice with environmentally relevant doses of BPA. Eggs from the dosed animals showed increases in problems of meiosis including disorganized or unaligned chromosomes, and an abnormal number of chromosomes, a condition known as aneuploidy.

Implication: The kinds of chromosomal abnormalities resulting from both the accidental exposure and the controlled experiment are leading causes of miscarriage, congenital birth defects and mental retardation in humans. Although no direct conclusions can be drawn on human health effects without further study, these results do raise concerns because another study in Germany indicated pregnant women are exposed to similar amounts of BPA. These findings provide the first conclusive link between mammalian aneuploidy and an accidental environmental exposure. The study also suggests that the mouse oocyte may provide a sensitive system for the study of reproductive toxins.

Citation: Hunt PA, Koehler KE, Susiarjo M, Hodges CA, Ilagan A, Voigt RC, Thomas S, Thomas BF, Hassold TJ. Bisphenol a exposure causes meiotic aneuploidy in the female mouse. *Curr Biol*. 2003 Apr 1;13(7):546-53.

Gene-Environment Interaction: Effect of Polymorphisms on Biomarkers in Coal Miners

Steven Kleeberger*
The Johns Hopkins University Bloomberg School of Public Health
P01ES09606

Background: Oxidative stress is a harmful condition that occurs when there is an excess of free radicals, not enough anti-oxidants, or both. Studies have shown that free radicals contribute to many diseases including asthma and chronic obstructive pulmonary disease (COPD), diseases of the aging, such as Alzheimer's and Parkinson's disease, and tissue damage resulting from diabetes.

Genetic factors may also play a part in the susceptibility to oxidative stress. Genetic polymorphisms have been implicated in differences in responses to environmental agents, but the interactions between genes and oxidative environmental agents involved in the development of human lung diseases have been largely unexplored. The overproduction of reactive oxygen species (ROS) from cigarette smoking and long-term exposure to dust and particles causes chronic airway inflammation. Inflammation is essential in the development of many airway diseases such as asthma, COPD, and coal workers' pneumoconiosis (CWP). This research team decided to investigate whether polymorphisms in two genes coding for tumor necrosis factor (TNF) and lymphotoxin (LTA), proinflammatory cytokines implicated in the progression of chronic lung disease, modify lung response to oxidants in an epidemiologic study of 253 coal miners.

Advance: A significant interaction was observed in miners with high oxidant exposure and a polymorphism in the *TNF* gene on red blood cell glutathione activity. No interaction was observed among workers with low exposure. Results also showed an association of CWP prevalence with a polymorphism in the *Lta* gene in workers with low catalase activity. Catalase is an enzyme that breaks down ROS like hydrogen peroxide. No association was seen in those with high catalase activity nor were any other significant associations observed.

Implication: These results provide the first demonstration of the involvement of genetic polymorphisms of two genes in the control of physiologic responses from exposure to oxidative stressors. The study suggests an interaction of genetic background with environmental exposure and intermediate responses are important in the development and progression of chronic pulmonary diseases such as coal worker's pneumoconiosis.

Citation: Nadif R, Jedlicka A, Mintz M, Bertrand JP, Kleeberger S, Kauffmann F. Effect of TNF and LTA polymorphisms on biological markers of response to oxidative stimuli in coal miners: a model of gene-environment interaction. *J Med Genet*. 2003 Feb;40(2):96-103.

* Present Address: Laboratory of Pulmonary Pathobiology, NIEHS

Surfactant Gene Expression Recovered after Inhibition of Nitric Oxide
George Leikauf
University of Cincinnati
R01ES10562 and P30ES06096

Background: Although one of the simplest biological molecules in nature, nitric oxide (NO) has found its way into nearly every phase of biology and medicine ranging from its role as a critical endogenous regulator of blood flow and thrombosis to a principal neurotransmitter mediating erectile function to a major pathophysiological mediator of inflammation and host defense. These major discoveries have stimulated intense and extensive research into a vast array of fields including chemistry, molecular biology, and gene therapy.

NO has been proposed as a therapeutic agent for acute lung injury. The administration of low levels of NO preferentially dilate vessels in the lung to improve oxygenation of the blood. However, the role of NO in acute lung injury remains controversial because overall mortality is not reduced in adults. NO formation in the lungs may be detrimental to recovery. This is illustrated by clinical studies where inhibitors of the NO synthase enzyme restored pulmonary function. These investigators explored the function of NO in mice with nickel-induced acute lung injury.

Advance: Nickel exposed mice with acute lung injury were given either a saline control treatment or a NO synthase inhibitor (N^G-nitro-L-arginine methyl ester; L-NAME). The saline treated mice exhibited multiple endpoints of acute lung injury while those that received the inhibitor had better survival, lower NO synthase activity, and lower levels of cytokine release, an indicator of inflammation. Surfactant protein gene expression initially decreased in both groups but recovered in the inhibitor group.

Implication: This work builds upon previous studies of acute lung injury that indicated inhibition of NO synthesis restores pulmonary function. These findings suggest inhibiting NO formation during acute lung injury may be protective possibly my limiting NO synthase-mediated vascular permeability, cytokine production, and causing later restoration of proper surfactant production.

Citation: McDowell SA, Gammon K, Zingarelli B, Bachurski CJ, Aronow BJ, Prows DR, Leikauf GD. Inhibition of nitric oxide restores surfactant gene expression following nickel-induced acute lung injury. *Am J Respir Cell Mol Biol*. 2003 Feb;28(2):188-98.

Identification of Possible Human Liver Tumor Suppressor Genes
Sharon L. Ricketts, Ph.D
UNC Lineberger Comprehensive Cancer Center
T32ES07017

Background: Several distinct regions of human chromosome 11 demonstrate loss of heterozygosity or confer tumor suppression in chromosome transfer studies in specific types of human tumors, including liver cancer, suggesting the presence of multiple tumor suppressor genes on this chromosome. By developing a model in which human chromosome 11 was introduced into a rat liver tumor cell line this laboratory was able to create a new cell line that exhibits suppression of tumorigenicity. Using this model system, the investigators were then able to employ a candidate gene approach to identify potential human liver tumor suppressor genes.

Advance: Thirty-eight genes have been mapped to this region of chromosome 11 by the Human Genome Project. Three of these genes were uniformly expressed by an index panel of suppressed microcell hybrid cell lines, which identified them as candidate liver tumor suppressor genes. In preliminary analyses in four human carcinoma cell lines, the transcript of one gene, p53-induced protein (PIG11), was lost or significantly decreased in two of the lines identifying this gene for potential involvement in some human liver carcinomas.

Implication: This study increased the knowledge of genes located in the liver tumor suppressor region of chromosome 11 and identified several candidate liver tumor suppressor genes from this region. Further characterization of these candidate genes may provide further insight into the role of this region of chromosome 11 in the pathogenesis of human liver cancer.

Citation: Ricketts SL, Carter JC, Coleman WB. Identification of three 11p11.2 candidate liver tumor suppressors through analysis of known human genes. *Mol Carcinog*. 2003 Feb;36(2):90-9.

Lead and Age Reduce The Fertilizing Ability of Sperm
Susan Benoff, MD, NYU School of Medicine, R01ES06100
Andy Wyrobek, Ph.D. and Brenda Ezkenazi, Ph.D., Univ. Cal. Berkeley, P42ES04705

Background: Human sperm are fragile creatures, but there are so many in a single ejaculate, odds are good that one will find the target—the egg. However, over the past 15-20 years, the scientific community has been alarmed over the drastic decreases in human sperm concentrations reported in some scientific publications. Environmental agents have been shown to reduce sperm concentrations and viability in laboratory animals. Increasing age is known to be a factor in fertility reduction in women in part because of the finite number of oocytes women have at birth. Lead is known to reduce fertility in animal models, but a direct link between lead exposure and human fertility has not been established.

Advance: In two separate studies published in the same issue of *Human Reproduction*, two NIEHS-supported teams reported the harmful effects of age and lead exposure on human sperm. In the study on age, semen volume, motility, and the ability to swim in a straight line declined with age. Although the sperm concentration remained relatively constant, these decreases in function suggest that fertility starts dropping when men are in their 20s and continues to diminish for the rest of their lives. In the lead exposure study, lead was measured in the seminal plasma of 140 partners of women undergoing *in vitro* fertilization. Men with higher levels of lead had decreases in sperm counts and were more likely to have damaged sperm less likely to fertilize an egg.

Implication: These results present clinicians with additional information to consider when evaluating couples with unexplained fertility. Given the need for sperm function tests to predict the outcome of *in vitro* fertilization attempts, and to help in determining the appropriate course of infertility treatment, infertility clinics should consider measuring lead in semen of the partners of women undergoing *in vitro* fertilization.

Citations:

- Benoff S, Centola GM, Millan C, Napolitano B, Marmar JL, Hurley IR. Increased seminal plasma lead levels adversely affect the fertility potential of sperm in IVF. *Hum Reprod*. 2003 Feb;18(2):374-83.

- Eskenazi B, Wyrobek AJ, Sloter E, Kidd SA, Moore L, Young S, Moore D. The association of age and semen quality in healthy men. *Hum Reprod*. 2003 Feb;18(2):447-54.

Destruction of Oxidized Proteins

Kelvin J. A. Davies
University of Southern California

R01ES03598

Background: The accumulation of damaged proteins is a characteristic of aging cells and many age-related conditions such as Alzheimer's disease. Unless these proteins are repaired or removed from cells, they often impair proper cell function and can lead to cell death. Cells contain complexes of enzymes designed to breakdown these proteins known as proteasomes. Proteasomes not only degrade harmful accumulations of damaged proteins, but they also breakdown short-lived regulatory proteins important in a variety of basic cellular processes.

Advance: For the most part, proteasomes recognize, unfold, and digest proteins that have been marked for degradation by the attachment of multiple molecules of ubiquitin. This ubiquitin-proteasome pathway functions widely in intracellular protein turnover. However, recent research sponsored by NIEHS at the University of Southern California has shown that at form of the proteasome known as 20 S can carry out protein degradation without the ubiquitinylation. This research, done in intact cells in culture, builds on previous findings in this laboratory.

