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
September 9-10, 2002

The National Advisory Environmental Health Sciences Council was convened for its one hundred seventh regular meeting on September 9, 2002, 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 5:00 p.m.. The meeting was closed for consideration of grant applications on September 10, from 9:30. a.m. until 12:00 p.m.. Dr. Kenneth Olden presided as Chair on September 9-10, 2002.

Members Present:

Daniel Baden, Ph.D.
Joan Cranmer, Ph.D.
Dale Eastman
George Friedman-Jimenez, M.D.
Michael Gallo, Ph.D.
Bernard Goldstein, M.D.
Barbara Hulka, Ph.D.
Phil Iannaccone, M.D., Ph.D.
Daniel W. Nebert, M.D.
Martyn T. Smith, Ph.D.
James G. Townsel, Ph.D.

Members Absent:

Deeohn Ferris, J.D.
Hon. Harriett M. Wieder
Frederick P. Guengerich, Ph.D.

Ex Officio Members Present:

Kelley Brix, Ph.D.

Ex Officio Members Absent:

Eric L. Stephens

Liaison Members Present:

Robert Spengler, Ph.D.

Members of the Public Present:

Debra Nickerson, Ph.D.
David Cox, Ph.D.
George Thurston, Ph.D.

NIEHS Staff:

Kathy Ahlmark
Janice B. Allen, Ph.D.
Beth Anderson
Lisa Archer
Trevor Archer, Ph.D.
Martha Barnes
Linda Bass, Ph.D.
Sharon Beard
PJ Blachshear, M.D.
David Brown
Gwen Collman, Ph.D.
Allen Dearry, Ph.D.
Dwight Dolby
Dorothy Duke
Sally Eckert-Tilotta, Ph.D.
Lerlita Garcia
Kimberly Gray, Ph.D.
William Grigg
Mike Humble, Ph.D.
Ethel Jackson, D.D.S.
Laurie Johnson
Marian Johnson-Thompson, Ph.D.
Annette Kirshner, Ph.D.
Cindy Lawler, Ph.D.
Charle League
Edith Lee
Francine Little
Carolyn Mason
Patrick Mastin, Ph.D.
Michael McClure, Ph.D.
Roseanne McGee
Sheila Newton, Ph.D.
Liam O'Fallon
Joan Packenham, Ph.D.
Jerry Phelps
Chris Portier, Ph.D.
Larry Reed
Susan Ricci

Jacqueline M. Russell
Anne P. Sassaman, Ph.D.
Carol Shreffler, Ph.D.
Shobha Srinivasan, Ph.D.
William Suk, Ph.D., M.P.H.
Anne Thompson
Claudia Thompson, Ph.D.
Fred Tyson, Ph.D.
Bennett Van Houten, Ph.D.
Jose Velazquez, Ph.D.
Charles Wells, Ph.D.
Brenda Weis, Ph.D.
Laura Williams-Boyd
Samuel Wilson, M.D.
Michelle A. Owens
Carolyn Winters
Geraldine Wolfle
Leroy Worth, Ph.D.

Other Federal Staff:

Patricia Greenwel, Ph.D. - CSR, NIH
Peggy Jones - FDA
Christine Hileman - FDA
Jeanne Radar - FDA
Paul M. Kuznesof - FDA

I. CALL TO ORDER AND OPENING REMARKS

TThe one hundred seventh regular meeting of the National Advisory Environmental Health Sciences Council was called to order by Dr. Olden.

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 MAY 20, 2002, MEETING

Council accepted the minutes without change.

FUTURE COUNCIL MEETING DATES

February 15-16, 2003 NIEHS
May 19-21, 2003 NIEHS (including Leadership retreat)
September 15-16, 2003 NIEHS

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

Dr. Olden began his report by commenting on the NIEHS push to have environmental health a top priority in NIH/DHHS and this looks like it will bode well for the future. The latest "road-mapping" exercise at NIH with Dr. Zerhouni and over 100 extramural individuals involved four different groups. When the results of this exercise are rolled out later, it is expected that environmental health will be prominent.

He noted that the doubling of the NIH budget is scheduled to end with the Fiscal Year 2003 budget. However, buying power has not increased with doubling, increasing by only 70%. The outlook for increases in future years is uncertain, and may be less than the cost of living. Interest groups are advocating 8-9% increases. In order to get an "above the NIH average" increase, institutes will have to have a compelling agenda. NIEHS will be convening a group of extramural investigators to focus on new opportunities in gene/environment interactions and what research is needed. This group will include the council members. We will need to frame in a new matrix and effectively communicate "game-changing research".

With regard to compelling issues in environmental health science, to be competitive in the current environment, we must think in term of deliverables. What are the advances and what is the impact of these on the public? NIEHS must be proactive as we have been for such programs as the Environmental Genome Project, mouse centers, environmental justice, toxicogenomics, the Parkinson's Disease Consortium, and the Children's Health Centers. Dr. Olden stated that most advances will occur at the interface of major disciplines, including math, information technology, chemistry, and engineering. Trans-NIH collaborations are essential for achieving this, and these collaborations are a priority for the new NIH Director. In addition, the National Academy of Sciences is looking at the NIH structure to see if most effective way of doing this.

The NIH director has asked the institutes to consider how to enhance synergy and novel ways of conducting research and also enabling technologies and methods. NIEHS has used this approach in setting our agenda. Communication to the public is important also. For example, estrogen is to be listed as known human carcinogen, and this will require explanation and clarification.

Dr. Olden then briefly mentioned items of interest from NIEHS programs and activities:

- The information system developed by the Program Analysis Branch, Division of Extramural Research and Training, is being used for tracking the publications of grantees and will be used to investigate the impact of our portfolio on the practice of medicine and public health in a number of areas.
- A press conference in August marked the roll-out of the Parkinson's Disease Consortium. NIEHS, particularly Dr. Cindy Lawler, has provided leadership in developing these programs, focused on gene-environment interactions.
- A recent Senate hearing was held, focusing on air pollution and health. NIEHS was able to provide information on the state of the science.

- Environmental Health Perspectives is now distributed in 137 countries. The June issue emphasized school safety.
- NIEHS has produced Public Service Announcements (PSAs) in both English and Spanish. These were distributed to 500 television stations and aired 2200 times in July alone.

V. Report of the Deputy Director, NIEHS - Dr. Samuel Wilson

Dr. Wilson began his report by commenting on future workshops and town meetings involving NIEHS: an NIEHS/USGS workshop as part of an interagency project on geospacial tools for exposure assessment; a Symposium on Functional Genomics in December, Marin County Town Meeting, October 7-8, 2002; the San Antonio Town Meeting, January 7-8, 2003, and the Miami Town Meeting in February 2003.

He noted that reports from the Institute of Medicine (IOM) Roundtable from meetings on Cancer and the Environment and also Gene/Environment Interactions are available. Topics to be considered for future meetings regarding genomics are biomarker validation (translational research), databases (trans-government effort) and animal models. Another publication, Biomarkers of Environmentally - Associated Disease, resulted largely from an NIEHS-sponsored meeting in Anchorage, Alaska.

The 1st US/Japan DNA Repair Meeting will be held October 27-31 in Sindai, Japan. This meeting will act as an outreach to stimulate research in Japan. NIEHS has an important role at this meeting.

VI. The Human Health Effects of Particulate Matter in Air Pollution - Dr. George D. Thurston, Sc.D.

Dr. Thurston published epidemiological and toxicological studies that collectively indicate that exposure to particulate matter (PM) air pollution can be associated with a wide range of adverse health effects, including increased risk of asthma attacks, hospital admissions, and mortality. The latest study answers questions raised by previous studies, such as inadequate follow-up and inadequate consideration of various gaseous exposures. The results have been published in the Journal of the American Medical Association, March 6, 2002, Vol. 287, No. 9.

People most affected by ambient pollen are older adults, persons with pre-existing lung disease, children, healthy people who work/exercise outdoors and person with inadequate health care. Coal fired power plants are the number one source of fine particulates.

Following Dr. Thurston's presentation, Council member Dr. Hulka made some prepared comments and Dr. Thurston responded with additional information on early measurement of fine particles and ecological variation, etc.

VII. SNPing in the Human Genome - Deborah A. Nickerson, Ph.D.

Dr. Nickerson was introduced by Dr. Jose Velazquez, who gave a brief history of the Environmental Genome Project, noting that the discovery process has now been expanded to include haplotypes.

One of the current major challenges in human genetics is identifying and correlating the patterns of genetic variation within and among individuals with risk to common disease. If common sequence variants confer at least a moderate risk for common disease, then it should be possible to identify susceptibility alleles via association studies in human populations using dense maps of single nucleotide polymorphisms (SNPs). Dr. Nickerson's group is performing large-scale, directed resequencing of human candidate genes to optimize association analysis of disease susceptibility in the human genome. To date, they have sequenced over two megabases of human reference sequence across more than 50 individuals, and have identified and genotyped over 10,000 novel SNPs. The NIEHS SNPs Program at the University of Washington is focused on the systematic identification of SNPs in environmental response genes for use in evaluating the complex interrelationship of genetic susceptibility, environmental exposure, and human disease. The first phase of this work has targeted the identification of common variations in human genes in the DNA repair and cell cycle pathways. These comprehensive data sets are revealing that only a minor fraction of sequence variation has been identified (~15%) although millions of SNPs are currently available in genome databases. They are also exploring the distribution of common haplotypes and linkage disequilibrium across candidate genes and clusters of candidate genes, and are finding that blocks of highly correlated sites can simplify the extraction of the minimal SNP marker sets for large-scale genetic analysis in human populations. These emerging resources are providing the information needed to identify optimal SNP marker sets, dramatically reduce genotyping costs and are increasing statistical power for large-scale genotype-phenotype analysis.

VIII. Identifying the Functional Information Encoded by Mammalian Genomes Using High-Density Oligonuecelotide Arrays - David R. Cox, MD, Ph.D.

Dr. Cox pointed out that genes, through the proteins they encode, interact with challenges from the environment, and that genetics form the basis of understanding these interactions. Variations in DNA sequence are responsible for all inherited aspects of our lives. Most variants are SNPs, and the more ancient the error, the more common the SNP. There is a commonality to variants in genome. Of the variants, 15% are group specific and 85% are common.

Dr. Cox described methodology being used for human high density oligo arrays-a way of resequencing the genomes of multiple individuals to identify human genome variation. The methodology includes chips which contain 400,000 probes and wafers that contain 60 million probes. There are whole wafer scanners and new software to analyze the ultrabytes of data. This was used to analyze chromosome 21 for haplotypes, and 36,500 SNPs were found. The researchers found limited haplotype diversity and also that the SNPs are not independent of one another.

Dr. Cox pointed out that this technology may be used to resequence the genome of multiple inbred mouse and rat strains to identify DNA variations. It may also be used to simplify genetic investigation and focus on environmental components of gene-environment interactions.

IX. Using Polymorphisms to Understand Disease Risk - Jack Taylor, Ph.D.

Dr. Taylor, a member of the Division of Intramural Research's Laboratory of Molecular Carcinogenesis and Epidemology Branch, presented some of his work combining research on exposure analysis and genetics. He pointed out that risk from an exposure may be evident only in genetically susceptible subgroups, and that risk of a particular genotype may by evident only in exposed populations. His presentation focused on risks for bladder and prostate cancer linked to specific exposures and genotypes. He described studies of smokers/non-smokers and NAT2/NAT2 genotype in risk for bladder cancer and of diet (as a measure of reactive oxygen species and oxidant damage) and DNA repair enzymes in prostate cancer.

X. Environmental Genome Project - Epidemiologic Opportunities and Challenges - Dr. Gwen Collman

Dr. Gwen Collman, Program Administrator, Chemical Exposures and Molecular Biology Branch, Division of Extramural Research and Training, began her presentation by quoting from Shulte and Perera in answer to the question: What can molecular epidemiology studies tell us? This answer includes 1) delineation of a continuum of events between an exposure and a disease; 2) identification of exposure to small amounts of toxicants and improved dose calculation; 3) identification of events earlier in the natural history of disease; 4) understanding susceptibility as an effect modifier; and 5) enhanced individual and group risk assessment. She then went through a series of issues to consider in epidemiologic studies as well as opportunities and challenges regarding haplotyping, data sharing, and translation between disciplines. Her presentation also included a discussion of ethical, legal and social issues and references to appropriate literature on each of these topics.

XI. Report of the Director, Division of Intramural Research (DIR) - Dr. Lutz Birnbaumer

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 as well as implementation of some of the recommendations from recent Board of Scientific Counselors reviews.

Dr. Birnbaumer noted that a new Laboratory of Neuroscience will be established with five existing staff with future recruitments to fill out the Laboratory. A focus will possibly be on the genetics of neurodegnerative diseases. Another area to be further developed is a clinical program in environmental medicine, which will include the training of clinicians in genomics. He noted that well-characterized clinical material will be needed for future research in genomic analysis.

Additional information from the DIR Director's Report can be found in Attachment B.

XII. Report of the Director, Division of Extramural Research and Training (DERT) - Dr. Anne P. Sassaman

Dr. Sassaman referred Council members to the written report from DERT (Attachment C). Her presentation focused on two topics: the Fiscal Year 2002 NIH Extramural Loan Repayment Program and a demonstration of the new DERT web site.

In Fiscal Year 2002, NIEHS entered into contracts with four investigators as part of the Loan Repayment Program, and these persons and their research projects were identified. There will be some major changes in the Program for Fiscal Year 2003, and these changes and clarification of eligibility and the application process have been announced in the NIH Guide to Grants and Contracts.

Dr. Sassaman thanked the team that developed the new DERT website, including staff of the Division led by Dr. Ben Van Houten, and staff and contractors from the Computer Technology Branch, led by Mr. Jerry Nehls. She then proceeded to demonstrate the site.
The Council expressed its appreciation for the work done
to make the information more user-friendly and to better convey the programs of the Institute.