Implications: The focus of this research team is the role of free radicals and oxidative stress in biology. In particular the lab is focused on oxidative stress during aging and aging pathologies such as Parkinson and Alzheimer's diseases. The results reported here describe a novel method for the destruction and removal of oxidatively damaged proteins. Although very basic in nature, this study provides insight into normal cell functioning, may lead to discoveries of how disease of aging impair these functions, and possibly provide clues to how these diseases may be prevented or treated.

Citation: Shringarpure R, Grune T, Mehlhase J, Davies KJ. Ubiquitin conjugation is not required for the degradation of oxidized proteins by proteasome. *J Biol Chem*. 2003 Jan 3; 278(1):311-8.

Chemical Driven Premature Ovarian Failure

Jonathan L. Tilly and James K. Pru,
Harvard Medical School/Mass. General Hospital

R01ES06999, R01ES08430, and F32ES11941

Background: Exposure to certain industrial chemicals has been shown to cause premature death of the stockpile of female germ cells mammals are born with. If this effect occurs in women as well, it could cause premature menopause leading to other hormonally related conditions. This was the focus of a previous report by these investigators (*Nat Genet*. 2001 Aug;28(4):355-60). In the earlier report compounds known as polycyclic aromatic hydrocarbons (PAHs), but not dioxin, were shown to signal through the Ah receptor/Bax-regulated pathway leading to oocyte death.

Finding: In a new report the investigators have expanded their work to include the environmental agent 4-vinylcyclohexene diepoxide (VCD). VCD is a by-product of the manufacture of plastics, rubber, flame retardants, and pesticides. VCD has also been shown to also cause premature death of immature

follicles from the ovaries of rats and mice. The current study shows that mice lacking the Bax gene retained more of their follicles than wild-type females when exposed to VCD. The same was true for mice lacking genes for the enzymes caspase-2 and caspase-3; enzymes essential in the life cycle of follicles.

Implication: These results add to the tremendous progress that has been recently made in understanding the cellular and molecular events responsible for oocyte death and follicle depletion under normal and pathological conditions. Future research aimed at finding natural substances that will modify or incapacitate these proteins may lead to methods to prevent oocyte loss in response to the natural aging process and from exposure to environmental agents.

Citation: Takai Y, Canning J, Perez GI, Pru JK, Schlezinger JJ, Sherr DH, Kolesnick, RN, Yuan J, Flavell RA, Korsmeyer SJ, Tilly JL. Bax, caspase-2, and caspase-3 are required for ovarian follicle loss caused by 4-vinylcyclohexene diepoxide exposure of female mice in vivo. *Endocrinology*. 2003 Jan;144(1):69-74.

Hand to Mouth—Ingestion of Pesticides by Children Living on the US/Mexico Border

Stuart Shalat and Kirby Donnelly
University of Medicine and Dentisty of New Jersey and Texas A&M University
P30ES05022 and P30ES09106

Background: Many studies have shown that children are exposed to environmental chemicals including pesticides through different mechanisms and sometimes in greater amounts than adults. This can be especially troublesome in agricultural communities where the potential for pesticide exposure is higher than in the general population. Clearly, the potential for toxicity is dependent on the dose children receive. Children in general spend more time in contact with surfaces prone to pesticide contamination such as floors and soils. The pesticides are transferred to the hands and then ingested when mouthing behavior occurs.

The purpose of this study was to evaluate relationships between exposure to organophosphate containing pesticides in children living in border agricultural communities and dose levels determined by measuring metabolites in urine.

Advance: Seventy-six percent of house dust samples and 50% of hand rinse samples contained pesticides. Urine samples from all 52 children contained at least one pesticide metabolite and 95% contained metabolites of two or more pesticides. Younger children and infants had higher concentrations of urinary metabolites than older children. Levels of pesticides on the childrens' hands were more closely associated with urine concentrations than were housedust samples.

Implication: This study demonstrates the elevation of pesticide contamination in children living in border communities. The levels were higher in younger children suggesting the need for study in younger infants. The level of pesticides found on the childrens' hands was correlated higher with urine concentrations than housedust samples suggesting it is a better estimate of exposure. Little is known about the health hazards from long-term exposure to these chemicals. The findings presented illustrate the importance of continued study of environmental pesticide exposure and its possible involvement in chronic illness among children living in agricultural communities along the US/Mexico border.

Citation: Shalat SL, Donnelly KC, Freeman NC, Calvin JA, Ramesh S, Jimenez M, Black K, Coutinho C, Needham LL, Barr DB, Ramirez J. Nondietary ingestion of pesticides by children in an agricultural community on the US/Mexico border: preliminary results. *J Expo Anal Environ Epidemiol*. 2003 Jan;13(1):42-50.1

Lead-Induced Learning Impairment Reversed by Environmental Enrichment

Tomas Guilarte and Christopher Toscano
The Johns Hopkins University Bloomberg School of Public Health
R01ES06189 and T32ES07141

Background: Lead is a potent environmental neurotoxicant with a long history of exposure in children. The developing brain is highly susceptible to lead exposure and long-term deficits in cognitive function are the principal effects of lead exposure in children. Despite significant and effective efforts to reduce lead levels in the environment, 1 in 20 children living in the U.S. have blood lead levels known to

Dr. McClure, COSPB, coathored with *Dr. Heindel, COSPB*, Dr. Philip Mirkes, University of Washington, and Dr. Miriam Saunders an article entitled: "Developmental Toxicology in the 21st Century: Multidisciplinary Approaches Using Model Organisms and Genomics" which appeared in the *Journal of Births Defects Research* (Part A, *Clinical and Molecular Teratology*), Vol.67:21-34, 2003.

The Advisory Committee for the National Center for Toxicogenomics held its first meeting at NIEHS on Mary 28. *Dr. Sassaman* presented the extramural Toxicogenomics Research Consortium component of the trans-institute program, and *Dr. Van Houten, PAB*, also participated in the discussion.

A Town Meeting on "Oceans and Human Health" was organized by the Marine and Freshwater Biomedical Research Center at the University of Miami was held February 27. This was followed the next day by a "Hollywood Squares" event at a local special high school which featured *Dr. Olden* as a "celebrity." Attending the events from DERT were *Drs. Sassaman, Tyson and Dearry* and *Mr. O'Fallon.*

Dr. McClure, COSPB, co-chaired the organizing committee for the NIEHS-supported 1st Annual International symposium entitled "Embryonic Stem Cell Biomedicine: the Journey from Mice to Patients," which was held May 15-17 at the Pittsburgh Development Center/Magee-Women's Research Institute following the "2003 Frontiers in Human Embryonic Stem Cells (lecture/laboratory) Training Course" supported by NIH

Dr. Suk, CRIS, co-chaired the "Children's Susceptibility to Environmental Agents Symposium" at the Enviromental Mutagen Society annual meeting in Miami, Florida, May 11th, and presented the paper entitled "Children's Health and the Environment."

Dr. Thompson, CRIS, presented the opening talk at the 12th International Symposium on Pollutant Responses in Marine Organisms (PRIMO^{12}) in Tampa Florida on May 9. The purpose of PRIMO^{12} was to provide a forum for presentation, discussion and publication of original research in mechanisms of toxicity, development of biomarkers, biotransformation, and assessment of biochemical, cellular, immunological and reproductive effects of chemical pollutants in aquatic organisms. The PRIMO^{12} Symposium was open to individual scientists, postdoctoral fellows and students interested in these areas of mechanistic aquatic toxicology.

Dr. Suk, CRIS, participated at the 4th International Conference on Environmental Mutagens in Human Populations (4th ICEMHP), May 4-8, in Florianopolis, Brazil. This Conference was designed to identify solutions to human environmental health problems and to facilitate the establishment of sustainable collaborative programs around the world. The overall goal is to reduce mutagen-induced environmental disease. Dr. Olden gave the keynote address. The conference participants included an international gathering of biomedical scientists. Dr. Suk co-chaired the Symposia "Children's Environmental Health,"and delivered the talk within that Symposia entitled "Environmental Hazards to Children's Health in the Modern World." The Symposia within this Conference provided a better understanding of the nature of environmental threats to assist in the development of strategies to prevent harmful exposures to children, and assisted in focusing the research community at looking at issues of childhood exposure and disease and prevention.

Dr. Weis, CRIS, and Dr. Heindel, COSPB, in conjunction with staff from DIR, organized an international conference on *Metabolic Profiling: Application to Toxicology and Risk Reduction* held at the NIEHS on May 14-15. The conference was designed to define the state of the science for the emerging technology of metabonomics/metabolomics and its application to environmental health research, and to identify future directions for exposure assessment, toxicology and clinical medicine.

Mr. Hughes, WETB, participated in and presented at the CDC Chemical/Radiation Preparedness Workshop in San Francisco, California on May 5-6. As part of the Interstate Chemical Terrorism

Working Group, NIEHS staff is assisting in a national survey of public health preparedness of state and local health departments for response to terrorist attacks and other disasters.

Dr. Gray, SPHB, participated in Effective Strategies of Asthma Interventions in Bethesda, Maryland, on April 4. This meeting was organized by CDC and hosted by NHLBI. It brought together a diverse group of participants such as asthma coalitions and Federal Agencies that conduct asthma interventions, as well as researchers actively implementing intervention studies of asthma, including clinical, community-based, and population-based research. The objectives of the meeting were to define criteria to successfully identify effective asthma strategies, to determine mechanisms that would facilitate the dissemination of these interventions, and to identify issues related to implementation and evaluation of successful asthma interventions.

Dr. Weis, CRIS, presented at a workshop entitled *Metrology and Standards Needs for Gene Expression Technologies: Universal RNA Standards* hosted by NIST-FDA on March 28-29 at Stanford University. Dr. Weis presented on "Applications of Standards to Harmonize Data Laboratories and Microarray Platforms."

Dr. Weis, CRIS, presented at two sessions of the Science Education Program's *Rx for Science Literacy* Workshop on March 25. The workshop was co-sponsored by the NIEHS and North Carolina Association for Biomedical Research. Her presentation was titled "Toxicogenomics: Genomic Science to Understand Biological Response to Environmental Stressors."

Dr. McClure, COSPB, accepted an NIH appointment to the NIH Office of Dietary Supplements Strategic Planning Committee for FY 2004-2009. He presented an invitational plenary lecture on "DHHS Public Health Perspectives of Dietary Supplement Risks and Safety Issues" at the May 8-9 stakeholder's meeting convened by the Office of the Director, NIH.

Dr. Heindel, COSPB, has been an invited speaker at three local SBIR workshops sponsored by the North Carolina Small Business Technology Development Center and the specific University Sponsored Activities Programs. These half day workshops were held February 13 at NC A&T, March 27 at UNC Wilmington and April 9 at East Carolina University. At each workshop Dr. Heindel gave two presentations, one entitled, "Overview of the NIH and NIEHS SBIR Programs and Interests" and another entitled, "SBIR grantsmanship: or How to Swim with the Sharks and Survive." Each workshop was attended by 30-40 university scientists and local small business representatives interested in generating SBIR funds.

Mr. Hughes and Mr. Winchel, WETB, presented at the EPA Emergency Support Function #10 Coordination for National Hazmat Disasters Committee in Washington, DC on April 9.

Drs. McClure, COSPB, and Sassaman, OD, co-organized and co-hosted, in conjunction with U.S.-EPA counterparts, the April 4 orientation meeting for the NIH Extramural Associates (EA) Program which provided an extensive research, training and career development programs orientation and facilities tour for the NIEHS and the EPA. NIH EAs are senior administrators/faculty of research capable Historically Black College or University (HBCU), Women's, or other minority organizations nominated in a grant application by an eligible organization"s President or CEO, who successfully complete either a short-term (1-3 Months) or long-term (6-12 Months) EA training program in sponsored research administration held annually at the NIH.

Dr. Shreffler, COSPB, participated in the Undergraduate Education Program for Minority Students on March 9 at the Society of Toxicology Meeting in Salt Lake City, Utah. She discussed the Graduate and Short Term Training programs supported by the NIEHS and provided information on fellowship opportunities for underrepresented minorities.

Mses. Duke, Mason, Garcia, Russell, Winters, Ricci and Mr. Dwight Dolby, GMB, attended the North Carolina Society of Research Administrators Annual Meeting, March 3-5 in Chapel Hill, North Carolina. At the meeting, Ms. Duke presented an NIH update. Mses. Garcia and Russell presented a Training Grants Update.

Dr. McClure, COSPB, co-organized with Dr. Straus, Director, NCCAM, NIH, and Dr. Coates, Director, ODS-OD/NIH, an independent external review committee evaluation of the Trans-NIH Botanical Research Centers program sponsored by NIEHS, NCI, NCCAM, NIDDK, NICHD and ODS-OD/NIH. The committee membership included senior NIH, USDA, and NIH supported expert consultants. Dr. Bernard Goldstein, NAEHS Council member, chaired the committee. Dr. Martin Philbert, an NIEHS grantee, served as a neurosciences expert consultant member. The committee review, held February 21, will yield a report to the Office of the Director, NIH.

Mr. Hughes, WETB, and staff conducted its Spring 2003 Awardee Meeting in Orlando, Florida on March 26. At the meeting over 100 individuals participated by interacting with NIEHS staff during the NIEHS Update and attending breakout sessions pertaining to specific grants and program topics such as A-133 Audits, Financial Management, Sub-Recipient Monitoring, Supplemental Awards, Life Skills/Remedial Education Training, Curricula Development and Redesign of the WETP Data Management System. As a result of recommendations from the WETP Strategic Plan (http://www.wetp.org/oldchfiles/awardee_mtgs/fall01/stratplanE.pdf), a new session format called Health and Safety Rounds was introduced. The Health and Safety Rounds are a new participatory series of sessions that are meant to address relevant topics on health and safety and management issues. Staff attending the workshop and participating in various activities included *Ms. Beard, Mr. Outwater, Ms. Thompson, WETB, and Ms. Duke, GMB*.

Mr. O'Fallon, SPHB, provided oversight in the development of an exhibit booth for the Community Outreach and Education Program. The booth made its debut at the National Science Teachers Association annual national convention in Philadelphia, Pennsylvania on March 24-27. The booth will be exhibited at the American Public Health Association conference this Fall and at the Society of Toxicology conference in March 2004. This booth helps NIEHS promote COEP as a larger program, as well as increase awareness of the outreach and educational materials offered by the 26 COEPs across the country.

Mr. Hughes, WETB, participated in and presented at the Federal Disaster Response Meeting in Arlington, Virginia on February 26. Representatives from EPA, NIOSH, OSHA, and RAND Corporation also shared their perspective on federal disaster response.

Dr. Tyson and Mr. O'Fallon, SPHB, organized the annual Environmental Health Sciences as an Integrative Context for Learning grantee meeting, held in Miami, Florida, February 26-27.

Dr. Gray, SPHB, was invited to the Veterans Administration ALS registry steering committee meeting and to present the data from the study, "Occurrence of Amyotrophic Lateral Sclerosis (ALS) Among Gulf War Veterans" in Washington, DC on February 13. She also presented on NIEHS' ongoing efforts to facilitate research in the area of environmental exposures and ALS.

Mr. Hughes, WETB, presented at the 13th Annual Construction Safety and Health Conference and Exposition in Chicago, Illinois on February 11. Mr. Hughes moderated a session entitled "Training Skilled Support Personnel at Federal Disaster Sites."

UPCOMING MEETINGS and WORKSHOPS

Ms. Beard WETB, will host a Brownfields Focus Meeting in Research Triangle Park, North Carolina on May 20. This meeting will focus on strengthening and promoting the strategic plan for Brownfields issues. All NIEHS/WETP Brownfields Minority Worker Training Awardees will participate in this meeting. Staff attending and participating in the meeting includes *Mr. Hughes, Mr. Outwater, and Ms. Thompson, WETB*.

Drs. Packenham, Gray, and Maull, SPHB, in conjunction with the Harvard Comparative Mouse Genomics Center, are organizing a scientific symposium under the auspices of the Environmental Genome Project titled “Genes, Environment and Disease.” This meeting will be held June 7-9 in Boston, Massachusetts at the Harvard School of Public Health. The scientific symposium is designed to examine the role of genetic variation in gene-environment interactions, emerging technologies used in the study of genetic variation, and examine issues of ethics and social consequences related to the discovery of environmentally responsive genes in human populations. This is an open meeting, with opportunities for other scientists to present their results at a poster session. The Comparative Mouse Genomics Centers Consortium and Molecular Epidemiology grantees are invited to attend a round-table discussion at the end of the meeting to foster dialogue and stimulate discussion among this multidisciplinary group of scientific experts about issues related to studying genetic susceptibility of environmentally induced diseases in the laboratory research to the human population studies, and to stimulate collaborative efforts that may lead to new directions of the EGP.

The National Institutes of Health (NIH) Director's scientific symposium/workshop entitled "NIH Research:

and Cornell Universities. He spent nine years as an assistant/associate professor in the Department of Microbiology at the University of Cincinnati College of Medicine where his research was focused on membrane bioenergetics in *Bacillus* and on chemically induced mutagenesis and transformation of mouse and human fibroblasts. Dr. Lang left academe in the early 90's to assume a leadership role in a start-up biotechnology company where he headed a research group that engineered *Bacillus* organisms to express foreign genes and to produce unique peptides from *in vitro* synthesized genes. His government service at NHLBI began in 1991.

Dr. Leslie Reinlib has joined SPHB as a Program Official. He is working with the forthcoming Breast Cancer and the Environment Research Centers and is the Overall Coordinator of the Environmental Genome Project. Dr. Reinlib received a Doctorate in Naturwissenschaften (Natural Sciences) from the Laboratory of Bichemistry at the distinguished ETH Zurich (Swiss Federal Institute of Technology). He has experience in cellular imaging and protein biochemistry and has applied basic science approaches to clinical questions, such as the cellular basis for Cystic Fibrosis, Crohn's Disease, heart failure, and the neuronal effects of alcoholism. After faculty positions at The Tufts University and The Johns Hopkins University Schools of Medicine, Dr. Reinlib moved to an administrative position with the NIH National Heart, Lung, and Blood Institute. There, he oversaw a broad spectrum of grants in basic and clinical research concerned with cardiovascular and lung diseases and was an Executive Council Member and NIH representative to the Heart Failure Society of America. At NHLBI, Dr. Reinlib was a Team Leader of the Programs for Genomic Applications and helped establish it as a national resource for future genomics studies as they apply to heart, lung, blood, and sleep disorders.

Mr.Rodney (Peppy) Winchel, Jr. MPH , has joined WETB for a three month rotation as part of the NIH Presidential Management Intern (PMI) Program. While in DERT, he is serving as the coordinator for the Weapons of Mass Destruction Supplemental Awards. Originally from Wisconsin, Mr. Winchel earned a BA in Biology from Illinois Wesleyan University. He served four years in the US Army as a Medical NCO. He earned his Masters of Public Health from Northern IL University. During the fall of 2001, Mr. Winchel served as the Illinois American Red Cross State Disaster Volunteer Coordinator, including being assigned as a local disaster volunteer coordinator in New York City. Immediately prior to his appointment to NIH as a PMI, he served as a program manager for a homeless/crisis services organization of North Chicago. Mr. Winchel is pursuing administration and management of biodefense and disaster research programs, especially communicating evidence-based knowledge to responders and the public.

Departures:
Dr. Allen Dearry, SPHB, departed from DERT on April 3 to join the NIEHS Office of the Director as the Associate Director for Coordination, Planning, and Translation. *Dr. Collman*, has been named Acting Branch Chief of the branch.

Ms. Laura Williams-Boyd, GMB, retired on March 31 after almost 35 years with the government. She had been with GMB nine years.

Ms. Sandi Manness, RCB, retired on March 31 after 32 years of government service. She had been in RCB for the past 17 years.

Ms. Helen Watson, GMB, retired on May 3 after more than 25 years of government service. She had been with GMB for almost 18 years.

DIVISION OF INTRAMURAL RESEARCH

NAEHS COUNCIL UPDATE

MAY 2003

DIR Recruitments

Chief, Laboratory of Computational Biology and Risk Analysis
An international search is being conducted for a tenured investigator to serve as Chief of the Laboratory of Computational Biology and Risk Analysis. The candidate will be expected to:

- Develop and maintain a strong personal research effort in the general area of bioinformatics, particularly as it relates to biological networks, proteomics and genomics.
- Provide overall leadership for the existing principle investigators within the LCBRA who study the development of laboratory methods for humans and animals combined with computational, statistical and mathematical methods to further our understanding of the mechanisms underlying environmental disease.
- Recruit talented investigators to the LCBRA and provide a focus for collaborations within the NIEHS.