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 232 applications requesting $53,714,185 in total cost. The Council recommended 137 applications with the total cost of $33,637,171.

XIV. ADJOURNMENT OF THE NAEHS COUNCIL

The meeting was adjourned at 12:30 on September 10, 2002.

ATTACHMENTS:

A. Council Roster
B. Report of the Director, Division of Intramural Research
C. Report of the Director, Division of Extramural Research and Training

DIVISION OF INTRAMURAL RESEARCH

NAEHS COUNCIL UPDATE

SEPTEMBER 2002

DIR Recruitments

Chief, Laboratory of Molecular Carcinogenesis
An international search is being conducted for a senior tenured investigator to serve as Chief of the Laboratory of Molecular Carcinogenesis. The candidate will be expected to:

- Develop and maintain strong personal research effort in the general area of molecular carcinogenesis, particularly as it relates to defining the critical target genes and cellular mechanisms in carcinogenesis and understanding how chemicals act upon these genes and cellular processes to influence cancer development.
- Provide overall leadership for the existing principle investigators within the Laboratory of Molecular Carcinogenesis, who study cell adhesion and migration, regulatory proteins, eicosanoid biochemistry, hormones and cancer, molecular and genetic epidemiology, metastasis, molecular toxicology and molecular mechanisms of gene regulation and metabolism.
- Recruit talented investigators to the Laboratory of Molecular Carcinogenesis and provide a focus for collaborations within the Institute.

The Candidate should have an international reputation in a specific area within the broad context of molecular carcinogenesis and it's relationship to the environment, an outstanding publication record, and a proven history of research leadership. The search committee, chaired by Dr. Thomas Kunkel, Chief of the Laboratory of Structural Biology has recently completed interviewing candidates and should have a recommendation in the very near future.

Chief, Laboratory of Computational Biology and Risk Analysis
An international search is being conducted for a senior 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 combined development of laboratory methods for humans and animals 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 specific 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 is currently being formed.

Tenured or tenure-track Reproductive Epidemiologist
The Epidemiology Branch is conducting an international search for a reproductive epidemiologist who will develop an outstanding research program on reproductive or perinatal health and the effects of environmental factors, including fertility, pregnancy loss, diseases of pregnancy, fetal development, birth defects, and other problems of the neonatal period. The position will be filled at the tenured or tenure-track level, dependant upon the qualifications of the applicant. The search

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committee, chaired by Dr. Mitch Eddy, Laboratory of Reproductive and Developmental Toxicology, has recommended a candidate to the Scientific Director.

Tenure-track or tenured Biostatistician--Statistical Genetics
The Biostatistics Branch is conducting 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. A search committee chaired by Dr. Michael Resnick, Laboratory of Molecular Genetics, is in the process of 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. The search committee, chaired by Dr. John Pritchard, Chief, Laboratory of Pharmacology and Chemistry, is interviewing candidates.

Staff Scientist-Head, Mass Spectrometry Protein Microcharacterization Core Facility
The Laboratory of Structural Biology is conducting a national search for a Staff Scientist to serve as Head of the Mass Spectrometry Protein Microcharacterization Core Facility in the Division of Intramural Research. The successful applicant will be a Staff Scientist in the Laboratory of Structural Biology under the supervision of Dr. K. Tomer and be responsible for the MALDI/MS and capillary HPLC/ESI/MS/MS identification of proteins isolated by 1-D and/or 2-D gel electrophoresis, in-gel digestion, determination of sites of post-translational protein modifications, identification of sites of interactions in protein complexes by limited proteolysis, protein purification by LC, and use of affinity techniques combined with mass spectrometry. Additional duties will include close interaction with DIR scientists, serving as a mass spectrometry expert during the planning and execution of experiments, and supervision of laboratory technicians. The search committee is chaired by Trevor Archer, Laboratory of Reproductive and Developmental Toxicology. Candidates are currently being interviewed.

Staff Clinician--Rheumatology
The Environmental Autoimmunity Group in the Office of Clinical Research has conducted a national search for a Staff Clinician to study the etiology, pathophysiology, and natural history of environmentally associated autoimmune diseases. Dr. Mark Gourley, currently at the Washington Hospital Center, has agreed to accept this position.

Staff Scientist--Veterinary Pathologist
The Laboratory of Experimental Pathology is seeking a toxicologic pathologist experienced in rodent toxicology and carcinogenicity studies to work within the National Toxicology Program (NTP). The successful candidate will be involved primarily in the management and oversight of the pathology peer review (evaluation), interpretation, and reporting of toxicology data. The candidate

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is also expected to identify and pursue special projects that will advance the understanding of various biological endpoints. Dr. David Malarky, currently at North Carolina State University, has agreed to accept this position.

Staff Scientist—Protein Expression

The Laboratory of Structural Biology is conducting a national search for a Staff Scientist to serve as Head of the Protein Expression Core Facility. The candidate will manage a facility the purpose of which is to provide proteins for structural and characterization studies conducted by intramural scientists. A search committee chaired by Dr. William Copeland, Laboratory of Molecular Genetics has been formed and will begin to evaluate applications after September 20, 2002.

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New DIR Recruits

Dr. Marilyn Diaz—Laboratory of Molecular Genetics

Dr. Marilyn Diaz recently joined the Laboratory of Molecular Genetics at the NIEHS as a tenure-track investigator. Dr. Diaz's research lies at the interface between DNA repair/replication and the immune system. Her research focuses on understanding the molecular basis of the process that deliberately introduces mutations into immunoglobulin genes during the course of an immune response (the process known as somatic immunoglobulin hypermutation). Dr. Diaz is also interested in the role that error-prone DNA polymerases play may in mutagenesis and ultimately, carcinogenesis. Because immunoglobulin hypermutation probably utilizes error-prone DNA polymerases, her research seeks to understand the components of the immunoglobulin mutator and its probable similarities to generalized processes of mutagenesis, particularly as these pertains to error-prone DNA polymerases.

While working with Dr. Martin Flajnik at the University of Miami, Dr. Diaz was the first to propose that a class of polymerases known to bypass DNA lesions (translesion synthesis) may play a role in immunoglobulin hypermutation. Her hypothesis has received strong support in the past two years. Both her own work with DNA polymerase zeta, and that of other laboratories with polymerases eta and iota have revealed links between this class of error-prone polymerases and immunoglobulin hypermutation. Her work has resulted in the development of a strain of mice that express antisense transcripts to the mRNA that encodes the catalytic subunit of DNA polymerase zeta. These mice have strongly reduced ultraviolet-induced mutagenesis and impaired immune responses, but are viable. Because the knockout model of this polymerase is an embryonic lethal, her antisense mouse is the only *in vivo* mammalian model available to study the impact of reduced mutagenesis by DNA polymerase Zeta.

Ultimately, Dr. Diaz's research is aimed at developing the immunoglobulin hypermutation mechanism as a model for the study of the role of translesion synthesis polymerases in mutagenesis, carcinogenesis, and aging. The recent availability of human cell line models of immunoglobulin hypermutation, and her continued development of mouse strains deficient in translesion synthesis polymerases will provide valuable tools for the study of mutagenesis in mammalian cells.

<u>Selected Publications</u>:

Diaz, M., and Flajnik, M.F. (1998) Evolution of somatic hypermutation and gene conversion in adaptive immunity. Immunol. Rev. 162: 13-24.

Diaz, M., Greenberg, A.S., and Flajnik, M.F. (1998) Somatic hypermutation of the new antigen receptor gene (NAR) in the nurse shark does not generate the repertoire: Possible role in antigen-driven reactions in the absence of germinal centers. Proc. Natl. Acad. Sci. USA 95: 14343-14348.

Diaz, M., Velez, J., Singh, M., Cerny, J., and Flajnik, M.F. (1999) Mutational pattern of the nurse shark antigen receptor gene (NAR) is similar to that of mammalian Ig genes and to spontaneous mutations in evolution: the translesion synthesis model of somatic hypermutation. Internat. Immunol. 11: 825-833.

Diaz, M., Verkoczy, L., Flajnik, M., and Klinman, N.F. (2001) Decreased frequency of somatic hypermutation and impaired affinity maturation, but intact germinal center formation in mice expressing antisense RNA to DNA polymerase Zeta. J. Immunol. 167: 327-335.

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Diaz, M., and Casali, P. (2002) Immunoglobulin somatic hypermutation. Curr. Opin. Immunol. 14: 235-240.

Dr. Jeffery Boyington—Laboratory of Structural Biology

Dr. Jeffrey Boyington will direct an x-ray crystallographic research group in the Laboratory of Structural Biology. His research goals are to investigate the structural aspects of interaction between cell surface receptors on phagocytes and various ligands using x-ray crystallography and binding studies. Professional phagocytic cells of the immune system as well as nonprofessional phagocytes such as vascular endothelium and certain epithelial cells encounter and interact with a variety of different targets including gram negative and gram positive bacteria, apoptotic cells, infected cells, cellular debris, oxidized lipoproteins, lipopolysaccharide (LPS), advanced glycation end products (AGE) and various types of environmental particles. A structural understanding of the mechanisms of ligand recognition and subsequent signal transduction through the plasma membrane is critical to elucidating how phagocytes interface with and respond to both organic and inorganic environmental challenges. Although many of these receptors have been and are currently being identified, few have been characterized on a structural level. Systems of immediate interest include a group of scavenger receptors (SR) involved in lipid transport, regulation of blood vessel relaxation/constriction and also in the specific internalization of low density lipoproteins (LDL) that have become oxidized. The recognition and internalization of oxidized LDL by SRs is of considerable interest since this appears pivotal to the pathology of atherosclerosis, a widespread disease responsible for over 50% of all deaths in western societies.

Selected Publications:

Boyington, J.C., Motyka, S.A., Schuck, P., Brooks, A.G. and Sun, P.D. (1999) Crystal structure of an NK cell Ig-like receptor in complex with its class I MHC ligand, Nature 405: 537-543.

Boyington, J.C., Riaz, A.N., Patamawenu, A., Coligan, J.E., Brooks, A.G. and Sun, P.D. (1999) Structure of CD94 reveals a novel c-type lectin fold: Implications for the NK cell-associated CD96/NKG2 receptors, Immunity 10: 75-82.

Boyington, J.C., Gladyshev, V.N., Khangulov, S.V., Stadtman, T.C. and Sun, P.D. (1997) Crystal structure of formate dehydrogenase H: Catalysis involving Mo, molybdopterin, selenocysteine, and an Fe_4S_4 cluster, Science 275: 1305-1308.

Boyington, J.C., Gaffney, B.J. and Amzel, L.M. (1993) The three-dimensional structure an arachidonate 15-lipoxygenase, Science 260, 1482-1486.

Dr. Manas K. Ray, Head, Transgenic Program

The DIR welcomes Dr. Manus Ray, who has been appointed to the position as the Head of the Core Facility for generation and analysis of transgenic. Dr. Ray received his PhD from the University of Nebraska-Lincoln in 1991 from the Department of Chemistry. His research thesis was focused on the mechanism of eukaryotic protein synthesis initiation, in particular, the role of a 67kDa protein (p67) in this process. Dr. Ray moved to Baylor College of Medicine in Houston, Texas for his postdoctoral training at the Department of Cellular and Molecular Biology and started to work on the molecular mechanism involved in the development and differentiation of pulmonary epithelium by identifying the regulation of Clara cell-specific protein (CC10) gene in mouse model using transgenic approach. Following his postdoctoral training at Baylor, Dr. Ray started his own

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laboratory in the Department of Surgery where he focused on developing mouse models to help elucidate the molecular basis of endocrine pancreas development by determining the role of transcription factors using transgenic and knock out techniques. Dr. Ray worked at the Eastern Virginia Medical School for a short period of time (2000-2002) prior to joining at the.

Dr. Ray is currently involved in several ongoing projects at the NIEHS, which includes generation of knock out and transgenic mice for transcription factors Glis1 and Glis2, zinc finger protein, required for skeletal development and organogenesis of various tissues and involved in a number of human diseases. The other projects involve tissue-specific over expression of Cox1, Cox2 and NAG1 using Cre-loxP system in transgenic mice, establishment of mutant ES cell line for MRCKS gene (Myristoylated Alanine-rich C kinase Substrate), and generation of floxed mice for BMP2 gene to inactivate in a tissue-specific manner. Dr. Ray also plans to begin his own line of research to study the role of transcription factors, in particular, GATA-4, in the development of pancreas and in insulin gene regulation in the near future.

Selected Publications:

Ray, M.K., Datta, B., Chakraborty, A., Chattopadhyay, A., Meza-Keuthen, S., and Gupta, N.K. (1992) The eukaryotic initiation factor 2 (eIF-1) associated 67-kDa polypeptide (p67) plays a critical role in regulation of protein synthesis initiation in animal cells. Proc. Natl. Acad. Sci. USA 89: 549-553.

Datta, B., Ray, M.K., Chakraborty, D., Wylie, D., and Gupta, N.K. (1992) Glycosylation of eukaryotic initiation factor 2 (eIF-2) associated 67kDa polypeptide (p67) and its possible role in the inhibition of eIF-2 kinase catalyzed phosphorylation of the eIF-2 subunit. J. Biol. Chem. 264: 20620-20624.

Ray, M.K., Chakraborty, A., Datta, B., Chattopadhyay, A., Saha, D., Bose, A., Kinzy, T., Wu, S., Hileman, R., Merrick, W.C. and Gupta, N.K. (1993) Characteristics of the eukaryotic initiation factor 2 (eIF-2) associated 67kDA polypeptide. Biochemistry 32: 5151-5159.

Ray, M.K., Magdaleno, S., O'Malley, B.W., and DeMayo, F.J. (1993) Cloning and characterization of the mouse Clara cell-specific 10kDa protein gene: comparison of the 5' flanking region with the human, rat and rabbit gene. Biochem. Biophys. Res. Commun. 197: 163-171.

Ray, M.K., Magdaleno, S., Finegold, M.J., and DeMayo, F.J. (1995) Cis-acting elements involved in the regulation of mouse Clara cell-specific 10-kDa protein gene: *in vitro* and *in vivo* analysis. J. Biol. Chem. 270: 2689-2694.

Ray, M.K., Chen, C., Schwartz, R.J., and DeMayo, F.J. (1996) Transcriptional regulation of the mouse Clara cell 10kDa protein (mCC10) gene by the members of the Nkx transcriptional factor family. Mol. Cell. Biol. 16: 2056-206

Ray, M.K., Finegold, M.J., and DeMayo, F.J. (1996) Immunohistochemical localization of mouse Clara cell-specific 10kDa protein using antibodies raised against the recombinant protein. Histochem. Cytochem. 44: 919-927.

Ray, M.K., Fagan, S.P., Moldovan, S., DeMayo, F.J., and Brunicardi, F.C. (1998) A mouse model for beta cell-specific ablation of target gene(s) using the Cre-loxP system. Biochem. Biophys. Res. Comm. 253: 65-69.

Ray, M.K., Fagan, S.P., Moldovan, S., DeMayo, F.J., and Brunicardi, F.C. (1999) Development of transgenic mouse model using rat insulin promoter to drive the expression of Cre recombinase in a tissue specific manner. Int. J. Pancreatology 25: 157-163.

Kleinman, R.M., Fagan, S.P., Ray, M.K., Adrian, T.E., Wong, H., Imagawa, D., Walsh, J., and Brunicardi, F.C. (1999) Differential inhibition of insulin and islet amyloid polypeptide secretion by intraislet somatostatin in the isolated perfused human pancreas. Pancreas 19: 346-352.

Ray, M.K., Fagan, S. and Brunicardi, F.C. (2000) Cre-loxP system: A versatile tool for targeting genes in a cell and stage-specific manner. Cell Transplantation 9: 805-815.

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DIR Research Accomplishments
FY2002

A New Genetic Defect That Results in Adverse Drug Toxicity of the Anticonvulsant Drug Phenytoin

A group of liver enzymes, known as cytochrome P450s (CYP) are responsible for metabolizing clinical drugs as well as environmental compounds like pesticides. CYP2C9 is one of the most important of these enzymes. It is responsible for the metabolism of many clinically important drugs such as the anticonvulsant, phenytoin (used for epilepsy); the anticoagulant, warfarin; antidiabetic drugs, such as tolbutamide; and most common nonsteroidal antiinflammatory drugs such as ibuprofen and Celebrex. A new genetic polymorphism was discovered by NIEHS scientists in an African-American individual who was found to be homozygous for a single base pair deletion in the gene coding for CYP2C9 resulting in the complete absence of this enzyme in the liver. As a result, standard recommended doses of drugs like phenytoin and warfarin was dangerous for this individual and for others carrying this trait. A preliminary screen of African-American individuals indicate that 1/60 African-Americans carry at least one gene for this defect. This is the first example of a null polymorphism (completely inactivating defect) in this medically important enzyme. A genetic test was developed for this genetic defect, which can identify an individual carrying this defect from less than a single drop of blood. Such genetic tests in the future can lead to individualized medicine in the future, alerting the physician to patients who cannot tolerate normal doses of medicines.

Kidd, R.S., Curry, T.B. Gallagher, S., Edeki, T.. Blaisdell, J.and Goldstein.J.A. (2001) Identification of a new null allele of *CYP2C9* in an African-American exhibiting toxicity to the anticonvulsant drug phenytoin**.** Pharmacogenetics 11: 803-809.

Fischer, T.L., Pieper, J.A., Graff, D.W., Rodgers, J.E., Fischer, J.D., Parnell, K.J., Goldstein, J.A., Greenwood, R., and Patterson, J.H. (2002) Evaluation of potential losartin-phenytoin drug interactions in healthy volunteers. Clin Pharm. Ther., in press.

Genes and Geography Influence Autoimmune Muscle Disease

Naturally occurring variations in genes and geography around the world allow for an evaluation of their roles in the development of autoimmune disease in different ethnogeographic groups. In order to exploit these differences, a group of 35 specialists at 16 centers on four continents, known as the International Myositis Collaborative Study Group, have been organized to collect clinical data and specimens on patients suffering from a potentially lethal form of autoimmunity affecting muscles known as myositis. This study focuses on findings from myositis patients in the United States and Mesoamerica, which includes Mexico and Guatemala. The investigation demonstrated that the specific clinical problems, autoantibodies and genes that predispose to and protect from myositis are different in these two ethnogeographic groups. Along with additional information on myositis patients from other global locations collected by this study group, these findings suggest that the expression of myositis is modulated by different genes and environmental exposures around the world.

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Shamim, E.A., Rider, L.G., Pandey, J.P., O'Hanlon, T.P., Jara, L.J., Samayoa, E., Burgos-Vargas, R., Vazquez-Mellado, J., Alcocer-Varela, J., Salazar-Paramo, M., Kutzbach, A.G., Malley, J.D., Targoff, I.N., Garcia de la Torre, I., and Miller, F.W. (2002). Idiopathic Inflammatory Myopathy Phenotypes and Genotypes Differ in Mesoamerican Mestizos and North American Caucasians: Ethnogeographic Influences in the Genetics and Clinical Expression of Myositis. Arthritis Rheum. 46: 1885-1893.

System Developed for the Rapid Modification of Genes Within Cells

The rapid modification of genes provides opportunities to study gene function and evaluate drug responsiveness. A new system demonstrated in yeast, referred to as *delitto perfetto* provides a simple, highly efficient system for changing genes and chromosomes. It relies on the placement of a "cassette" in a gene and the subsequent removal with very small seqeuences (oligonucleotides) that provide the desired changes in the gene. Using this yeast-based system, it has been possible to study changes in the most prominent tumor suppressor gene p53 to understand better the consequences of the tumor associated mutations. The system can easily be applied to many genes that are associated with human disease.

Storici, F., Lewis, K.L., and Resnick, M.A. (2001) *In vivo* site-directed mutagenesis using oligonucleotides: a versatile approach based on recombination in yeast. Nature Biotech. 19: 773-776.

Development of an Immunoassay-Based Procedure for the Detection of Free Radicals

NIEHS scientists have developed and validated an imunoassay for the detection of nitrone free radical adducts. This result brings the power of immunological methods into the field of free radical biology and opens way for simple, rapid tests for human exposures to potentially damaging free radicals.

Detwiler, C.D., Deterding, L.J., Tomer, K.B., Chignell, C.F., Germolec, D., and Mason, R.P. (2002) Immunological identification of the heart myoglobin radical formed by hydrogen peroxide. Free Radical Biol. Med. 33: 364-369.

Novel G Protein-Dependent Signaling Mechanism For Thyroid

Researchers at the NIEHS have discovered a new molecular mechanism for thyroid hormone action, which has important implications for several neurological disorders in humans with thyroid hormone deficiency. Iodine-deficient diets have been known to cause deafness and severe brain malformation and retardation, called cretinism, by preventing thyroid hormone synthesis. More recently less severe disruptions of thyroid signaling by mutations in the receptor proteins, or by environmental toxins that block thyroid hormone transport, have also been linked to increases in the incidence of attention deficit disorder in children and to malformations during metamorphosis in frogs. Newly published data indicate that at least one effect of thyroid hormone, rapid stimulation of potassium channels, and possibly many more effects of thyroid hormone and other nuclear hormones, are mediated by a G protein of the *Rac* family. Because Rac is essential for normal neurite outgrowth during brain development in rodents and potassium channel mutations produce

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deafness through the same cellular mechanism as thyroid hormone receptor deletions, this study of potassium channel regulation in a rat pituitary cell line has opened a new window into brain development. It also provides new impetus for identifying industrial chemicals, such as the halogenated aromatic hydrocarbons, that disrupt thyroid hormone signaling.

Storey, N.M., O'Bryan, J.P., and Armstrong, D.L. (2002) Rac and rho mediate opposing hormonal regulation of the ether-a-go-go-related potassium channel. Curr. Biol. 12: 27-33.

Demonstration of the capability to identify signature profiles of altered gene expression in liver mRNA of animals exposed to specific chemical classes using microarray technology

The application of these seminal studies to predict exposures to specific chemical classes was reported in a publication in which mRNA obtained from animals exposed to specific chemicals/drugs were analyzed under code in a blinded manner by similar microarray techniques The results demonstrated that even with a minimum learning set, it was possible to correctly determine patterns of altered gene expression which were concordant with previously determined patterns for 22 of 23 samples analyzed. They were followed by a subsequent study to relate altered patterns of gene expression to specific toxic effects, a concept referred to a phenotypic anchoring of gene expression patterns. This study demonstrated a methodology for analysis of the hepatotoxicity of metharyraline revealed by cDNA microarray analysis of samples of livers from exposed animals. This study demonstrated that it is possible to "phenotypically anchor" the results of microarray analysis of the tissues exposed animals to specific parameters of toxicity.

Hamadeh, H.K., Bushel, P.R., Jayadev, S., DiSorbo, O., Sieber, S., Bennett, L., Li, L., Tennant, R.W., Stoll, R., Barrett, J.C., Blanchard, K., Paules, R.S., and Afshari, C. A. (2002) Gene expression analysis reveals chemical-specific profiles. Tox. Sci. 67: 219-231.

Hamadeh, H.K., Bushel, P.R., Jayadev, S., DiSorbo, O., Bennett, L., Li, L., Tennant, R.W., Stoll, R., Barrett, J.C., Paules, R.S., Blanchard, K., and Afshari, C. A. (2002) Prediction of compound signature using high density gene expression profiling. Tox. Sci. 67: 232-240.

Hamadeh, H.K., Knight, B.L., Haugen, A.C., Sieber, S., Amin, R.P., Bushel, P.R., Stoll, R., Blanchard, K., Jayadev, S., Tennant, R.W., Cunningham, M.L., Afshari, C.A., and Paules, R.S. (2002) Methapyrilene toxicity: Anchorage of pathologic observations to gene expression alterations. Tox. Path. 30: 470-482.

Cellular Pathways Involved in the Regulation of Inflammation

NIEHS scientists have been investigating signaling pathways in mice that mediate the body's response to loss of an important cellular regulatory protein, tristetraprolin or TTP. They have found that most of the inflammatory response that occurs in mice lacking this protein is mediated through the type 1 receptors for the well-known proinflammatory hormone, tumor necrosis factor (TNF). The presence of type 2 receptors actually confers a protective effect. They also found that, in the absence of both of the known TNF receptors, the mice still showed evidence for excessive white blood cell production, supporting a previously identified function of TTP to regulate production of

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an important hormone that stimulates white cell production, granulocyte-macrophage colony stimulating factor (GM-CSF). Phosphorylation of TTP by the cellular protein kinase p38 inhibited the binding of TTP to the TNF mRNA, inhibiting its. This provides one possible mechanism for the well-known ability of p38 kinases to regulate TNF production and inflammation. Finally, NIEHS scientists have described novel pathways by which TTP proteins are cycled between the nucleus and cytoplasm of the cell, and identify some of the key amino acid sequences involved in this intracellular movement. These studies may provide novel targets for the development of novel, highly specific anti-inflammatory drugs.

Carballo, E. and Blackshear, P.J. (2001) Roles of tumor necrosis factor α receptor subtypes in the pathogenesis of the tristetraprolin-deficiency syndrome. Blood, 98:2389-2395.

Carballo, E, Cao, H, Lai, W.S., Kennington, E.A., Campbell, D and Blackshear, PJ. (2001) Decreased sensitivity of tristetraprolin-deficient cells to p38 inhibitors suggests the involvement of tristetraprolin in the p38 signaling pathway. J. Biol. Chem., 276: 42580-42587.

Phillips, RS and Blackshear, PJ. (2002) Members of the tristetraprolin family of tandem CCCH zinc finger proteins exhibit CRM1-dependent nucleocytoplasmic shuttling. J. Biol. Chem.277: 11606-11613.

Potential Improvement for Therapy for Asthma and Bronchitis

NIEHS scientists have identified the gene responsible for controlling synthesis of inositol 3,4,5,6-tetrakisphosphate, an intracellular signal that regulates a class of chloride ion channels that control salt, fluid and mucous secretion from epithelial cells. These scientists have also discovered how the synthesis of inositol tetrakisphosphate inside cells is tightly coupled to hormone action. As a consequence of these studies, work is now progressing to genetically and pharmacologically manipulate the synthesis of inositol 3,4,5,6-tetrakisphosphate to see to what extent this controls the over-production of airway mucus that results from allergic asthma and bronchitis. This represents a completely novel therapeutic approach to these debilitating pathological conditions.

Ho, M.W.Y., Yang, X., Carew, M.A., Zhang, T., Hua, L., Kwon, Y.-U,, Chung, S.-K., Adelt, S., Vogel,G., Riley, A.M., Potter, B.V.L. and Shears, S.B. (2002) Regulation of Ins(3,4,5,6)P4 signaling by a reversible kinase/phosphatase. Curr. Biol. 12: 477-482.

Identification of a Disease Caused by Defective Mitochondrial DNA Polymerase

Progressive external ophthalmoplegia (PEO), is a late onset mitochondrial disease characterized by the accumulation of large deletions in the mitochondrial DNA. Recently, a genetic mutation coding for a conserved tyrosine in the nuclear gene for the mitochondrial DNA polymerase, DNA polymerase gamma, was shown to be associated with the disease. NIEHS scientists showed that this mutation decreases the fidelity of DNA replication and accelerates the accumulation of point mutations, frameshifts, and deletions in mitochondrial DNA. The steady accumulation of mutations is what accounts for the late onset and progressive nature of PEO. As a result, researchers now know that an errant mitochondrial DNA polymerase can cause human disease.

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Ponamarev, M.V., Longley, M.J., Nguyen, D., Kunkel, T.A., and Copeland, W.C. (2002) Active Site Mutation in DNA Polymerase γ associated with progressive external ophthalmoplegia causes error-prone DNA synthesis. J. Biol. Chem. 277: 15225-15228.