The Candidate should be a senior investigator with an international reputation in a research area within the broad context of bioinformatics and its relationship to the environment. Possible research areas include but are not limited to mathematics, statistics, genetics, bioengineering and molecular biology. The successful candidate will also have an outstanding publication record and proven history of research leadership. A search committee chaired by Dr. Clarice Weinberg, Chief, Biostatistics Branch is reviewing applications.

Tenure-track Bioinformaticist
The Biostatistics Branch is conducting a nationwide search for a tenure-track investigator with training and experience in bioinformatics. The person selected will focus activities upon developing novel methods related to toxicogenomics, such as developing and evaluating data mining approaches for elucidating characteristic patterns in gene expression array or proteomic data in order to facilitate searches for functionally-coordinated families of genes related to disease processes or response to toxicants. Improved quantitative methods for functional genomics and data mining are needed to make full scientific use of the toxicogenomics data being produced by the NIEHS Microarray Center and the National Center for Toxicogenomics. A search committee chaired by Dr. Douglas Bell, Laboratory of Computational Biology and Risk Analysis has begun interviewing candidates.

Tenure-track Immunologist
The Laboratory of Pulmonary Pathobiology is conducting a national search for a cellular/molecular immunologist. The candidate will be expected to establish a high-quality independent research program in pulmonary immunology in a laboratory with diverse research interests and backgrounds. The successful candidate will have research strengths in, but not necessarily limited to, pulmonary biology (such as mechanisms of tolerance, allergy, adaptive and/or innate immune response to respiratory infections, etc).

A search committee chaired by Dr. John Drake, Chief or the Laboratory of Molecular Genetics is interviewing candidates.

Tenure-track Environmental Epidemiologist
The Epidemiology Branch has conducting a national search for an environmental epidemiologist. This person will be expected to develop an outstanding research program on the effects of environmental exposures and risks of chronic disease. Applicants with demonstrated research interests in biological mechanisms and etiology of (not limited to) neurodegenerative diseases, diabetes, multiple sclerosis, renal disease, cardio-respiratory diseases; and such exposures as pesticides, metals, and/or solvents are most welcome. A search committee chaired by Dr. Steven Kleeberger, Chief of the Laboratory of Pulmonary Pathobiology is interviewing candidates.

Staff Scientist Biostatistician
The Biostatistics Branch is conducting a national search for a statistician to collaborate closely with the National Toxicology Program. The successful candidate will provide statistical leadership and consulting support for the National Toxicology Program and will also develop methods related to design and analysis of toxicology studies. Applicants should have with experience in statistical consulting and a demonstrated ability with problems in applied statistics. A selection has been made, pending approval by NIH.

Tenure-track or tenured Biostatistician--Statistical Genetics
The Biostatistics Branch has conducted an international search for a tenure-track or tenured statistician to conduct independent research on methods development in statistical genetics. The successful candidate will be expected to develop statistical methods for family-based studies aimed at identifying and mapping genes that influence risk modifying quantitative traits or diseases or that interact with the environmental agents that cause human disease. An offer has been extended to a leading candidate.

Staff Scientist--Toxicologic Pathologist
The Laboratory of Experimental Pathology is conducting a national search for a toxicologic pathologist to provide support and peer review for the National Toxicology Program toxicity and carcinogencity studies and to provide support for NIEHS researchers. A search committee chaired by Dr. Rick Hailey, Toxicology Operations Branch, has been formed and review of applications will begin before the end of May.

Staff Scientist—Pathologist/Laboratory Animal Vertinarian
The Laboratory of Experimental Pathology is conducting a national search for a laboratory animal veterinarian to provide management, oversight, production support, genetic monitoring and disease surveillance of laboratory animals for the National Toxicology Program. A search committee chaired by Dr. Joseph Roycroft, Toxicology Operations Branch, has been formed and review of applications will begin before the end of May.

Staff Scientist—Bioethics

The Office of Clinical Research is conducting a national search for a bioethicist to be involved with health policy research on the effectiveness of federal and Institutional Review Board regulations in addressing clinical studies and clinical genetics issues. A search committee chaired by Dr. Stephanie London, Epidemiology Branch, has been formed and the position has been advertised.

DIR Recruits

Dr. Trevor K Archer
Chief, Laboratory of Molecular Carcinogenesis

Dr. Archer directs a research group in Chromatin and Gene Expression. The overall goal of his research over the last 10 years has been to understand the mechanisms by which gene expression is initiated in response to physiological and environmental signals and how those signals are mediated by steroid receptors within the context of chromatin. Dr. Archer and colleagues have pursued two highly interactive objectives. The first is to provide a molecular definition of the relationship between nuclear receptors, chromatin remodeling machines and promoter chromatin structure in the regulation of steroid receptor activity using the mouse mammary tumor virus (MMTV) system as a model for a steroid hormone activated promoter. The wealth of prior information, extensive reagents and resources on this model will allow Dr. Archer to pursue a series of goals that are not possible in other systems. The second objective is the development of additional model systems to understand glucocorticoid, progesterone and estrogen receptors (GR, PR, ER). This objective has resulted in initial characterization of the human cathepsin D and inhibitor of nuclear factor Kappa B alpha genes. The research efforts of the Archer lab are informed by the overwhelming evidence that a full understanding of transcriptional control requires an appreciation for roles played by the chromatin structure of target genes and the molecular machines that are required to unleash the regulatory potential of steroid receptors. The approach has been bi-directional with efforts geared to understanding transacting proteins and the protein architecture of chromatin that is subject to post-translational modifications. Studies have focused on the mammalian chromatin remodeling complex that is the homologue of the yeast SWI/SNF complex and its interactions and regulation by the glucocorticoid and progesterone receptors. The activity of this complex has been evaluated in the context of the chromatin within human and mouse cells. Using the MMTV promoter as the primary model system, Dr. Archer and colleagues have paid particular attention to the phosphorylation of histone H1 and the acetylation of the core histones. The nature of many of the models, human and mouse beast cancer cells, is also indicative of Dr. Archer's active interest in women's health and breast cancer. Additional research in the Archer group examines the epigenetic regulation of the human breast cancer susceptibility gene BRCA1 (initially identified at the NIEHS) and the estrogen receptor regulation of the protease cathepsin D, the over-expression of which, is closely associated with a poor clinical outcome for patients with breast cancer. Dr. Archer has served as chair of the National Cancer Institute of Canada Peer Review Panel on Cell Cycle, Hormone/Steroid Receptors and Signal Transduction, as a member of an NIH Study Section (CDF-6) and as a reviewer for the National Research Foundation of South Africa.

<u>Publications</u>:

Fryer, C.J. and Archer, T.K.: Chromatin remodeling of the glucocorticoid receptor requires the BRG1 complex. Nature 393: 81-91,1998.

Bhattacharjee, R.N., Banks, G.C., Trotter, K.W., Lee, H-L, and Archer, T.K.: Histone H1 phosphorylation by Cdk2 selectively modulates MMTV transcription through chromatin remodelling. Mol. Cell. Biol. 21: 5417-5425, 2001.

Mancini-DiNardo, D.N., Butcher, D.T., Robinson, D.P., Archer T.K., and Rodenhiser, D.I.: Functional analysis of CpG methylation in the BRCA1 promoter region. Oncogene 20: 5331-5340, 2001.

Banks. G.C., Deterding, L.J., Tomer, K.B., and Archer, T.K.: Hormone mediated dephosphorylation of specific histone H1 isoforms. J. Biol. Chem., 276: 36467-36473, 2001.

Deroo, B.J. and Archer, T.K.: Glucocorticoid receptor activation of the IkBa promoter within chromatin. Mol. Biol. Cell. 12: 3365-3374, 2001.

Deroo, B.J., Rentsch, C., Sampath, S., Young, J., DeFranco, D.B. and Archer T.K.: Proteasomal inhibition enhances glucocorticoid receptor transactivation and alters its sub-nuclear trafficking. Mol. Cell Biol., 22: 4113-4123, 2002

Hebbar, P.B. and Archer T.K.: Nuclear factor 1 (NF1) is required for both hormone dependent chromatin remodeling and transcriptional activation of the MMTV promoter. Mol. Cell Biol., 23: 887-898, 2003

Dr. Rachel Neal
Head, Protein Microcharacterization Facility

Dr. Rachel Neal recently joined the Mass Spectrometry Group as the Head of the Protein Microcharacterization Facility. She received a PhD in chemistry on the catastrophic biological effects of high dose radiation from the University of Missouri-Rolla in 1999. Concurrently, she published several papers on the biological effects of low-level Pb-exposure. As a post-doctoral fellow at the National Eye Institute, NIH, she demonstrated that alterations in protein post-translational processing occurred in the lens following low-level oral Pb exposure in rats and induced opacities in lens organ culture following modifications in cytoskeletal and crystallin proteins. She also created a topographical map of proteins expressed in the human vitreous humor.

The Microcharaterization Facility is currently involved in multiple collaborations involving identification of protein-protein interaction sites and mapping of post-translational protein modification sites. In addition, the facility provides DIR scientists with cutting-edge mass spec services for the identification of proteins from gels and solutions.

Publications:

Neal, R., Matthews, R.H., Lutz, P., and Ercal, N.: Antioxidant role of N-acetyl cysteine isomers following high dose irradiation. Free Radic. Biol. Med., 34: 689-95, 2003.

Neal, R, Zigler, J.S., Jr., and Bettelheim, F.A.: On the equilibrium between monomeric alpha-lactalbumin and the chaperoning complex of alpha-crystallin. Biochem. Biophys. Res. Commun., 280: 14-18, 2001.

Neal, R., Cooper, K., Kellogg, G., Gurer, H., and Ercal, N.: Effects of some sulfur-containing antioxidants on lead-exposed lenses. Free Radic. Biol. Med. 26: 239-243, 1999.

Neal, R., Cooper, K., Gurer, H. and Ercal, N. Effects of N-acetylcysteine and 2,3-dimercaptosuccinic acid on lead induced oxidative stress in rat lenses. Toxicology 130: 167-174, 1998.