The Genetic Control of Toleration to Radiation and Other Types of Chromosomal Damaging Agents is Much Larger Than Anticipated

Many genes are known to affect the responses to chromosomal damaging agents. Using a methodical examination of most genes in yeast, the number of genes that are important in protecting cells from radiation was found to be at least 3-fold more than previously expected. The genes are important in a variety of normal cellular processes. Since most of the genes responsible for protecting human cells from chromosomal damage were first identified in yeast and many of the newly discovered genes have human homologues, these findings provide a rich new base of information for further investigation of human cells. Moreover, many of the yeast genes correspond to human genes associated with cancer.

Bennett, C. B., Lewis, L. K., Karthikeyan, G., Lobachev, K., Jin, H. J., Snipe, J., Sterling, J., and Resnick, M. A. (2001) Diverse cellular activities required for ionizing radiation resistance in yeast: A functional genomics approach. Nature Genet. 29: 426-434.

Arrangements of Human Common Repeat Elements Can Determine Potential Sites of Chromosome Instability

The genomes of all organisms contain motifs that are at-risk (ARMs) for change and they are frequently a source of genetic disease. In humans, the frequently repeated Alu element has been suggested as a source of instability. Using a yeast-based assay along with an analysis of Alu repeat distribution in humans, it is now established that closely-spaced inverted Alu's are highly unstable and may cause double-strand breaks and lead to severe chromosome mutations. The yeast study also revealed that a gene MRE11, identified with a rare DNA repair metabolic disease, may be a guardian of the genome against novel DNA structures known as hairpins.

Lobachev, K., Gordenin, G., and Resnick, M.A. (2002) The Mre11 complex is required for repair of hairpin-capped double-strand breaks and prevention of chromosome rearrangements. Cell 108: 183-193.

Stenger, J. , Lobachev, K., Gordenin, D.A., Darden, T. Jurka, J., and Resnick, M. A. (2001) Biased distribution of inverted and direct *Alus* in the human genome: implications for insertion, exclusion and genome stability. Genome Res. 11: 12-27.

A Yeast Model for the Human Disease Friedreich's Ataxia Reveals Important Role of Mitochondria in Protecting Against Nuclear Damage

The mitochondria organelles within a cell are the source of cellular energy. Defects in mitochondrial function have been identified with a variety of human diseases including Friedreich's ataxia (FA), the most common inherited ataxia disease. FA is associated with a deficiency in the mitochondrial iron-binding protein frataxin. Using a yeast-based system, the absence of frataxin was shown to lead to considerable chromosome instability via the production of reactive oxygen

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species. This is the first demonstration of a direct role of the mitochondria in protecting the nucleus. The system is now being modified to be more representative of the course of the disease and to provide opportunities for addressing other mitochondrial diseases as well as therapies.

Karthikeyan, G., Lewis, K., and Resnick, M.A. (2002) The mitochondrial protein frataxin prevents nuclear damage. Human Mol. Genet. 11: 1351-1362.

Mutations in the p53 Tumor Suppressor Gene Often Alters the "Chords" on the p53 Piano

Alterations in the tumor suppressor and stress-responsive p53 gene are associated with nearly all tumors. Using a yeast-based system, the ability of p53 to activate its many target genes responsible for cellular growth, death, and development was found to vary by over 1000-fold. With this system, many p53 mutants were found to have altered ability to activate the many target genes. In the same way that a hand can create a chord on the piano, the p53 gene creates "chords" of target genes that are turned on or off depending on the cellular stress. Many of the mutations in p53 can lead to new chords that may determine the course of disease. A hotspot for p53 mutation in a mouse skin model cancer system is consistent with this newly developed "p53 piano" hypothesis.

Inga, A., Nahari, Dorit, Friedberg, E.C., and Resnick, M. A. (2002) A novel mutational hotspot in the *Trp53* gene in skin cancers from UV Irradiated *Xpc* mutant mice encodes a protein with altered transactivation functions. Oncogene (in Press).

Monti, P.,Campomenosi, P., Ciribilli, Y., Iannone, R., Inga, A., Abbondandolo, A., Resnick, M. A., and Fronza, G. (2002) Tumour p53 mutations exhibit promoter elective dominance over wild type p53. Oncogene 21: 1641-1648.

Epitope Recognized by In-Vivo Neutralizing HIV Antibody Determined

Using a combination of mass spectrometry and enzymatic cleavages, NIEHS scientists have determined the functional epitope recognized by one of the five human antibodies that can neutralize the AIDS virus in vivo. Initial experiments using alternative methods had concluded that the antibodyrecognized a short 6 amino acid sequence while the MS-based technique showed that the functional epitope contains 16 residues.

Parker, C.E., L.J. Deterding, C. Hager-Braun, J.M. Binley, N. Schuelke, H. Katinger, J.P. Moore, and K.B. Tomer (2001) Fine definition of the epitope on the gp41 glycoprotein of human immunodeficiency virus type 1 for the neutralizing monoclonal antibody, 2F5. J. Virol., 75: 10906-10911.

The First National Survey of Lead and Allergens in the U.S. Reveals That a Significant Proportion of Homes Have Lead-Based Paint Hazards and Allergen Levels That Exceed Clinically Relevant Thresholds

The National Survey of Lead and Allergens in Housing (NSLAH) is the first study to provide estimates of lead and allergen exposure in the U.S. population. The survey includes approximately 2500 individuals living in 831 homes in 75 different locations throughout the country. Analysis of

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dust mite allergen data indicate that approximately 24% of U.S. homes have beds that contain >10 microgram dust mite allergen/gram dust (a level previously associated with symptomatic asthma) and approximately 46% of U.S. homes have beds that contain >2 microgram dust mite allergen/gram dust (a level previously associated with mite allergen sensitization). The likelihood of having a high dust mite allergen level in the bed is greater for older homes, non-western region homes, lower income households and bedrooms with higher humidity. Analysis of lead data demonstrate that 38 million housing units have lead-based paint and 25 million had significant lead-based paint hazards. The prevalence of lead-based paint hazards increases with age of housing, is highest in the Northeast and Midwest, and is particularly high in homes occupied by low-income families with children.

Vojta, P.J., Friedman, W., Marker, D., Clickner, R., Rogers, J.W., Viet, S.M., Muilenberg, M.L., Burge, H.A., Thorne, P.S., Arbes, S.J., and Zeldin, D.C. (2002) First National Survey of Lead and Allergens in Housing: Survey Design and Methods for the Allergen and Endotoxin Components. Env. Health. Perspect. 110: 527-532.

Jacobs, D.E., Clickner, R.P., Zhou, J.Y., Viet, S.M., Marker, D.A., Rogers, J.W., Zeldin, D.C, Broene, P., and Friedman, W. (2002) The Prevalence of Lead-Based Paint Hazards in U.S. Housing. Env. Health Perspect., in press.

Hormonal and Reproductive Risk Factors for Systemic Lupus Erythematosus

NIEHS scientists have been studying cotential causes of the autoimmune disease systemic lupus erythematosus (SLE). SLE is a chronic inflammatory disease that disproportionately affects women and African Americans. In this study, little or no evidence was found that estrogen-related exposures (e.g., hormone replacement therapy or oral contraceptive use) could be associated with SLE. However, breast-feeding was associated with a decreased risk of SLE.

Cooper, G.S., Dooley, M.A., Treadwell, E.L., St. Clair, E.W., and Gilkeson, G.S. (2002) Hormonal and reproductive risk factors for development of systemic lupus erythematosus. Arthritis Rheum. 46: 1830-1839.

A Mutation in Drosophila Causes Telomeres to Grow

A new mutation, Tel, has been discovered in Drosophila that causes telomeres on all chromosome ends to grow. The mutation occurred spontaneously and was discovered in a strain that had been taken from a wild population several years earlier. It maps to about 69 on the recombination map of chromosome 3. In the presence of the Tel mutation telomeres accumulate telomere-specific HeT-A and TART DNA sequences, but bind normal amounts of the telomeric HP1 protein. Independent of the presence of Tel the long telomeres associate with each other at interphase, but these associations are broken duing mitosis. This is the first demonstration of genetic control of telomere length in *Drosophila*.

Siriaco, G.M., Cenci, G., Haoudi, A., Champion, L., Zhou, C., Gatti, M., and Mason, J.M. (2002) Telomere elongation (Tel), a new mutation in Drosophila melanogaster that produces long telomeres. Genetics; 160: 235-245.

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A Strategy to Enhance the Effectiveness of Chemotherapeutics in Treating Brain Tumors
Paclitaxel (Taxol) and derivatives are active against a variety of cancers. However, because of limited entry into the central nervous system, they are largely ineffective in treating brain tumors. Here we demonstrate that the primary barrier to Paclitaxel entry into the brain is a xenobiotic efflux pump at the blood-brain barrier, p-glycoprotein. In mice given intravenous Paclitaxel, coadministration of a pump inhibitor substantially increases brain Paclitaxel levels and dramatically reduces the volume of a human glioblastoma implanted in the brain; without the p-glycoprotein inhibitor, Paclitaxel is ineffective. This study identifies one mechanism responsible for the limited effectiveness of chemotherapeutics in treating malignant brain tumors and tests one strategy to overcome it. Since a wide range of drugs, including chemotherapeutics, HIV protease inhibitors, antivirals and antibiotics, are p-glycoprotein substrates and poorly penetrate into the brain, the strategy should have wide applicability. This study follows the development of confocal imaging-based techniques to identify mechanisms of xenobiotic transport at the blood-brain barrier that were carried out at the NIEHS

Fellner, S., Bauer, B., Miller, D.S., Schaffrik, M., Spruss, T., Bernhardt, G., Graeff, C., Farber, L., Gschaidmeier, H., Buschauer, A., and Fricker, G. (2002) Transport of Paclitaxel (Taxol) across the blood-brain barrier in vitro and in vivo. J. Clin. Invest., in press.

Fricker, G., Nobmann, S., and Miller, D.S. (2002) Permeability of porcine blood brain barrier to somatostatin anlogs. Brit. J. Pharmacol., 135: 1308-1314.

Miller, D.S., Graeff, C., Droulle, L., Fricker, S., and Fricker, G. (2002) Xenobiotic efflux pumps in isolated fish brain capillaries. Am. J. Physiol., 282: R191-R198.

Absence of Either COX-1 or COX-2 is Associated with Reduced Skin Tumorigenesis
NIEHS scientists have found that both cyclooxygenase-1 (COX-1) and COX-2 play roles in epidermal cell differentiation and in skin Tumorigenesis. Mice deficient in either COX-1 or COX-2 showed premature onset of keratinocyte differentiation and decreased skin tumorigenesis after exposure to dimethylbenz(a)anthracene.

Tiano, H.F., Loftin, C.D., Akunda, J., Lee, C.A., Spalding, J., Sessoms, A., Dunson, D.B., Rogan, E.G., Morham, S.G., Smart, R.C., and Langenbach, R. (2002) Deficiency of either cyclooxygenase (COX)-1 or COX-2 alters epidermal skin differentiation and reduces mouse skin tumorigenesis. Cancer Res. 62: 3395-3401.

Researchers Discover Mechanism for Common Post-Translational Modification
Researchers at the NIEHS have identified a sequence in proteins which directs the covalent attachment of ubiquitin, a process referred to as ubiquitination. This research has found that a short peptide sequence, termed the ubiquitin-interacting motif or UIM, is both necessary and sufficient to direct mono-ubiquitination of a protein containing this sequence. Although poly-ubiquitination of proteins frequently leads to their rapid degradation, this work demonstrates that UIM-directed mono-ubiquitination does not lead to protein degradation. Given the presence of UIMs in wide variety of proteins, these findings suggest a general role for UIMs and mono-ubiquitination

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regulation of protein function. Furthermore, the presence of UIMs in several proteins associated with neurodegeneration suggests a potential role for this motif in the pathogenesis of neurodegeneration.

Oldham, C. E., Mohney, R. P., Miller, S. L. H., Hanes, R. N., and O'Bryan, J. P**.** (2002) The ubiquitin-interacting motifs (UIMs) target the endocytic adaptor protein epsin for ubiquitination. Curr. Biol. 12: 1112-1116.

Development of Immortalized Human Uterine Leiomyoma and Myometrial Cell Lines

NIEHS scientists have developed immortalized human uterine leiomyoma and myometrial cell lines by inducing expression of active telomerase in normal human smooth muscle cells and in a benign smooth muscle neoplasm cell line. Both immortallized cell lines showed no phenoypic alternations when compared to the respective parental cells. These cells will be usful in the study of cellular mechanisms involved in the development and growth of utermine leiomyomas. These studies are expected to yield improved treatment and intervention regimens for clincal cases of uterine leiomyomas.

Carney, S.A., Tahara, H., Swartz, C.D., Risinger, J.I., He, H., Moore, A.B., Haseman, J.K., Barrett, J.C., and Dixon, D. (2001) Immortalization of human uterine leiomyoma and myometrial cell lines after induction of telomerase activity: Molecular and phenotypic characteristics. Lab Invest. 82: 719-728.

New Potential Drugs to Alleviate Pain

Antagonist ligands for the δ-opioid receptor, the receptor that is not linked to addiction (which is the μ-opioid receptor), were converted into potent δ-agonists with or without μ agonism by simple alteration to their structure. Reversion back to an antagonist also had dual receptor action properties in some cases depending on the particular compound.

Balboni, G., Guerrini, R., Salvadori, S., Bianchi, C., Rizzi, D., Bryant, S.D., and Lazarus, L.H. (2002) Evaluation of the Dmt-Tic Pharmacophore: conversion of a potent δ-opioid receptor antagonist into a potent δ-agonist and ligands with mixed properties. J. Med. Chem. 45: 713-720.

Balboni, G., Salvadori, S., Guerrini, R., Negri, L., Yunden, J., Bryant, S.D., and Lazarus, L.H. (2002) New potent δ-opioid agonists based on the Dmt-Tic pharmacophore. *J. Med. Chem*. in press.

Assessment of the Binding Pocket in a Membrane Receptor.

Through the use of X-ray crystallography data on several distinct opioid ligands of the Dmt-Tic pharmacophore family and computer modeling techniques, the spatial requirements for the δ-opioid receptor can now be determined. The structural data permit the investigator to define the parameters required for both antagonists and agonists for this receptor molecule.

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Bryant, S.D., George, C., Flippen-Anderson, J., Salvadori, S., Balboni, G., Guerrini, R., and Lazarus, L.H. (2002) Solid-state structure of analogues containing the Dmt-Tic pharmacophore. J. Med. Chem., in press.

Synthesis and Bioactivity of Unique Analogues for the Morphine Receptor.
A series of peptides containing two aromatic residues at the N-termini were synthesized containing either the pyrazinone platform or an alkyl chain. The only active residue was 2',6'-dimethyl-L-tyrosine (Dmt) which yielded potent μ-receptor opioid ligands with potency equal to or greater than morphine in tissue samples or in rodents. The results were submitted for patent protection in Japan. Another series of compounds, the endomorphins, natural occurring μ-opioid peptide in brain, was modified to produce an array of ligands with unusual properties.

Okada, Y., Fujisawa, Y., Morishita, A., Shiotani, K., Miyazaki, A., Fujita, Y., Tsuda, Y., Yokoi, T., Bryant, S.D., and Lazarus, L.H. (2002) Deamination of 2(1H)-pyrazinone derivatives during catalytic hydrogenation. Tetrahedron Lett., in press.

Highlights from the National Toxicology Program
September 2002

NTP Testing Program

Hexavalent Chromium
The NTP held a public meeting on July24, 2002, at the NIEHS to review and discuss 1) data from studies designed to assess the absorption of chromium by rats, mice and guinea pigs receiving hexavalent chromium as sodium dichromate dihydrate in drinking water; 2) the design and data from 90-day oral toxicity studies in rats and mice receiving hexavalent chromium in drinking water; and 3) a proposed design for 2-year rodent cancer studies of hexavalent chromium in drinking water. The NIEHS invited scientific experts to participate in this review and time was set aside for oral public comments. The NTP felt this meeting was highly productive and identified issues for its consideration relative to the design and conduct of the 2-year rodent cancer studies. The panel's recommendations are posted on the NTP web site. The NTP is proceeding with these studies using design modifications recommended by the panel.

New Nominations for NTP Toxicology Studies
Nineteen new nominations have been evaluated for possible toxicology and/or carcinogenesis studies. The NTP Interagency Committee for Chemical Evaluation and Coordination (ICCEC) examined this group of nominations in April 2002 after which the ICCEC's testing recommendations were published in the Federal Register and pubic comment was solicited. The NTP Executive Committee reviewed the nominations, ICCEC recommendations, and public comments at its meeting August 22. The NTP will design and implement appropriate studies as resources and time permits.

The 14 nominations for which one or more types of toxicological studies are recommended:
Abrasive blasting agents – 5 different industrial materials used as alternatives to sand
5-Amino-*o*-cresol – permanent hair dye ingredient
***tert*-Butyl hydroperoxide** – high production volume industrial catalyst
Chloramine-T and *p*-Toluenesulfonamide – active ingredient and metabolite of therapeutant used in aquaculture to control bacterial infections
Cobalt metal dust – important industrial material linked to lung problems in workers
Ephedrine alkaloid dietary supplements – widely used herbal dietary supplements with numerous reports of adverse effects in consumers
Ethanone, 1-(1,2,3,4,5,6,7,8-octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-(Iso-E-Super) – high production volume fragrance material
Hexafluorosilicic acid and Sodium hexafluorosilicate – primary agents used to fluoridate public drinking water supplies
Ketamine hydrochloride – approved anesthetic drug that causes brain lesions in developing rats
Mercury, ((o-carboxyphenyl)thio)ethyl-, sodium salt (Thimerosal) – organomercury-based preservative used in vaccines and other biological products
Nitrogen trifluoride – cleaning and etching agent in the semiconductor industry
Sodium metasilicate – industrial cleaning agent

Turpentine – high production volume industrial solvent and raw material
Welding fume – variable composition mixture responsible for respiratory and other adverse effects in exposed workers

The 5 nominations for which no studies are recommended at this time:
1,3-Hexachlorobutadiene – industrial by-product and persistent environmental contaminant
Infrasound – low-frequency acoustic energy present at low levels in community and occupational settings
Magnesium oxide – high production volume chemical with numerous industrial uses
Methylolurea – starting material for and impurity in urea-formaldehyde resins
4-Methylquinoline - environmental pollutant structurally related to the carcinogen quinoline

Further information, including supporting documents for each nomination, is available on the NTP website or by contacting Dr. Scott Masten,
Office of Chemical Nomination and Selection, NIEHS, P.O. Box 12233, MD A3-01, Research Triangle Park, NC 27709; T; 919- 541-5710

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NTP Collaborations

The NTP has developed or is developing formal collaborations with a number of non-U.S. agencies to develop efficient means to avoid duplication of efforts in toxicology testing. The Ramazzini Foundation (Bologna, Italy) has been conducting chronic exposure carcinogenicity studies in Sprague-Dawley rats for over 25 years. The NTP has begun collaboration with the Ramazzini Foundation to create sufficiently similar protocols, similar quality assurance and similar reporting between the two agencies. The first phase of this collaboration has begun with the Ramazzini Foundation using the NTP's data acquisition and analysis databases to store and analyze some of their data. In addition, a researcher from the Ramazzini Foundation is visiting NIEHS for two years to gain expertise in the use of molecular biology tools in cancer testing.

The World Health Organization (WHO) International EMF Project has been coordinating research on the health effects of electric and magnetic fields including those generated by cellular telephone technologies. The NTP is developing exposure systems and study designs to test for potential toxicity from radio-frequency emissions in the cellular range. A collaboration has been set up with the WHO to coordinate our activities with other groups world-wide and to facilitate workshops to keep all interested parties updated on current research.

The Korean Government has begun the development of a National Toxicology Program. The lead scientists for this program visited the NIEHS September 4-11 to meet with NTP scientific staff and attended the September 5-6 NTP Board of Scientific Counselors Technical Reports Review Subcommittee meeting. NTP Scientists will be visiting Korea in November to formalize discussions on our collaboration and coordination efforts.

NTP Board of Scientific Counselors

The NTP Board provides primary scientific oversight to the NTP Director regarding the program and the scientific merit of its intramural and collaborative programs.

Technical Reports Review Subcommittee
The Subcommittee met September 5-6, 2002, at the NIEHS to review the findings and conclusions of draft technical reports from NTP toxicology and carcinogenesis studies. The reports under review included: cinnamaldehyde, decalin, dipropylene glycol, elmiron, pentaerythritol triacrylate (PETA), trimethylolpropane triacrylate (TMPTA), and urethane + ethanol. The studies of PETA and TMPTA are 6-month dermal studies conducted in the Tg.AC mouse model. The NTP did not request that the Subcommittee make a final recommendation regarding the carcinogenicity of TMPTA or PETA, but asked the Subcommittee to consider broader issues with regard to the interpretation and reporting of these studies. Additional information about this meeting, including the agenda, is available on the NTP web site. Electronic copies of
draft technical reports are available from Environmental Health Perspectives.
The deliberations of the Subcommittee will be available at the NAEHS Council Meeting.

Board of Scientific Counselors
The Board will meet September 17-18, 2002, at the Radisson Governors Inn in Research Triangle Park, NC. Information about this meeting, including a preliminary agenda, was published in the Federal Register and is available on the NTP web site. Primary agenda items include:

- The format of the NTP brief included in each NTP-CERHR monograph prepared for chemicals evaluated by the Center for the Evaluation of Risks to Human Reproduction (see the CERHR below for information about the briefs and monographs).
- The role of genetically altered mouse models in the NTP testing program.
- Toxicogenomics and links between the NIEHS National Center for Toxicogenomics and the NTP.
- Updates on current research initiatives and new nominations to the NTP for study.
- Concept reviews for using contract mechanisms to provide support to the NTP for genetic toxicity, chemical mechanisms of toxicity, and MRI and multimodality imaging.

NTP Centers

Center for the Evaluation of Risks to Human Reproduction (CERHR)
The CERHR serves as an environmental health resource to the public and health, research and regulatory agencies for scientifically based, uniform assessments of the potential for adverse effects on reproduction and development caused by agents to which humans are exposed. The following is an update of scientific peer review activities of the CERHR. All expert reports from these reviews and other CERHR information are posted on its web site.

The availability of a prototype NTP-CERHR Monograph was announced through the Federal Register (August 19, 2002: Vol. 67, No. 160, pages 53808-53810). The prototype monograph is for di-n-butyl phthalate (DBP) and includes the CERHR Phthalates Expert Panel Report on DBP, public comments on the DBP expert panel report, and the NTP-CERHR Brief on DBP. The NTP-CERHR brief provides the NTP's interpretation of the potential for exposure to DBP to adversely affect reproduction and/or development in humans. The CERHR is requesting input from the NTP Board of Scientific Counselors at its upcoming meeting September 17-18, 2002 (see above) on the structure, clarity, and utility of the brief. When the final format of the NTP-CERHR brief is agreed upon, briefs will be prepared on all chemicals evaluated by expert panels.

NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)
The NICEATM and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) facilitate the development, scientific review, and validation of new and revised toxicological test methods that may predict human health risks better than currently used methods and may improve toxicity characterization, increase savings in time and cost, and refine, reduce, or replace animal use. The NICEATM also promotes information sharing and communication among stakeholders.

Expert Panel Review on *In Vitro* Assays
ICCVAM and NICEATM held an expert panel review meeting, May 21-22, 2002, at the Sheraton Imperial Hotel in Research Triangle Park, NC to assess the validation status of *in vitro* assays proposed for use in the EPA's Endocrine Disruptor Screening Program (EDSP). The proposed estrogen receptor (ER) and androgen receptor (AR) binding and transcriptional activation (TA) assays are relevant for screening purposes because some substances may alter natural endocrine processes in the body by binding with estrogen and/or androgen receptors and either initiating or inhibiting sex hormone dependent gene activation. NICEATM prepared background review documents on *in vitro* ER and AR binding and TA assays that provided comprehensive reviews of available data and related information necessary to evaluate the validation status of these assays. Availability of the final expert panel report will be announced in an upcoming Federal Register notice and relevant materials used for this review can be found on the ICCVAM/NICEATM website.

Corrosivity Report

NICEATM has released the report entitled, "ICCVAM Evaluation of EPISKIN™, EpiDerm™ (EPI-200), and the Rat Skin Transcutaneous Electrical Resistance (TER) Assay: *In Vitro* Test Methods for Assessing the Dermal Corrosivity Potential of Chemicals," NIH Publication 02-4502. The report contains test method summary reports, protocols, and the ICCVAM's final recommendations on the three methods. The report is available electronically (PDF and HTML) on the NICEATM/ICCVAM web site.

The ICCVAM recommends that EPISKIN™, EpiDerm™ (EPI-200), and the Rat Skin TER assay can be used to assess the dermal corrosivity potential of chemicals and chemical mixtures in a weight-of-evidence approach using an integrated testing scheme for dermal irritation/corrosion. In this approach, positive *in vitro* corrosivity responses will not generally require further testing and the results can be used for classification and labeling without the need for animal testing. Accordingly, these methods provide for the replacement of animal use when positive results are obtained.

In accordance with Public Law 106-545, NCEATM will forward the ICCVAM test recommendations through the Secretary, Health and Human Services to Federal agencies for their consideration and appropriate action. Agency responses to ICCVAM test recommendations will be made available on the ICCVAM/NICEATM web site. Inquiries or comments about the report should be addressed to Dr. William S. Stokes, Director, NICEATM, NIEHS, P.O. Box 12233, MD EC-17, Research Triangle Park, NC, 27709; fax: 919-541-0947; telephone: 919-541-2384.

In Vitro Validation Study

In a joint effort with the U.S. EPA and in collaboration with the European Centre for the Validation of Alternative Methods (ECVAM), NICEATM has begun a multi-laboratory validation study to evaluate the usefulness of the BALB/c 3T3 and the Normal Human Keratinocyte (NHK) NRU assays to estimate acute oral toxicity of chemicals and to serve as the basis for selecting starting doses for animal studies. A list of 72 chemicals, which represent the 6 acute toxicity hazard classifications in the Globally Harmonized Classification Scheme, has been compiled for testing. Two U.S. laboratories and one European laboratory are participating in the study. Testing will proceed in three phases that are designed to facilitate standardization and optimization of the test method protocols before the majority of the chemicals are tested. Phase I began in August 2002 and all testing is expected to be completed by December 2003.

FEATURED ACTIVITIES of the DIVISION OF EXTRAMURAL RESEARCH AND TRAINING
October 2002

MEETINGS

The Role of Environmental Agents in Cardiovascular Disease
August 6-7, 2002
Durham Marriott at the Civic Center, Durham, NC

Cardiovascular disease represents the primary source of mortality in the industrialized world. The etiologies of cardiovascular diseases (CVDs) are multifactorial and include diet, genetics, and lifestyle. Findings over the last several years have made it clear, however, that environmental factors, such as ambient particulate matter, aldehydes, and polyaromatic hydrocarbons are also associated with pathophysiological changes in the cardiovascular system, cardiac malformations, and death due to CVD. However, relative to other organ systems, there has been little research into the effects of environmental agents on the cardiovascular system.

Recent advances in the fields of signal transduction, genetics, molecular biology, and epidemiology make expansion of research efforts in this area very timely. In addition, some environmental health science and cardiovascular researchers have successfully bridged the gap between these disciplines, resulting in innovative approaches to the study of environmentally induced CVD. Therefore, enhanced collaboration between these disciplines is seen as vital to the success of these efforts.