Dr. Robert Petrovich
Head Protein Expression Core Facility

Dr. Robert Petrovich has recently joined the NIEHS as Head of the Protein Expression Core Facility. He received his Ph.D. in biochemistry from the University of Wisconsin-Madison in 1992. His thesis research focused on identifying the metal cofactors for lysine-2,3-aminomutase, and determining their role in the mechanism of the enzyme. Dr. Petrovich then moved on to a post-doctoral research position in the Chemistry Department at the University of Wisconsin-Madison where he studied the method for the activation of soluble guanylyl cyclase by nitric oxide. Dr. Petrovich then moved to the laboratory of Dr. Eileen Jaffe at the Fox Chase Cancer Center, where he studied the mechanism of porphobilinogen synthase. In 1999, Dr Petrovich was hired by Novartis/Syngenta to develop high throughput screens for the discovery of new agro-chemicals. In the course of this work he directed the Syngenta effort to establish a rapid parallel method for the expression of protein targets. The output of this effort was 100 new soluble proteins per year from genetically validated targets.

Dr. Petrovich's current role in the Laboratory of Structural Biology is to establish a protein expression core facility. Current ongoing projects include setting up a method to rapidly determine the best method to express a protein from a gene. This effort includes *E. coli*, baculovirus/insect cell and mammalian cell expression systems. Dr. Petrovich also plans to pursue the implementation of general refolding techniques to produce soluble proteins.

Publications:

Petrovich, R.M. and Jaffe, E.K.: Magnetic resonance studies on the active site and metal centers of Bradyrhizobium japonicum porphobilinogen synthase. Biochemistry 36: 13421-13427, 1997.

Petrovich, R.M., Litwin, S., and Jaffe, E.K.: Bradyrhizobium japonicum porphobilinogen synthase uses two Mg(II) and monovalent cations. J. Biol. Chem. 271: 8692-8699, 1996.

Petroivch, R.M., Ruzicka, F.J., Reed, G.H., and Frey, P.A.: Characterization of iron-sulfur clusters in lysine 2,3-aminomutase by electron paramagnetic resonance spectroscopy. Biochemistry 31: 10774-10781, 1992.

Petroivch, R.M., Ruzicka, F.J., Reed, G.H., and Frey, P.A.: Metal cofactors of lysine-2,3-aminomutase. J. Biol. Chem. 266: 7656-7660, 1991.

TRAINING AND MENTORING

2003 NIEHS/NTA Science and Career Fair

The Sixth Annual NIEHS/NTA Biomedical Science and Career Fair was held on April 25, 2003 in the Rodbell Conference Center, NIEHS. The keynote speaker was Dr. Yvonne T. Maddox, Deputy Director, National Institute of Child Health and Human Development, NIH, DHHS. The panel discussion this year focused on "Career Opportunities in Science." It was moderated by Dr. Thomas Kunkel, Chief, Laboratory of Structural Biology, and Scientific Program Director, Environmental Biology Program, NIEHS. Panel participants included Dr. Maddox; Dr. Mohammed Bourdi, Staff Scientist, Laboratory of Molecular Immunology, NHLBI, NIH; Dr. Allison Chausner, Health Scientist Administrator, Division of Neuroscience and Behavioral Research, Translation Research Branch, NIDA, NIH; Dr. Adnan Hammad, Director, Community Health and Research Center, Detroit, MI; Dr. J. Eric McDuffie, Senior Scientist, Toxicologic Pathology, Pfizer Global Research and Development, Ann Arbor, MI; Dr. Viviana Simon, Program Manager, Society for Women's Health Research, Washington, D.C.; and Dr. Abdelkrim Smine, Senior Research Scientist, Global Assistance Initiative, United States Pharmacopeia, Rockville, MD. Other events at the Science and Career Fair included a poster session with 62 posters and a Career Fair with 17 participating companies.

There were more than 270 registered attendees from universities and research institutions in the Triangle Area and the rest of North Carolina. The NIEHS, CIIT Centers for Health Research, the Burroughs Wellcome Fund, S & M Separation Technologies, Inc., Taylor and Francis, Merck and Co., and Sigma Xi, cosponsored this event.

International Activities in the DIR 2002

Dr. Kamel Abdo (Toxicology Operations Branch) has a collaborative study with scientists at the Department of Community, Environmental, and Occupational Medicine, Faculty of Medicine, Ain Shams University, Cairo, Egypt to investigate the association between pesticide use in Egypt and occurrence of different cancers among Egyptians and a collaboration with scientists at the Center for Environmental and Occupational Health Sciences, Birzeit University, Ramallah, Palestine to determine indices of nutritional status of children.

Dr. Steven Akiyama (Laboratory of Molecular Carcinogenesis and Deputy Scientific Director) served on a Grant Review Committee for the Italian Ministry for Education University and Research and as an external grant reviewer for the Michael Smith Foundation for Health Research, Vancouver, BC, Canada.

Dr. Trevor Archer (Chief, Laboratory of Molecular Carcinogenesis) has collaborative research projects with scientists at the Child Health Research Institute, University of Western Ontario, London, Ontario, Canada to perform a functional analysis of CpG methylation in the BRCA1 promoter region.

Dr. David Armstrong (Acting Chief, Laboratory of Environmental Neuroscience) has a collaboration with scientists in the Department of Physiology at the University of Edinburgh to study the structural basis for potassium channel regulation by the cAMP-dependent protein kinase and is serving on a Grant Review Committee for the Italian Ministry for Education University and Research for the topics of calcium signaling and signal transduction.

Dr. Donna Baird (Epidemiology Branch) is working with Drs. Clare Weinberg (Biostatistics Branch), Allen Wilcox (Epidemiology Branch), and Donna Baird (Epidemiology Branch) in collaboration with researchers at the Department of Epidemiology and Social Medicine, University of Arhus, Denmark, to study pre-eclampsia and infertility through the Danish National Birth Cohort, an ongoing prospective study of pregnancies in Denmark.

Dr. Perry Blackshear (Director, Office of Clinical Research and Laboratory of Signal Transduction) has collaborations with scientists at McGill University, Montreal, Canada to study genetic modifiers of insulin action with PHAS-I knockout mice (which were developed at the NIEHS); with scientists at the Institute of Immunology, Biomedical Sciences Research Center 'Alexander Fleming', Vari, Greece to study interactions between TTP knockout mice and TNF and TNF receptor knockout and knock-in mouse lines; with scientists at the Institute of Clinical Biochemistry and Pathobiochemistry, Medical University Clinic, Würzburg, Germany to study P38 kinase – TTP interactions using TTP knockout mice (which were developed at the NIEHS); with scientists at the University of Manchester, UK to resequence the promoter and exons of the ZFP36 gene, encoding TTP, in University of Manchester population of patients with well-characterized forms of juvenile rheumatoid arthritis; at the University of Udine, Italy to

resequence the promoter and exons of the ZFP36 from patients with rheumatoid arthritis who either responded or didn't respond to anti-TNF therapy; with scientists at the University of Zurich, Switzerland to study interstitial cell MARCKS and MLP expression in the normal kidney and in kidneys of mice with fibroproliferative diseases; with scientists in the Department of Applied Biochemistry and Biology, Faculty of Agronomy, Gembloux, Belgium to study interactions between bovine leukemia virus, HTLV, and TTP in the pathogenesis of bovine leukemia; with scientists in the Division for Immunology, Zurich University, Switzerland to evaluate TTP and TNF mRNA kinetics and responses in farm children exposed to low or high endotoxin levels; with scientists in the Department of Molecular Genetics, The Weizmann Institute of Science, Rehovot, Israel to work on MARCKS and MLP in animal models of lissencephaly syndromes; with scientists in the Department of Veterinary Microbiology, University of Saskatachewan, Saskatoon, Canada to work on *Trypanosoma congolense* infections in TTP deficient mice; with scientists at the University of British Columbia, Canada to evaluate telomere length in mice deficient in a RECQL helicase, which may have a cancer-susceptible phenotype; with scientists at the Zentrum für Molekulare Neurobiologie, Universität Hamburg, Germany to study MARCKS interacting proteins and peripheral nerve migration; and with scientists in the Department of Pathology, Yonsei University, College of Medicine, Seoul, Korea to work on mononucleotide repeats in MARCKS sequences in colon cancer. Dr. Blackshear also has a Cooperative Research and Development Agreement with Oxford Glycosciences, Abingdon, UK to look at proteomics modifications in diabetes as indicators of disease status and status of complications.

Dr. Rajendra Chhabra (Toxicology Operations Branch) was invited by the International Program on Chemical Safety to participate in, and serve as a WHO Temporary Adviser to, the Tenth Final Review Board Meeting for Concise International Chemical Assessment Documents held in Monks Wood, UK, September, 2002 to review and finalize draft Concise International Chemical Assessment documents (CICADs), which are intended to provide an assessment of the health and environment hazards and risks of chemicals, together with advice on prevention of exposure and protective measures. The draft CICADs finalized in this meeting were arsine, 1,1-dichloroethane, ethylene oxide, hydrogen sulphide and sulphides, thiourea, and trichloropropane.

Theodora Devereux (Laboratory of Molecular Carcinogenesis) hosted the sabbitical of a scientist from Queens University, Kingston, Ontario, Canada in her lab to collaborate on a study to examine global expression changes in sets of mouse lung tumor cell lines with different invasiveness based on movement through Matrigel.

Dr. John Drake (Chief, Laboratory of Molecular Genetics) is serving as the DHHS mentor and a collaborator with scientists in Tbilisi, Georgia at the G. Eliava Institute of Bacteriophages, Microbiology and Virology. This is a Biotechnology Engagement Program (BTEP) project entitled "Study of Phage-Specific "Killer" Proteins" to understand just how bacteriophages used as antibiotics kill at the molecular level. He also has a collaborative research program with scientists at the Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw investigating the structural basis

of DNA polymerse fidelity and serves on the Executive Board of the International Genetics Federation, an umbrella organization of numerous national genetics societies.

Dr. E. Mitch Eddy (Laboratory of Reproductive and Developmental Toxicology) served as an external examiner for grant applications to the Michael Smith Foundation for Health Research, Vancouver, BC, Canada; the Wellcome Trust, London, England; Comitato Telethon Fondazione ONLUS, Rome, Italy; and the National Health and Medical Research Council, Australia and is serving as the NIH mentor for an MD-PhD student in the Tel Aviv University – NIH Program for Israeli Predoctoral Biomedical Researchers on a project is to isolate cDNAs for the ubiquitously expressed calpain 1 and calpain 2 and the spermatogenic cell-specific calpain 11. Dr. Eddy has collaborations with researchers at the Department of Anatomy and Reproductive Cell Biology, Miyazaki Medical College, Miyazaki, Japan to clone cDNAs for proteins involved in fertilization; with scientists in the Department of Life Sciences, Kwangju Institute of Science and Technology (K-JIST), Kwangju, Korea to produce a conditional mutant for protamine 2; with scientists in the Department of Bioenvironmental Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan to target sequences for the spermatogenic cell-specific form of type 1 hexokinase; with scientists in the Department of Embryology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel to express and localize calpain-1, -2, and –11 in spermatogenic cells; with scientists at the Instituto de Biologia y Medicina Experimental, Buenos Aires, Argentina to produce a targeted mutation of the gene encoding epididymal protein DE; with scientists at the Monash Institute of Reproduction and Development, Monash University, Clayton, Victoria, Australia to study genetics of human male infertility; with scientists in the Laboratory of Experimental Animals, Department of Molecular Biology and Immunology, National Institute of Agrobiological Sciences, Tsukuba, Japan to study regulation of expression of genes essential for male fertility.

Dr. John French (Laboratory of Molecular Toxicology) organized and participated the International Union of Toxicology workshops in Nanjing, China in October 2001 and organized and participated in the International Workshop on Genetic Toxicology in Plymouth England in June 2002.

Dr. Dori Germolec (Laboratory of Molecular Toxicology) is currently serving on the World Health Organization International Program on Chemical Safety (IPCS) Task Group to compose the Environmental Health Criteria “Scientific Principles and Methods for Assessing Autoimmunity Associated with Exposure to Chemicals;” and on the organizing committee for the US/Japan joint meeting entitled “Arsenic in Biology and Medicine,” the purpose of which was to discuss the dose-response relationship for cancer and non-cancer endpoints for target organs of arsenic toxicity and to determine the relevance of low-level effects relating to these endpoints in light of increased worldwide

Dr. Beth Gladen (Biostatistics Branch) has collaborations with investigators at the Institute of Pediatrics, Obstetrics, and Gynecology, Kyiv, Ukraine; the National Medical University, Kyiv, Ukraine; Kyiv Medical Academy of Post-Diploma Education, Kyiv, Ukraine, and the University of Bristol, Bristol, UK to examine pollution and reproductive outcomes in two cities in Ukraine; with scientists at Health Canada, Ottawa, Canada to examine patterns of exposure to different polychlorinated biphenyl congeners in milk samples collected from women across Canada in 1992 in order to determine whether health effects of different congeners could be examined separately; with scientists at the University of Southern Denmark, Odense, Denmark; DK-Teknik Energy and Environment, Søborg, Denmark; Erasmus University, Rotterdam, The Netherlands; University Hospital, Groningen, The Netherlands; Heinrich-Heine-University, Düsseldorf, Germany; Institute of Environmental Toxicology, Kiel, Germany; and Laval University, Beauport, Canada on studies of neurodevelopmental effects of transplacental exposure to polychlorinated biphenyls have used different techniques to assess exposure; and with scientists at the Instituto Nacional de Salud Pública, Cuernavaca, México to study effects of the antiandrogen DDE on anthropometric measures at birth.

Dr. Joyce Goldstein (Laboratory of Pharmacology and Chemistry) has a collaboration with scientists from the Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Thailand on an analysis of CYP2C19 polymorphisms in Thai populations.

Dr. Traci M.T. Hall (Laboratory of Structural Biology) has a collaboration with scientists at the Agricultural Biotechnology Center, Plant Biology Institute in Gödöllõ, Hungary to determine the three-dimensional structures of plant viral proteins that suppress post-transcriptional gene silencing.

Dr. Ronald Herbert (Laboratory of Experimental Pathology) attended the World Health Organization/International Agency for Research on Cancer (IARC) Monograph Committee and Chaired the Carcinogenesis Working Group, IARC Working Group Meeting to prepare Volume 82 of IARC Monographs Series on Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene, Lyon France, in February, 2002.

Dr. William Jameson (National Toxicology Program) was the National Toxicology Program representative at the International Agency for Research on Cancer (IARC) Working Group meeting in Lyon, France in February to review the cancer data for some traditional herbal medicines, some mycotoxins, naphthalene and styrene. The reviews and evaluations of this Working Group resulted in the publication of the IARC Monograph on the Evaluation of Carcinogenic Risks to Humans, Vol. 82, Some Traditional Herbal Medicines, some Mycotoxins, Naphthalene and Styrene.

Dr. Anton Jetten (Laboratory of Pulmonary Pathobiology) had collaborations with scientists from the Department of Molecular Medicine, University of Osaka, Osaka, Japan to study the function of the nuclear orphan receptor RORgamma; with scientists at the Department of Mucosal Immunology, University of Tokyo, Tokyo, Japan to study the role of the nuclear orphan receptor in the immune system; with scientists at the Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel to

study the function of p63 in the differentiation of esphageal and tracheal epithelium; and with scientists at the Department of Structural Biology and Structural Genomics, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France to study the structure of the RORgamma protein.

Dr. Steven Kleeberger (Chief, Laboratory of Pulmonary Pathobiology) has a collaboration with researchers at the National Institute of Health and Medical Research, INSERM, Paris to investigate the genetic basis for susceptibility to the effects of coal dust in miners.

Dr. Thomas Kunkel (Chief, Laboratory of Structural Biology) was an organizer of the Symposium on "Structural Biology of Replication and Its Relevance to Mutation Research" at the Eighth International Conference on Environmental Mutagens, Shizouka, Japan, October, 2001; and has collaborations with researchers at the Institute for Molecular and Cellular Biology,Osaka University, Japan to study the efficiency and fidelity of DNA synthesis by human DNA polymerase eta; and with scientists at Centro de Biología Molecular Severo Ochoa (CSIC-UAM) Universidad Autónoma, Madrid, Spain to study the biochemical properties and function of human DNA polymerase lambda.

Dr. Larry Lazarus (Laboratory of Computational Biology and Risk Analysis) has collaborations with researchers at the Department of Pharmaceutical Sciences and Biotechnology Center, University of Ferrara, Ferrara, Italy, the Department of Toxicology, University of Cagliari, Cagliari, Italy, and the Department of Human Physiology "Vitorio Erspamer," Unviversity La Sapienza, Rome, Italy to develope highly specific antagonists and agonists for the δ- and μ-opioid receptors, or bifunctional compounds for both receptors; and with researchers on the Faculty of Pharmaceutical Sciences and High Technology Research Center, Kobe Gakuin University, Kobe, Japan; and the Tohoku Pharmaceutical University, Sendai, Japan to develope unique analogues for the μ-opioid receptor based on simple structural motifs.

Dr. Stephanie London (Epidemiology Branch) has collaborations with scientists at the National Institute of Public Health, Cuernavaca, Mexico to study the genetics of childhood asthma in Mexico City; with investigators at the National University in Singapore and the University of Southern California to investigate the relation between diet and the incidence of asthma and chronic bronchitis in a cohort of 63,000 adult Singaporeans of Chinese ethnicity; and with scientists at the Wuhan Public Health and Anti-Epidemic Station and the University of Southern California to study indoor air pollutants in relation to childhood respiratory symptoms.

Dr. Matthew Longnecker (Epidemiology Branch) has collaborations with scientists at the Institute of Public Health, University of Southern Denmark, Odense, Denmark; the Department of Pediatrics, Division of Neonatology, Erasmus University and University Hospital/Sophia Children's Hospital, Rotterdam, The Netherlands; the Department of Social and Preventive Medicine, Laval University and Public Health Research Unit, CHUQ Research Center (CHUL), Beauport, Quebec, Canada; the Medical Institute of

Environmental Hygiene at Heinrich-Heine-University Duesseldorf, Duesseldorf, Germany; the Perinatal Nutrition and Development Unit, Department of Obstetrics/Pediatrics, University Hospital Groningen, Groningen, The Netherlands; the Institute of Environmental Toxicology, Kiel, Germany; and DK-TEKNIK Energy & Environment, Soeborg, Denmark to study the comparison of polychlorinated biphenyl (PCB) levels across studies of human neurodevelopment; and with researchers at the National Institute of Public Health in Cuernavaca, Mexico to examine the relation between maternal serum levels of the androgenic DDT metabolite DDE in relation to anthropometric measures in 200 male newborns in Tapachula, Mexico, where there has been recent, high-level exposure to DDT.

Dr. James Mason (Laboratory of Molecular Genetics) has collaborations with scientists at the Institute of Science History and Technology, Russian Academy of Sciences, St. Petersburg, Russia, to characterize telomere-telomere interactions in *Drosophila*; with scientists at the University of Rome, Italy, to characterize a mutation in *Drosophila* that causes telomeric repeat arrays to grow to great lengths; and with scientists at the Institute of Gene Biology, Russian Academy of Sciences, Moscow, Russia, to identify and clone a second mutation that increases telomere length in *Drosophila*.

Dr. B. Alex Merrick (National Center for Toxicogeniomics) was an invited to the Human Proteome Organization (HUPO) Liver Proteome Conference in Beijing, China, October 22-24, 2002.

Dr. David Miller (Laboratory of Pharmacology and Chemistry) has collaborations with scientists at the Department of Pharmacology & Toxicology, Nijmegen Center for Molecular Life Sciences, Nijmegen, The Netherlands to characterize the regulation of xenobiotic export pumps in renal proximal tubule; and with scientists at the Institute for Pharmacy and Biotechnology, University of Heidelberg, Heidelberg, Germany to characterize the role of drug export pumps in blood-brain barrier function. Dr. Miller also hosted a scientist from the National Institute of Toxicological Research, Seoul, Korea in his laboratory and provided training in the conduct of assays of renal cell function (transport in renal slices, measurement of slice respiration, confocal imaging of single renal tubules) that can be used to assess effects of nephrotoxicants.