The format of the workshop, with emphasis on 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.

The workshop was cosponsored by NIEHS, EPA, NHLBI, and the American Heart Association Council on Epidemiology and Prevention and the AHA Expert Panel on Population and Prevention Science. Members of the organizing committee included Drs. Pat Mastin (NIEHS), Robert Devlin, Stacey Katz, and Gail Robarge (US EPA), Ken Ramos (Texas A&M University), Aruni Bhatnagar (University of Louisville), Wayne Cascio (UNC-Chapel Hill), John Godleski (Harvard School of Public Health), Murray Mittleman (Beth Israel Deaconess Medical Center), and Eser Tolunay (NHLBI).

Meeting Highlights
The workshop had more than 110 attendees. Most of the participants were environmental health scientists, but a good representation of cardiologists and cardiovascular researchers also attended. The major activities of the workshop were the six breakout sessions, which covered the following topics: Cardiovascular Epidemiology, Particulate Air Pollution and Myocardial Infarction, Environmental Agents and Vascular Disease, Environmental Modulation of Myocardial Excitability, Cardiovascular Oxidative Stress and Environmental Pollutants, and Environmental Toxicity and Cardiovascular Development. The workshop was very successful in bringing researchers from different fields together to discuss these issues. There was universal agreement that inclusion of multiple perspectives greatly enhanced the discussions. Research needs that were identified by the participants included more epidemiology and screening programs to identify agents, research to define the characteristics of toxicants and exposures, new animal models (or new application of existing models), identification of susceptibility factors (such as age and pre-existing diseases), and research to better understand the molecular targets in the cardiovascular system and to identify potential biomarkers. The workshop will hopefully act as a starting point for more activities, and collaborations, in this relatively understudied area.

The products of the meeting will include publication of the identified research needs and development of a document to serve as a framework for future program planning in this area.

Comparative Mouse Genomics Centers Consortium Symposium 2002
Human Gene Variation: From SNPs to Phenotypes
July 28-30, 2002
Seattle, Washington

The NIEHS Division of Extramural Research and Training initiated the Comparative Mouse Genomics Centers Consortium (CMGCC) Program in May 2001. The CMGCC scientific program falls under the auspices of the NIEHS Environmental Genome Project (EGP), a multidisciplinary, collaborative program that is focused on examining the relationships between environmental exposures, inter-individual sequence variation in human genes and disease risk in U.S. populations. The EGP, as it is organized today, has three phases. Phase 1 involves the systematic identification and genotyping of single nucleotide polymorphisms (SNPS) in cell cycle and DNA repair environmental response genes. Phase 2, of which CMGCC is a part, involves functional analysis of human DNA polymorphisms. Phase 3 will involve population-based epidemiology studies of human DNA polymorphisms. The CMGCC program is designed to develop transgenic and knockout mouse models based on

human DNA sequence variants in environmentally responsive genes. These mouse models will be used as tools to improve our understanding of the biological significance of the human DNA polymorphisms identified during Phase 1. This cooperative agreement program is comprised of an NIEHS Extramural team, including Drs. Velazquez and Packenham, Ms. Winters and Ms. McDuffie, and five University Centers with close to one hundred individuals.

CMGCC Centers:
MD Anderson Cancer Center, University of Texas, Dr. David Johnson – Center Director
Harvard Medical School, Dr. Raju Kucherlapati – Center Director
University of Washington, Dr. Warren Ladiges – Center Director
University of Cincinnati, Dr. Peter Stambrook – Center Director
University of Texas at San Antonio, Dr. Jan Vijg – Center Director

This Symposium was the first annual meeting of the CMGCC. The meeting was organized to introduce the CMG Centers to the Scientific Community and to promote strong Consortium interactions between the Centers principal investigators, post – doctoral fellows, staff and students. The symposium addressed how genetically engineered mouse models can be used to study environmentally induced human diseases

Meeting Highlights:
Over 100 individuals attended the meeting, including scientists from the U.S. and abroad. The meeting was two and one-half days. The topics for the first day of the meeting were DNA repair, cell cycle control, and gene function and regulation. Each Center director gave a general overview of his center followed by scientific presentations from Center Investigators. The evening session of the first day included a scientific poster session. The poster session consisted of projects from graduate students, post-docs and junior faculty. During each day of the symposium, notable scientists presented keynote addresses:

- "History of Mouse Genetics" - *Dr. Muriel Davisson*, Senior Staff Scientist, The Jackson Laboratory, Bar Harbor Maine
- "Natural Genetic Variation" - *Dr. Leland Hartwell*, Nobel Laureate, President and Director, Fred Hutchinson Cancer Research Center, Seattle, Washington
- "Our Changing Perception of the Terms "Mutation" and "Haplotype" - *Dr. Daniel W. Nebert*, Professor of Environmental Health and Pediatrics and Developmental Biology, University of Cincinnati Medical Center

During the second day of the meeting, distinguished speakers presented scientific lectures in the following areas:

SNP Variants
- "SNPing in the Human Genome" – *Debbie Nickerson*, University Of Washington
- "Patterns of Human DNA Sequence Variation" – *J. Claiborne Stephens*, Genaissance Pharmaceuticals
- "SNP Assessment in the Human Population" – *Leonid Kruglyak*, Fred Hutchinson Cancer Research Center

SNP Epidemiology
- "Folate, DNA Repair and Colorectal Neoplasia" **–** *Cornelia Ulrich*, Fred Hutchinson Cancer Research Center
- "Winnowing Seeds from the Chaff: Do any of the Large Number of Polymorphic Variants in DNA Repair Genes Impact Individual Cancer Risk" **–** *Harvey Mohrenweiser*, Lawrence Livermore National Laboratories

ENU Mutagenesis
- "Mouse ENU Mutagenesis" – *Monica Justice*, Baylor College of Medicine

Pheonomics
- "Image Based Phenotyping – The Visible Mouse" **–** *G. Allen Johnson*, Duke University Medical Center
- "Mouse Models of Diabetes and Atherosclerosis" **–** *Renee Le Boeuf*, University of Washington
- "Small Animal Imaging for Serial Characterization of Developing Mouse Embryos" – *Kenneth Krohn*, University of Washington

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Built Environment - Healthy Communities, Healthy Homes, Healthy People: Multilevel, Interdisciplinary Research Approaches
July 15-16, 2002
Research Triangle Park, NC

The built environment is defined as part of the overall ecosystem of our earth. It includes land-use planning and policies that impact our communities in urban, rural and suburban areas. It encompasses all buildings, spaces and products that are created, or modified, by people. It includes our homes, schools, workplaces, parks/recreation areas, business areas and roads. It extends overhead in the form of electric transmission lines, underground in the form of waste disposal sites and

subway trains, and across the country in the form of highways (adapted from Health Canada, 1997).

Dr. Srinivasan, Mr. O'Fallon and Dr. Tyson, CEMBB/DERT, organized this meeting, which was co-sponsored by the NIH Office of Behavioral and Social Science Research and the NIH Office of Rare Diseases. The purpose of the conference was to focus on the state of the science and explore future directions in conducting research on the built environment and health. It built upon the past workshops convened by NIEHS and the knowledge the agency has garnered from Health Disparities projects it has supported. The meeting was well attended, with over 100 participants. It was held in conjunction with the Health Disparities Grantee Meeting. A report based on the proceedings of this meeting will be available October 2002.

Meeting Highlights

The meeting was divided into three sessions. The first session titled "Environmental Health and Sustainable Communities" highlighted the importance of including environmental health in policy deliberations that in the long term create communities that are sustainable. The presentations focused on providing some broad based framework for the discussion on built environment and the creation of sustainable communities which incorporate improved environmental and public health. Sustainable communities are defined as those that seek to balance the social, economic, cultural, and the ecological infrastructure with human health and development. The second session entitled "Health Impacts" discussed the creation of communities that are environmentally healthful that requires an understanding of the impact of the structure of the built environment and urban ecosystems on air and water quality in homes, offices, and industry, the system of transportation and the emissions from automobiles, etc. This session highlighted the importance of planning that is cognizant of environmental health in the creation of healthy communities, healthy homes and healthy people. The final session titled "Partnerships for Environmentally Healthful Communities" focused on the creation of communities that are cognizant of the environment and the health of its citizens that require partnerships among policy makers, governments, researchers, communities, and health specialists who have an interdisciplinary perspective. This session highlighted several programs that have developed partnerships to create sustainable communities and that have a positive impact on public health.

Each session involved three grantees from the Health Disparities program responding to the speakers in relation to their project funded by NIEHS.

Meeting Recommendations

- The need for more effective measures of built environment, such as indicators for sustainable communities.
- Health impacts of better planning and use of more efficient or alternative energy (in areas such as, transportation, agriculture, land use, architecture, community design, etc).
- Need to incorporate cost effectiveness of adopting environmentally sustainable technologies.
- Need to develop an interdisciplinary program approach for training, within agencies and in research.
- Improve communication strategies which promoting community participation.
- Develop multi-levels of measurement and analyses that incorporate longitudinal models (including SES, biology, neighborhoods, physical environment etc).
- Identify factors that mediate and moderate built environment health effects.
- Methods and channels to translate research findings to policy.

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Arsenic in New England: A Multidisciplinary Scientific Conference
http://www.dartmouth.edu/~cehs/ArsenicConference/IndexAS.html
May 29-31, 2002
Manchester, New Hampshire

The Superfund Basic Research Program (SBRP) has an established history in supporting multi-disciplinary conferences and workshops developed by SBRP grantees that focus on research topics important to the mission of the Program. SBRP sponsored scientific conferences are considered an integral component of the program's mission to disseminate scientific information. Conferences are just one of several approaches used by the Program for dissemination of research findings not only to the scientific community but to the many different audiences/stakeholders that may use information generated by the Program for decision-making.

The Dartmouth Program with its research emphasis on arsenic has been very proactive in developing the New Hampshire Arsenic Consortium. Arsenic exposure is of particular concern in New England, where soils and waters in many regions naturally contain levels of arsenic that are substantially higher than those found in other areas of the United States. The New Hampshire Arsenic Consortium brings together university scientists and the New Hampshire Departments of Environmental Services and Health and Human Services and the US Geological Survey. Formation of this group had led to increased communication among the agencies and has resulted in the design and undertaking of inter-agency projects to collect data to support risk assessments. One of the outcomes of this collaboration was the "Arsenic in New England" conference, which the NIEHS SBRP supported.

Exposure to arsenic in drinking water represents a significant health problem for people around the world. Arsenic tops the list

of U.S. Environmental Protection Agency's list of hazardous chemicals at toxic waste sites. Though exposure to arsenic has been linked to increased risk of cancer, heart disease, diabetes and reproductive disorders in humans, most studies have involved people exposed to elevated levels in the workplace or in parts of the world where drinking water contamination is exceptionally high. Scientists have little direct information about the effects of arsenic at levels found commonly in the United States. In addition, the way arsenic interacts with other substances in biological systems--such as the cells in human bodies -- is poorly understood.

This two-and-a-half day, multidisciplinary scientific conference provided participants with an overview of new findings regarding arsenic in New England by researchers in disciplines ranging from geology to molecular biology. Scientific presentations and discussions focused on arsenic's natural occurrence; patterns of anthropogenic use and disposal in New England; mechanisms of action as a toxin; effects on human health; environmental impact and movement through ecosystems; and regulation and remediation strategies. One goal of this cross-disciplinary forum was to provide an opportunity for synthesis - a more comprehensive view of arsenic and its impact on human health.

Meeting Highlights
The conference had more than 170 attendees representing a diverse community including academicians, State Health and Environmental Department officials from New Hampshire, Massachusetts, Maine, Vermont, and Wisconsin, Federal agency representatives from EPA and USGS, environmental consultants and private industry representatives. The two-and-a-half meeting consisted of five scientific platform sessions and an evening poster session. The platform sessions covered the following topics: Arsenic Occurrence and Geochemistry, Local Arsenic Issues – Controls and Mechanisms, Biology and Epidemiology of Arsenic, Epidemiology of Environmental Arsenic Exposure and Remediation and Regulation of Arsenic.

The first session on occurrence and geochemistry summarized the prevalence of arsenic in ground water in the New England states and the likely sources for the arsenic. In general, the predominate source of arsenic in groundwater and stream sediments is rock-based arsenic and the levels seen appear to be strongly correlated with water chemistry, geologic provinces and rock chemistry while there is a weak correlation with past agriculture land-use. Moreover, well by well measurements for arsenic is still the most reliable method to quantitate arsenic levels. Studies presented demonstrated that even with significant geochemical and geospatial data, the microenvironment has significant influence over arsenic levels making model predictions very unreliable as currently developed.

The second session on controls and mechanisms provided more in depth discussions on the influence of water and rock chemistry and geologic formations on the fate and transport of arsenic through the aquifer.

The third and fourth sessions focused on biology and epidemiology. These sessions provided the audience with the latest scientific advances concerning the potential impact of arsenic on human health. Presentations focused on molecular and cellular mechanisms for cardiovascular effects, expression of DNA repair genes and arsenic as an endocrine disruptor; its role in cancer, vascular disease and diabetes. Epidemiology studies discussed arsenic exposure and reproductive effects and cancer risks. In addition, there was a public health discussion on arsenic exposure through indirect water pathways such as bathing and issues that communities face when told there is arsenic in the water.

The last session of the conference focused on primary prevention; what remedial steps can be taken to reduce arsenic levels in drinking water and the regulatory policies that govern drinking water standards for arsenic. The meeting ended with a panel discussion that grounded all of the participants in the reality of the arsenic problem. Discussion by state health officials reminded us that the public would like answers to their questions "What does this arsenic concentration in my drinking water mean to the health of me and my family?" and "What can we do about it?" There are still no easy answers to these questions.