Dr. Fred Miller (Office of Clinical Research) co-chaired with Dr. Lisa Rider (Office of Clinical Research) the International Workshop on Myositis Outcome Measures and Clinical Trial Design Issues. Dr. Miller is also a member of The International Myositis Collaborative Study Group with scientists from Montreal, Canada; Santiago, Chile; Guatemala City, Guatemala; Mexico City, Mexico; Guadalajara, Mexico; Aachen, Germany; Nijmegen, The Netherlands; Warsaw, Poland; Glasgow, Scotland; Barcelona, Spain; Stockholm, Sweden; New Delhi, India; Tokyo, Japan; and Seoul, South Korea which has been organized to collect standardized data and specimens on myositis patients.

Dr. Yuji Mishina (Laboratory of Reporductive and Developmental Toxicology) has a collaboration with scientists at the Brain Science Institute, RIKEN, Saitama, Japan

Group to uncover the function of bone morphogenic protein signaling in brain development.

Dr. Elizabeth Murphy (Laboratory of Signal Transduction) is serving as a member of the International Council of the International Society for Heart Research.

Dr. Masahiko Negishi (Laboratory of Reproductive and Developmental Toxicology) is a member of the International Advisory committee on the 14th International Symposium on Microsomes and Drug Oxidation and has a collaboration with scientists at Kobe Pharmaceutical University, Japan to perform an X-ray crystallographic analysis of glycosyltransferases involved in heparan sulfate synthesis.

Retha Newbold (Laboratory of Molecular Toxicology) worked with DES Action International providing scientific information on DES exposure and animal models and with the World Wildlife Fund reviewing proposals and providing scientific information on endocrine disrupting chemicals. Ms. Newbold also has collaborations with scientists at the University of Rome "La Sapienza," Italy to study effects of environmental estrogens on development of bone tissue; with scientists at the University of Karlsruhe, Germany to study effects of genistein and daidzein on the developing reproductive tract; with scientists at the University Hospital of Copenhagen, Denmark to study effects of genistein on the developing ovary; with scientists at Bar-ilan University, Ramat-Gan, Israel to test a natural antioxidant found in spinach for hormonal activity; and with scientists at the Okazaki National Research Institute, Japan to study effects of endocrine disrupting chemicals on the developing reproductive tract using fetal or neonatal mouse models.

Dr. Christopher Portier (Chief, Laboratory of Computational Biology and Risk Analysis) participated as a plenary speaker and session chair at the conference on "Light, Endocrine Systems and Cancer" on May 2-3, 2002 in Cologne, Germany sponsored by the German Research Council (DFG); participated in a workshop on "Hepatic preneoplasia: quantitative evaluation in carcinogenesis biossays and relevance for human hepatocarcinogenesis" on June 29-30, 2002 in Heidelberg, Germany; served as the chair of the Science Advisory Board for one aspect of the Finnish Academy of Sciences, Centers of Excellence Program; was asked by the German Cancer Research Insitute (DKFZ) to review research directions on the use of the rat liver focus bioassay by the DKFZ; and represented the Department of Health and Human Services at the Global Mercury Assessment Working Group in Geneva, Switzerland, at the request of the Office of Science Policy within the Office of the Secretary, DHHS, to outline options for consideration at the twenty-second session of the Governing Council/Global Ministerial Environment Forum of the United Nations Environment Program addressing any significant global adverse impacts of mercury, inter alia, by reducing and or eliminating the use, emissions, discharges and losses of mercury and its compounds; improving international cooperation; and ways to enhance risk communication. Dr. Portier also has a collaboration with researchers at the University of Bern, Switzerland on the analysis of data pertaining to receptor-mediated activation of a number of different cellular constructs.

Dr. Michael Resnick (Laboratory of Molecular Genetics) has collaborations with researchers at the National Institute for Cancer Research in Genoa, Italy to study partial-function p53 mutations; with researchers in the Unit of Molecular Carcinogenesis, International Agency for Research on Cancer (WHO), Lyon, France to analyze partial function mutations of p53 and develope a p53 database that includes functional alterations and clinical features; with researchers in the Molecular Immunology Unit, Institute of Child Health, University College London, England to develop an inducible enzyme system in mammalian cells that provides for the production of a single, unique double-strand break in cells of humans; with researchers at the Institute of Veterinary Biochemistry and Molecular Biology, University of Zürich-Irchel, Switzerland to conduct an extensive structure-function investigation of human FEN1 nuclease; and with researchers in Chromosome Replication Group, Laboratories for Biomolecular Networks, Graduate School of Frontier Biosciences, Osaka University, Japan to study a mutation in yeast DNA polymerase epsilon that results in hypermutation and characterize effects of hypermutation on the proofreading (error-correction) function of this protein. Dr. Resnick has also organized an international meeting “Functional consequences of TP53 mutations" to be held at IARC, in Lyon, France, from June 30 to July3, 2003 to explore the importance of various p53 functional mutations and their relevance to cancer as well as to develop further the existing p53 database as IARC.

Dr. John Roberts (Laboratory of Molecular Carcinogenesis) has a collaboration with scientists at the Institute of Cell Biology and Immunology, University of Stuttgart, Germany to work on PKC-mu and its role in tumor cell adhesion to the extracellular matrix.

Dr. Walter Rogan (Epidemiology Branch) was an organizer and host of a multinational meeting in Hanoi to study people affected by Agent Orange, a defoliant used by US troops during the Vietnam War and help to edit the proceedings of the meeting. Dr. Rogan continued 18 years of work in collaboration with scientists at the National Cheng Kung University Hospital in Taiwan to follow children transplacentally exposed to high levels of heat-degraded PCBs.

Dr. Dale Sandler (Acting Chief, Epidemiology Branch) has a collaboration with researchers at the Prague Institute of Advanced Studies, Prague, Czech Republic and the Center for Epidemiological Studies, Pribram, Czech Republic to study cancer risk among underground uranium miners in the Czech Republic.

Dr. Roel M. Schaaper (Laboratory of Molecular Genetics) has collaborations with scientists at the Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland to study mechanisms of DNA replication fidelity; and with scientists in the Department of Genetics, St. Petersburg State University, St. Petersburg, Russia to study base analog detoxification by molybdenum-dependent activities, research that is supported by a Collaborative Linkage Grant awarded by NATO.

Dr. James Selkirk (National Center for Toxicogenomics) organized and hosted the US - Japan Panel on Environmental Genomics and Carcinogenesis. This meeting centered around the use of expression array technologies for genes, and new developments in proteomics and emphasized mutual areas of public health interest to both countries and serves as a venue to exchange ideas and collaborative research.

Dr. Steven Shears (Laboratory of Signal Transduction) has collaborations with researchers in the Department of Chemistry, Pohang University of Science and Technology, Korea to characterize a novel, physiologically-relevant reversible kinase/phosphatase with the goal of developing new therapy for both enhancing and promoting mucous secretion (for the common cold, bronchitis and cystic fibrosis); with researchers in the Department of Physiological Sciences, Lund University, Sweden to study the regulation of insulin secretion by inositol phosphates and the relevance to the etiology of type II diabetes; and with researchers at the Institut de Recherches Microbiologiques Jean-Marie Wiame, Université Libre de Bruxelles, Belgium to study yeast as a model for understanding the participation of inositol phosphates in cell responses to environmental stress.

Dr. William Stokes, (National Toxicology Program and Director, NTP Interagency Center for the Evaluation of Alternative Toxicological Methods) participated in a European Centre for the Validation of Alternative Methods scientific symposium on the status of alternative methods convened to recognize ECVAM's tenth anniversary in June 2002 and presented an overview of current and proposed collaborations between NICEATM, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), and ECVAM; and NICEATM and ICCVAM, in partnership with the International Life Sciences Institute (ILSI), served on the organizing committee for an international training workshop on *in vitro* and *in vivo* alternative acute toxicity testing methods, which met on February 19-21, 2002, to provide practical information and case studies to facilitate understanding and the implementation of *in vivo* and *in vitro* alternative methods for acute toxicity.

Dr. Kenneth Tomer (Laboratory of Structural Biology) served as an expert in separations for the EU 5th Microproteomics Consortium meeting, July, 2002 in Konstanz, Germany, coordinating development of microfluidic/ma

the session on base excision repair. He also made a presentation on "Protection against genomic damage by base excision repair and DNA polymerase beta" at the 32nd Annual Meeting of European Environmental Mutagen Society, "DNA Damage and Repair Fundamental Aspects and Contribution to Human Disorders" in Warsaw, Poland.

Dr. Jerrel Yakel (Laboratory of Signal Transduction) has collaborations to study co-assembly of nicotinic acetylcholine receptor α7 and β2 subunits to form functional heteromeric nicotinic receptor channels with researchers from the Department of Pharmacology, University College London, England; and with researchers form the Johannes A. van Hooft, University of Amsterdam, Swammerdam Institute for Life Sciences, Amsterdam, the Netherlands to characterize the function and structure of serotonin 5-HT_3 receptors in rat CA1 hippocampal interneurons.

Dr. Darryl Zeldin (Laboratory of Pulmonary Pathobiology) had a collaboration with scientists at the University of Bochum and St. Josef Hospital, Bochum, Germany to study variants in the human *CYP2J2* gene and with scientists at the Tongji Medical Center, Tongji, Peoples Republic of China to study the regulation of endothelial nitric oxide synthase (eNOS) by endothelium-derived hyperpolarizing factors (EDHF) and the relevant signaling pathways involved.

The NIEHS had two projects as part of the Congressionally mandated Agent Orange initiative in Vietnam. The first project, a workshop on the health and environmental effects of Agent Orange/Dioxin in Vietnam was held in March in Hanoi. The second project is ongoing and is focused on the validation of cell-based assays for measuring dioxin levels in soils.

The NIEHS and the National Toxicology Program (NTP) have signed a Memorandum of Understanding (MOU) with the Ramazinni Foundation. The Ramazinni Foundation supports a toxicology program in Bologna which conducts large carcinogenesis bioassays similar to those conducted by the NTP. This MOU sets up a coordinating body between the two agencies to avoid duplication of effort and to provide computing and statistical support to the Ramazinni Foundation by the NTP.

The Korean Government just initiated a new National Toxicology Program. The NIEHS and the US NTP began the development of joint programs with the Korean NTP.