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Bioremediation and Biodegradation: Current Advances in Reducing Toxicity, Exposure and Environmental Consequences
July 9-12, 2002
Pacific Grove, California

The United States has thousands of hazardous waste sites, most of which are legacies of many decades of industrial development, mining, manufacturing and military activities. Biodegradative processes and bioremediation solutions form a large part of the current science and technology directed at treatment of environmental contaminants at these hazardous waste sites. As there has been an explosion of cutting-edge basic research in these areas over the past several years and, as the SBRP has a long history of supporting research in area of bioremediation, it was timely for the Program to bring together leaders in the field to discuss innovations in the field of monitoring and remediating as well as specific advances in the degradation of PCE's, PAHs, MTBE and nitrophenol. Reoccurring themes of discussion centered on the implications of improved analytical technologies and the bioavailability of contaminants in the context of biodegradative processes.

The conference was sponsored by the Superfund Basic Research Program and organized and chaired by SBRP grantees Jerome Kukor, (University of Michigan program) and Lily Young (New York University). Dr. William Suk presented the

keynote address, “The Superfund Basic Research Program: A Model for Meeting the Interdisciplinary Research Needs of the Nation.” Scientists and students from across the country met to address current issues at the interface areas of toxicity reduction, exposure assessment, and evaluation of environmental consequences. The conference included a mixture of formal (platform lectures) and less-formal (roundtable discussions and poster sessions) sessions, allowing all participants opportunities to express their views. Sessions included:

I. Approaches to Overcome Bioavailability Limitations in Bioremediation;
II. New Discoveries in Microbial Degradation of Persistent Environmental Contaminants;
III. Biological Activity and Potential Toxicity of the Products of Biodegradation;
IV. New Methods to Monitor and Assess the Effectiveness of Remediation Processes; and
V. Strategies for Remediation of Mixed Contaminants.

Conference proceedings will be available on CD, and it is anticipated that the platform lectures will be published in *Environmental Health Perspectives.*

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DERT PAPERS OF NOTE

Martyn T. Smith
University of California Berkeley
P30ES01896

Gene Polymorphisms and Altered Risk of Adult Acute Lymphocytic Leukemia

Background: Although the clinical and pathological aspects of leukemia are well known, little is understood about the genes that influence susceptibility to this complex disease. Certain gene polymorphisms have been shown to alter the risk of development of leukemia and these variations can interact with diet, other environmental exposures, and individual immune function to be major determinants of susceptibility. This researcher has previously reported polymorphisms in a folate metabolizing gene with a decreased risk of acute adult lymphocytic leukemia (ALL). The research hypothesis is that the protective effect is due to an increase in the flux of folate compounds available for DNA synthesis and subsequent reductions of uracil in the DNA. Accumulation of uracil in DNA and its subsequent removal during excision repair processes can result in DNA double strand breaks which are necessary for chromosomal translocations and deletions.

Advance: Polymorphisms in methionine synthase (*MS*), cytosolic serine hydroxymethyltransferase (*SHMT*), and a double or triple 28-base pair tandem repeat in the promoter region of thymidylate synthase (*TS*) were studied and all were found to reduce ALL risk dramatically. When individuals had both the SHMT polymorphisms and the triple repeats in *TS* or the *MS* and *SHMT* polymorphisms the ALL risk was even further reduced.

Implications: This research illustrates an association between changes in folate metabolic pathways which affect *TS* and lymphocytic leukemia risk that may underscore the importance of compromised DNA fidelity and insufficient folate intake in the development of ALL. DNA integrity is dependent on the bioavailability of deoxynucleotides, particularly in cells with high replication rates such as those found in the hematopoetic system and epithelium. Moreover, low intake of folic acid and other factors such as vitamins B2, B6, and B12 may increase ALL risk in persons with high risk genotypes. A combination of unfavorable genotypes, diet, and vitamin B intake and balance may conceivably be the key factor in susceptibility to ALL. Continued research may lead to dietary and nutritional modifications to decrease the risk of genetically susceptible individuals.

Citation: Skibola CF, Smith MT, Hubbard A, Shane B, Roberts AC, Law GR, Rollinson S, Roman E, Cartwright RA, and Morgan GJ. Polymorphisms in the thymidylate synthase and hydroxymethyltransferase genes and risk of adult acute lymphocytic leukemia. *Blood* 2002; 99, 10:3786-3791.

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Patricia A. Buffler and Martyn T. Smith
University of California, Berkeley
R01ES09137 and P42ES04705

Discovery of Gene Translocations in Childhood Acute Myeloid Leukemia

Background: The cause and progression of childhood leukemia is of great interest to patients and their families and physicians as well as basic and epidemiologic researchers. Understanding how the disease starts and proceeds has implications on how it can be prevented and/or treated. Recent studies have shown that the genetic changes necessary to allow the development of the disease occur *in utero*. Leukemia may not develop for several years suggesting additional molecular events that are necessary for disease development. Childhood acute myeloid leukemia (AML) comprises about 20% of all childhood leukemia and represents a group of diseases with a variety of molecular subtypes. After a peak incidence of leukemias in infants with translocation of the *MLL* gene, children with AML exhibit the same range of abnormalities as adults, the most frequent of which is a fusion of the *AML1* and *ETO* genes.

Advance: Unlike the age associated peak in leukemia incidence seen with the *MLL* translocation, the *AML1-ETO* fusion increases slowly during childhood and is constant throughout life. Similar leukemias sometimes develop after chemotherapy for other cancers adding additional evidence that further triggers are necessary for leukemia development. These researchers analyzed genomic sequences for 5 AML patients. Two of the patients were older than 10 years at the time of diagnosis indicative of a protracted postnatal latency period. Further studies showed that the genomic fusion sequences persist during remission.
Implications: These studies indicate that the genetic alterations leading to AML in children occur *in utero*, possibly as an initiating event that requires secondary genetic alterations to cause leukemia. This raises the question of whether translocation positive-preleukemic stem cells formed *in utero* may persist into adulthood providing a lifetime supply of cells that may progress to AML given the correct secondary genetic alteration.

Citation: Wiemels JL, Xiao A, Buffler PA, Maia AT, Ma X, Dicks BM, Smith MT, Zhang L, Feusner J, Wiencke J, Pritchard-Jones K, Kempski H, and Greaves M. In utero origin of t(8;21) *AML1-ETO* translocations in childhood acute myeloid leukemia. *Blood*, 15 May 2002; 99, 10:3801-3805.

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Bruce J. Aronow
University of Cincinnati College of Medicine
R01ES08822

Microarray Techniques Used to Identify Genes Controlling the Development of the Mouse Gastrointestinal Tract

Background: Understanding the molecular basis of gene expression along the anterior-posterior (A-P) axis of the mammalian gastrointestinal (GI) tract is a critical need for determining the genes that are involved in the embryonic development of the GI tract, how gene expression occurs, and how development progresses. This understanding has been lacking, but using novel microarray gene expression techniques can lead to rapid discovery of candidate genes responsible for GI development along the length of the GI tract.

Advance: This team of investigators hypothesized that patterns of gene expression along the A-P axis could be defined at the gene and molecular level by analyzing expression profiles of large numbers of genes. Microarrays containing over 8,600 complementary DNA (cDNA) sequences were used to define expression profiles for mouse stomach, duodenum, jejunum, ileum, cecum, proximal colon, and distal colon. Highly expressed cDNAs were classified based on segmental expression patterns and protein function. The investigators found 571 cDNAs which were expressed at least 2-fold higher than reference in at least one GI region. Most of the genes displayed sharp boundaries at anatomically defined locations. Boundaries were especially sharp for genes encoding proteins that function in intermediary metabolism, transport, and cell-cell communication–functions critical for the proper coordination of GI function. Genes with distinct expression profiles were compared with mouse and human genomic sequence for promoter analysis and gene discovery.

Implications: The anatomically defined regions of the GI tract can also be defined by the pattern of expression profiles from genes located in and controlling functions in these regions. Distinctions in gene expression patterns between the small and large intestines were much less striking than those between the stomach and the small and large intestines. The investigators also identified new genes not previously known to be expressed in GI tissue. Identification of genes co-regulated along the A-P axis provides a basis for new insights, research, and gene discovery relevant to GI development, differentiation, function, disease, and therapeutic interventions.

Citation: Bates MD, Erwin CR, Sanford LP, Wiginton D, Bezerra JA, Schatzman LC, Jegga AG, Ley-Ebert C, Williams SS, Steinbrecher KA, Warner BW, Cohen MB, and Aronow BJ. Novel genes and functional relationships in the adult mouse gastrointestinal tract identified by microarray analysis. *Gastroenterology* 2002;122:1467-1482.

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Harold F. Hemond

Massachusetts Institute of Technology
P42ES04675

Water Nitrate Effects Arsenic Valence and Concentration in an Urban Lake

Background: Anyone who has ever read a mystery novel knows that arsenic is acutely toxic and that its harmful effects have been known and exploited for hundreds if not thousands of years. However, at doses found in some drinking water supplies and private wells in parts of the United States and other parts of the world such as Chile, Taiwan, and Bangladesh, arsenic can have chronic, long-term effects such as causing skin, bladder, and prostate cancer. In the environment, inorganic arsenic is found in oxidized and reduced states. The oxidized form is known as arsenate and this valence state is much more carcinogenic than the reduced form known as arsenite.

When lakes and streams become anoxic, underlying sediments tend to release arsenic and iron compounds. In most cases, the arsenic originates from industrial pollution. Arsenate can be bound up by iron particles rendering the arsenic unavailable to wildlife and humans who may drink or otherwise come into contact with the water. Therefore, any agent that changes this intricate balance or the valence state of the arsenic compounds may have important public health consequences.

Advance: This publication describes how nitrate contamination alters the balance of arsenic and iron complexes. Nitrates accumulate in water systems through agricultural and lawn fertilizer use and from animal waste runoff. Nitrate is a powerful oxidant and thus has the potential to oxidize arsenite released from lake and stream sediments to the more toxic arsenate. Depending on the concentration of iron in the water, arsenate may accumulate in the water.

Implication: With the new drinking water standard for arsenic of 10 µG/l, some municipalities will have to pay high costs to bring their water supplies into compliance to protect the health of the people they serve. Understanding the causes of arsenic contamination and other factors influencing the concentration of arsenic and its oxidation-reduction cycle may influence the method used to reduce the arsenic concentration. Methods to prevent the introduction of nitrates into drinking water supplies may be important in keeping the arsenic in the lake and stream sediments and out of the water supply.

Citation: Senn DB, Hemond HF. Nitrate controls on iron and arsenic in an urban lake. *Science* 2002, vol. 296:2373-2376.

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Richard D. Kolodner
University of California at San Diego
R01ES11040

New Discoveries in the Development of Colon Cancer

Background: Several forms of human colorectal cancer exist with the most frequent being the sporadic form. A variety of genetic mutations have been implicated in the development of this deadly disease. Sporadic defects in DNA mismatch repair genes play a role in either the initiation or progression of a number of tumor types. This observation suggests that other DNA repair genes could be involved in the development of colon cancer. One such gene known as *Flap endonuclease 1* (*Fen1*) was investigated in this study. *Fen1* is required for DNA replication and repair, and defects in the gene encoding Fen1 are known to cause accumulation of mutations and genomic rearrangements. Using gene knockout techniques, the investigators introduced a mutation into *Fen1*.

Advance: Genetic analysis of the mice used in this study showed that none were homozygous for the *Fen1* mutation. This suggests that absence of *Fen1* expression leads to embryonic lethality. Most of the mice heterozygous for the *Fen1* mutation appeared normal, but further studies showed that when combined with a mutation in the adenomatous polyposis coli gene, double heterozygous animals have increased numbers of adenocarcinomas and decreased survival.

Implication: This study suggests that insufficiency of *Fen1* expression may not make a difference to a cell undergoing normal replication, but if the cell cycle is perturbed by mutations in oncogenes or tumor suppressor genes, additional levels of a least some gene products might be necessary to accommodate the change in rates of cell division. If one or more products necessary for replication and repair is not present in the right quantities, the result may be detrimental to genomic stability. These results imply that a quantitative measure of the expression of some of these gene products may be useful as prognostic indicators of disease.

Citation: Kucherlapati M, Yang K, Kuraguchi M, Zhao J, Lia M, Heyer J, Kane MF, Fan K, Russell R, Brown AMC, Kneitz B, Edelmann W, Kolodner RD, Lipkin M, and Kucherlapati R. Haploinsufficiency of Flap endonuclease (Fen1) leads to rapid tumor progression. *PNAS*, July 23, 2002; 99; 15:9924-9929.

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Scott Ballinger
University of Texas Medical Branch
R03ES09318

Mitochondrial Damage Leads to Atherosclerosis

Background: Reactive species (RS) are made up of a group of reactive oxygen and nitrogen species that can alter the biological functions of essential molecules such as lipids, proteins, and DNA. Numerous studies have linked RS with the development of atherosclerotic disease which remains the leading cause of death in the Western world. Although the exact sequence of events in this process is yet to be determined, RS likely play an important role in vascular cell dysfunction and atherosclerosis probably through oxidative damage to the mitochondrial genome. In this study, the investigators examined the contribution of mitochondrial oxidant generation and DNA damage to the progression of atherosclerotic lesions in human arterial specimens and a mouse model prone to atherosclerosis.

Advance: Mitochondrial DNA damage correlated with the extent of atherosclerosis in the human tissue and in the susceptible mice. The DNA alterations were seen prior to the development of atherogenesis in the mice suggesting a causative relationship. In addition, mice deficient in manganese superoxide dismutase, a mitochondrial antioxidant enzyme, exhibited early increases in mitochondrial DNA damage and a phenotype of accelerated atherogenesis.

Implications: These studies suggest that mitochondrial DNA damage may result from RS production in vascular tissues and may also be an early event in the development of atherosclerosis. Additional research confirming these results may lead to earlier detection of individuals at risk for atherosclerotic disease and improved methods to prevent of reverse the formation of atherosclerotic plaques through the use of antioxidant therapies.