The NTP is collaborating with the World Health Organization to coordinate and summarize research on radiofrequency electric and magnetic fields. This collaboration is aimed at reducing duplication of effort and identifying data gaps in the research on cellular phones that might be met by the NTP.

NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) initiated a joint collaborative validation study in July 2002 with the European Centre for the Validation of Alternative Methods (ECVAM) on *in vitro* methods for assessing acute systemic toxicity. Two U.S. labs and one European lab are conducting the studies, which will evaluate the usefulness of cytotoxicity data for

estimating the acute systemic toxicity potential of chemicals. Preliminary data indicate that *in vitro* cytotoxicity data will reduce the number of animals required and reduce the number of deaths that occur from acute toxicity studies.

NICEATM and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) organized an international expert panel meeting to assess the validation status of *in vitro* assays proposed for use in the EPA's Endocrine Disruptor Screening Program (EDSP) on May 21-22, 2002, which involved scientists from the UK, Canada, Japan, and Denmark, to review estrogen receptor (ER) and androgen receptor (AR) binding and transcriptional activation (TA) assays, provide recommendations on test methods and technologies that should be given priority for further development and validation, and recommend substances that should be used for validation studies, and minimum procedural standards that should be incorporated in the various assays.

National Toxicology Program (NTP)
Update May 2003

New Database Available

The NTP is creating a new database to allow searches of its study data using the web. Currently the NTP is developing programming tools for accessing the data, and is interested in obtaining feedback from the public on its use of these new searches.

To access the database and do a search go to the NTP homepage (http://ntp-server.niehs.nih.gov) and select "NTP Study Information." On this page are new options for accessing the database and doing a search. (Hyperlinks on this page are shown here in italics.) Go to:

SEARCH THE NTP STUDIES DATABASE
Available Data on Individual Studies
Pathology
Incidence rates for completed chronic and prechronic studies collected in the Toxicology Database Management System (TDMS)
Individual animal pathology data for completed chronic studies collected in the NTP's Toxicology Database Management System (TDMS) and Carcinogenesis Bioassay Data System (CBDS)

The first search category "Available Data on Individual Studies" allows the user to enter a chemical name or CAS # and retrieve information about the types of studies (*e.g.*, chronic exposure studies, reproductive/development, genetic toxicity, etc.) that are completed and to determine which studies have data available in electronic format. The types of data that might be available are clinical chemistry, hematology, organ weights, body weights, survival, clinical observations and pathology.

The second category, "Pathology" includes two links to search through the NTP pathology databases. Under this heading the first link retrieves the incidence rates and the second link retrieves individual animal evaluations.

The NTP welcomes receiving input from persons who try the database. Please send your queries, comments, and suggestions to: ntpwm@niehs.nih.gov

NTP Board of Scientific Counselors

The next meeting of the NTP Board of Scientific Counselors Technical Reports Review Subcommittee is scheduled for May 22, 2003 at the NIEHS. This subcommittee of the NTP Board of Scientific Counselors meets regularly to review the findings and conclusions of NTP toxicology and carcinogenesis studies.

The primary agenda topic is the peer review of six draft Technical Reports (TR) of rodent toxicology and carcinogenesis studies conducted by the NTP. At this meeting the NTP will unveil a new technical report series for studies using genetically modified models. The first two reports in this series are on aspartame and acesulfame potassium.

Chemical (Primary Uses)	**Report #**
Propylene glycol mono-*t*-butyl ether (Solvent)	TR 515
2-Methylimidazole (Chemical and pharmaceutical intermediate)	TR 516
Triethanolamine (Industrial and manufacturing applications)	TR 518
Stoddard solvent IIC (Paint and dry cleaning solvent)	TR 519
Aspartame (Artificial sweetener)	GMM 1
Acesulfame potassium (Artificial sweetener)	GMM 2

Draft reports, agenda and roster of subcommittee members will be available for public prior to the meeting and summary minutes will be available following the meeting. (See "What's New?" on the NTP web homepage at http://ntp-server.niehs.nih.gov)

Satellite Symposium at Society of Toxicologic Pathology Meeting

NTP is co-sponsoring a satellite symposium with EPL, Inc. entitled “An Exercise In Peer Review: The Pathology Working Group”. It will be held at the annual meeting of the Society of Toxicologic Pathology on Saturday, June 14, 2003 in Savannah, Georgia.

The objective of this symposium is to provide continuing education on some basic and common lesions seen in toxicity and carcinogenicity studies and to generate lively and productive conversation about controversial and/or uncommon lesions.

This satellite symposium will present a mock pathology working group with audience participation. After cases are presented, the audience will vote on the diagnosis, and a brief discussion will follow. The audience will be equipped with voting units allowing for the instantaneous collection and display of responses. Cases will be available to registered attendees on May 15, 2003 via a link on the Society of Toxicologic Pathology web page: http://www.toxpath.org/

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

NTP-CERHR Monograph on Phthalates

The CERHR has a new monograph series and the first is "NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-*n*-Butyl Phthalate (DBP)." The monograph is available to the public and was sent to appropriate federal and state health and regulatory agencies.

This monograph includes three parts:

1) the NTP brief, which presents the NTP's interpretation of the available data and its conclusions on the potential for DBP to cause adverse developmental and reproductive effects in humans,
2) the expert panel report, and
3) all public comments on the expert panel report.

The CEHRH convened expert panels to evaluate seven phthalates. The monographs for the other six are in process and will be posted on the CERHR web site when completed.

Ethylene Glycol and Propylene Glycol Exposures Panel Convened

The CERHR convened an expert panel on February 11-13, 2003, in Alexandria, Virginia, to evaluate whether or not exposure to ethylene glycol or propylene glycol is a reproductive and/or developmental hazard.

Ethylene glycol was selected because

1) it is a high production volume chemical,
2) there is the potential for widespread occupational and general population exposures due to its use in heating and cooling systems (e.g., automotive antifreeze), and
3) there is published evidence from laboratory studies of developmental toxicity resulting from its exposure.

Propylene glycol is used commercially as an intermediate in the manufacture of unsaturated polyester resins and in the production of plasticizers. It was selected for evaluation because of

1) its similarity in structure to ethylene glycol and
2) the potential for widespread human exposure through its use in food, tobacco, pharmaceutical products, cosmetics, various paints and coatings and as an antifreeze and de-icing solution.

The expert panel reviewed and evaluated the available scientific evidence on ethylene glycol and propylene glycol in three primary areas: human exposure, reproductive and

developmental toxicity, and metabolism. They considered the quality, quantity and strength of the evidence in their deliberations about the potential for either chemical to cause adverse effects on human reproduction and/or development.

For ethylene glycol, the expert panel concluded that there was "negligible concern" for developmental toxicity and reproductive toxicity at current estimated levels of human exposure.

For propylene glycol, the expert panel concluded "that current estimated exposures to propylene glycol are of negligible concern for [causing] reproductive or developmental toxicity in humans."

The reports from the evaluations of ethylene glycol and propylene glycol will be posted on the CERHR website (http://cerhr.niehs.nih.gov) and made available from the CERHR in printed text. The CERHR will solicit public comment on the reports through an announcement in the Federal Register. Following this comment period, the CERHR will prepare an NTP-CERHR monograph on each chemical.

Workshop on Reproductive Effects of Thyroid Toxicants

The CERHR sponsored a workshop "Thyroid Toxicants: Assessing Reproductive Health Effects" on April 28 - 29, 2003 at the Holiday Inn Old Town Select Hotel in Alexandria, Virginia. The objectives of this workshop are two-fold:

1) To discuss the optimal design of tests to detect adverse reproductive and developmental effects resulting from chemical-induced thyroid dysfunction.
2) To discuss the relevance of thyroid-related adverse reproductive and developmental effects observed in rodents for predicting adverse effects in humans.

The agenda included plenary talks with time set aside for general discussion. Additional information is available on the CERHR web site (http:://cerhr.niehs.nih.gov).

Future Expert Panel Evaluations

The CERHR plans to conduct expert panel evaluations on the potential reproductive and/or developmental toxicity of fluoxetine hydrochloride (Prozac®; Sarafen™, and acrylamide. Dates for the two expert panel meetings are not yet set, but are tentatively planned for late 2003 and early 2004.

Fluoxetine hydrochloride (Prozac®; Sarafem), an antidepressant, was selected due to sufficient reproductive and developmental animal data, human exposure information, and public concern. Under the name Sarafem™, it is being prescribed to treat premenstrual dysphoric disorder (PMDD), potentially increasing the number of exposures to women of childbearing age. The FDA recently approved it for use in 7-17 year-olds.

Acrylamide (CAS RN 79-06-1) is used in the production of polyacrylamide, in molecular biology procedures such as electrophoresis, and in the synthesis of dyes, adhesives, contact lenses, soil conditioners, and permanent-press fabrics. It is a neurotoxicant and in animal studies has been shown to be a carcinogen, germ cell mutagen, and reproductive toxicant. Acrylamide was selected due to the recent public concern for human exposures through its presence in starchy foods treated at high temperatures, *e.g.*, french fries, potato chips. There are recent data available on occupational exposure, bioavailability, and reproductive toxicity.

Special Session at the SOT 42nd Annual Meeting and ToxExpo 2003

"Medicinal Herbals and Dietary Supplements"

Dr. Cynthia S. Smith of the Laboratory of Pharmacology and Chemistry was a co-presenter at a well-attended continuing education course on herbals and dietary supplements offered at the annual meeting of the Society of Toxicology (SOT) annual meeting in March 2003. Her topic was the characterization and use of herbal medicines and dietary supplements in bioassays. The NTP is presently conducting studies on the following medicinal herbals and herbal components: aloe vera gel, black cohash, comfrey, ginseng and ginsenosides, goldenseal, kava kava, pulegone, thujone, and extracts of grape seed, pine bark, black walnut, *Echinacea purpurea*, *Ginkgo biloba* and milk thistle.

Medicinal herbals and other dietary supplements are consumed by an estimated one-third of the U.S. population. Over 1500 botanicals are sold as dietary supplements, or ethnic traditional medicines. Their use has increased substantially since passage of the 1994 Dietary Supplement Health and Education Act. Herbal formulations are not subjected to FDA pre-market toxicity testing to assure their safety or efficacy. However, there is an increased public awareness of the need to conduct toxicity studies on herbs and herbal ingredients and many government and private laboratories are contributing to this effort.