Citation: Ballinger SW, Paterson C, Knight-Lozano CA, Burow DL, Conklin CA, Hu A, Reuf J, Horaist C, Lebovitz R, Hunter GC, McIntyre K, and Runge MS. Mitochondrial Integrity and Function in Atherogenesis. *Circulation* 2002; 106:544-549.

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David A. Schwartz
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R01ES07498, P01ES09607, and U19ES11375

Receptor Variant that Confers Decreased Immune Function is a Marker for Resistance to Atherosclerosis

Background: The ability to mount a prominent inflammatory response to a bacterial challenge confers an advantage in innate immune defense; however, the effects of intravascular inflammation lead to proatherogenic effects. The focus of this study was to determine if genetic variants in the toll-like receptor 4 (TLR4) that confer differences in the inflammatory response due to bacterial lipopolysaccharide are related to the development of atherosclerosis. The hypothesis tested was that efficient immune defense offers an early advantage but at a cost of chronic vascular damage in later years.

Advance: An epidemiologic study was carried out in 810 persons in which the team screened for TLR4 polymorphisms. The extent and progression of atherosclerosis was also assessed. Fifty-five individuals were found to have the Asp299Gly TLR4 polymorphism. These individuals had lower levels of certain proinflammatory cytokines and other inflammatory agents. While these subjects were more susceptible to severe bacterial infections, they had an almost 50% reduction in the risk of carotid arterial atherosclerosis.

Implications: The polymorphism identified in this study attenuates receptor signaling and diminishes the inflammatory response to gram negative bacteria along with decreasing the risk of atherosclerosis. This study provides further evidence that an efficient innate immune defense against bacteria is associated with long-term intravascular inflammatory stress leading to the development of atherosclerosis.

Citation: Kiechl S, Lorenz E, Reindl M, Wiedermann CJ, Oberhollenzer F, Bonara E, Williet J, Schwartz DA. Toll-like receptor 4 polymorphisms and atherogenesis. *New England Journal of Medicine* 2002, 347; 3:185-192.

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Joseph Kiesecker
Pennsylvania State University

R01ES11067

Frog Limb Deformities: Synergism Between Pesticide Exposure and Parasite Infection

Background: For the past ten years, biologists have been puzzled by the apparent epidemic of frog limb deformities seen in Canada and the United States especially in the West, Midwest, and Northeast. Researchers are unsure whether the dramatic increase relates to increased awareness or environmental changes, but a variety of studies have sought to link the deformities and the dwindling number of frogs to ultraviolet radiation exposure, chemical pollution, predation, parasites, or disease outbreaks. Earlier reports by Kiesecker have suggested that extremely dry climatic conditions cause increased exposure to ultraviolet radiation which in turn causes immune dysfunction leading to decreases in population density resulting from increased susceptibility to diseases and parasite infection.

Advance: In the current study, Kiesecker reports field and laboratory studies that conclusively demonstrate that trematode infection was required for the development of limb deformities in wood frogs. Deformities were more common at sites adjacent to agricultural runoff. The laboratory studies corroborate the association between pesticide exposure and increased infection with pesticide-mediated immunocompetency as the apparent mechanism.

Implication: The immune effects in the laboratory studies were seen at pesticide levels within the current EPA drinking water standards. Similar adverse effects are seen in other amphibians in other regions and may be explained by the widespread use of pesticides. Whether similar adverse effects are seen in other animal species remains to be determined, but the fact that these effects are seen at relatively low pesticide concentrations, suggests major public health implications and the possibility of the need for more stringent pesticide regulation.

Citation: Kiesecker JM. Synergism between trematode infection and pesticide exposure: A link to amphibian limb deformities in nature? *PNAS*, July 23, 2002, v. 99:15, 9900-9904.

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E. William Spannhake
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P30ES03819

Adding Environmental Insult to Injury: Oxidant Pollutants Add to Inflammatory Cytokine Release in Response to Rhinoviral Infection

Background: Cold viruses and environmental pollutants cause respiratory cells to release inflammatory cytokines which contribute to the general malaise people feel when they have respiratory symptoms. Cytokines are cellular inflammatory components that cause inflammation, which leads to the release of fluids, swelling, and other symptoms. Not everyone reacts the same way to the combined insult of respiratory infection and pollutant exposure. Asthmatics are particularly susceptible to the combined adverse effects.

Advance: Researchers at The Johns Hopkins Bloomberg School of Public Health determined that the combined effects of NO_2 or O_3 and rhinoviral infection in cultured respiratory cells rapidly increased the release of the inflammatory cytokine interleukin-8 through oxidant-dependent mechanisms. The combined effects ranged from 42% to 250% greater than additive for NO_2 and from 41% to 67% for O_3. The effect was lessened by treatment of the cells with the antioxidant *N*-acetylcysteine.

Implication: These results indicate that oxidant pollutants can increase the production of proinflammatory cytokines by rhinoviral infected cells and suggest that viral-induced inflammation in upper and lower airways may be exacerbated by concurrent exposure to ambient levels of oxidants commonly encountered in the indoor and outdoor environments.

Citation: Spannhake EW, Reddy SPM, Jacob DB, Yu X-Y, Saatian B, Tian J. Synergism between rhinovirus infection and oxidant pollutant exposure enhances airway epithelial cell cytokine production. *Environmental Health Perspectives*, 110:7, 665-670.

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Robert E. Gawley - University of Miami
Daniel G. Baden - University of North Carolina at Wilmington
T32ES07320 and P30ES005705

Does *Pfiesteria* Produce Toxins? New Research Suggests Not

Background: The bacteria *Pfiesteria piscicida* has received a lot of attention since it was first reported to be responsible for

fish kills and human illnesses along the Atlantic coast in 1992. The dinoflagellate was reported to produce one or more toxins in certain strains and at certain periods in a proposed intricate life cycle which is said to contain "amoeba-like" stages. Other researchers in this field have been unable to reproduce these results and thus a major controversy has grown. Defenders of the original hypothesis say that these researchers are using the wrong strain of bacteria. The detractors say that the toxins are produced by another microbe that has contaminated the *Pfiesteria* cultures and thus, *Pfiesteria* is not the culprit.

Advance: A team led by researchers at the University of Miami has found that a close relative to *P. piscicida, Pfiesteria shumwayae*, does not produce the ichthyotoxins postulated to kill fish and harm humans. Simple removal of the bacteria from contaminated water by centrifugation resulted in water that was non-toxic to healthy fish. When the bacteria itself is applied to the fish, sores similar to those reported as evidence of toxin exposure are seen. The researchers speculate that similar infections may make fish more susceptible to other microbes such as a highly pathogenic fungus.

Implication: While not definitively proving that *Pfiesteria* species are or are not responsible for large fish kills along the east coast, this work suggests that the original hypothesis of the bacteria producing ichthyotoxins responsible for the kills may not be the actual pathogenic mechanism. Additional research soon to be published documenting the chemical structure and potency of a *Pfiesteria* toxin will provide additional information surrounding this controversy.

Citation: Berry JP, Reece KS, Rein KS, Baden DG, Haas LW, Ribeiro WL, Shields JD, Snyder RV, Vogelbein WK, Gawley RE. Are *Pfiesteria* species toxicogenic? Evidence against production of ichthyotoxins by *Pfiesteria shumwayae*. *PNAS*, U.S.A. Early Addition. 2002 Aug 5.

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T32ES07051

Enzyme Deficient Mice Display Hyperactivity and Impaired Learning Ability

Background: The family of calcium/calmodulin dependent phosphodiesterases (CaM-PDE) act as a potential point of interaction between the Ca^{2+} and cyclic nucleotide signaling pathways. The three known CaM-PDE genes *PDE1A-C*, are expressed in the central nervous system. *PDE1A* is expressed throughout the brain with high levels in the cerebellum and lower levels in the striatum. *PDE1B* is expressed predominantly in regions of the brain with high dopaminergic innervation such as the striatum and cerebellum. *PDE1C* is also expressed predominantly in the striatum. The expression of these genes in the striatum and other evidence that cyclic nucleotides and calcium are principal second messengers of the signal transduction pathways in the striatum suggest that CaM-PDEs may play a role in motor control. To investigate this hypothesis, these investigators generated knock-out mice lacking the *PDE1B* gene.

Advance: These mice showed increased hyperactivity, as compared to normal mice, after acute exposure to D-methamphetamine. Since the mice lacked the enzyme, hydrolysis of the cyclic nucleotides could not occur and was confirmed with analysis of tissue slices. The knock out mice and mice with one copy of the functioning gene demonstrated spatial-learning deficits in experiments employing a Morris maze.

Implication: These results indicate that enhancement of cyclic nucleotide signaling by inactivation of *PDE1B*-mediated cyclic nucleotide hydrolysis plays a major role in dopaminergic function. The results of these experiments support the conclusion that regulation of intracellular cyclic nucleotide concentration is important in the cellular processes that underlie learning and memory.

Citation: Reed TM, Repaske DR, Snyder GL, Greengard P, Vorhees, CV. Phosphodiesterase 1B knock-out mice exhibit exaggerated locomotor hyperactivity and DARPP-32 phosphorylation in response to dopamine agonists and displayed impaired spatial learning. Journal of *Neuroscience*, June 15, 2002; 22(12):5188-5197.

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David C. Christiani
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P01ES06409 and P30ES00002

Glutathione S-Transferase and p53 polymorphism Are Associated with Increased Lung Cancer Risk

Background: Genetic susceptibility is one of the primary hypotheses used to explain why a minority of smokers develop lung cancer. Polymorphisms of genes involved in metabolism of carcinogens have been studied as possible attenuators of risks for lung cancer. The family of glutathione S-transferase (GST) enzymes are important components of carcinogen metabolism so polymorphic differences in these enzymes may be responsible for at least part of the differences in risk. GST π is the most

expressed metabolic enzyme in the lung and it is encoded by a polymorphic gene *GSTP1*. These polymorphisms are a consequence of a single base-pair substitution which leads to a single amino acid change in the enzyme. This substitution results in lower enzymatic activity and is associated with higher DNA adduct formation in human lymphocytes. A polymorphism in another GST gene, *GSTM1-null*, results in the total loss of enzymatic function.

Polymorphic genes involved in cell cycle regulation, apoptosis, and tumor suppression have also been studied as possible risk factors for lung cancer. *p53* is one of these genes and is one of the most commonly mutated genes in all human cancers. Several studies have shown that the polymorphic *p53* gene is associated with a higher risk of lung cancer.

Individually, functional polymorphisms of these genes have been studied as risk factors for lung cancer; however, small sample sizes have prevented the investigation of possible increases in risk associated with having two or more at risk polymorphisms. Christiani and colleagues have carried out such an investigation of "double variants" in a large Caucasian population. Because these double variants may promote lung cancer at an earlier age, a subgroup of people 55 and younger was examined separately.

Advance: In the whole population, those with double variants had a higher risk of lung cancer compared with controls. The *GSTP1* and *GSTM1-null* double variants had a relative risk only slightly higher (60%) than controls; however the *GSTP1* and *p53* double variant were twice as likely to develop lung cancer. In the younger group, the relative risks were 4- and 5-fold higher respectively.

Implication: Specific double variants of *GSTP1*, *GSTM1-null*, and *p53* double variants are associated with higher lung cancer risks. This susceptibility is highest among younger individuals. Additional studies will help elucidate the possible mechanisms involved in the steps leading to carcinogenesis. Although the combined double variant risks generally had a greater than additive effect, larger sample sizes are needed to consider differences in gender and to clarify the association with early onset lung cancer.

Citation: Miller DP, Liu G, De Vivo I, Lynch TJ, Wain JC, Su L, Christiani DC. Combinations of the variant genotypes of *GSTP1, GSTM1*, and *p53* are associated with an increased lung cancer risk. *Cancer Res*. 2002 May 15;62(10):2819-23.

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GRANTEE HONORS and AWARDS

NIEHS-supported investigator, Dr. Brendan H. L. Lee, M.D., Ph.D., of Baylor College of Medicine, has been awarded a prestigious Howard Hughes Investigator Award. The announcement was made on May 28. Dr. Lee is one of 12 of the nation's top physician-scientists to be appointed as Howard Hughes Medical Institute investigators in an innovative program to improve the translation of basic science discoveries into enhanced treatments for patients.

Dr. Peter Stacpoole, Professor of Medicine, Biochemistry and Molecular Biology at the University of Florida, was inducted as a Fellow of the Royal College of Physicians of London, on July 25, 2002, in London, England.

STAFF HONORS and AWARDS

Dr. Allen Dearry, OPD/CEMBB, and Mr. Joseph Hughes, OD/WETP, were recipients of the Secretary's Award for Distinguished Service, which was presented at the DHHS awards ceremony Wednesday, June 12. A group award was bestowed on the pair "For dedicated support to the health and safety of emergency responders, remediation workers and the community at the World Trade Center disaster." The ceremony was held in the Great Hall, Hubert H. Humphrey Building, Washington, DC.

The NIEHS Toxicogenomic Research Consortium Group received the 2002 NIH Director's Award at a ceremony held Wednesday, June 19, at NIH. The award was "For Outstanding Efforts Conceptualizing, Initiating, and Implementing the NIEHS Toxicogenomics Research Consortium." Awardees included *Dr. Anne Sassaman , OD; Dr. William Suk, OPD; Drs. Michael McClure and Jerrold Heindel, OPD/OSTB; Dr. Bennett Van Houten, OPD/PAB; Drs. Jose Velazquez and Claudia Thompson, OPD/CEMBB; Dr. Linda Bass and Ms. RoseAnne McGee and Ms. Michelle Mayo, OPO/SRB; Ms. Jackie Russell, OPO/GMB; Ms. JoAnn Lewis, OPO/RCB*, Drs. Raymond Tennant, Richard Paules, Pierre Bushell, and Cynthia Afshari, and Mr. Stan Stasiewicz from DIR, and Dr. Samuel Wilson, Deputy Director.

Ms. Martha Barnes, OPD/PAB, is a member of a trans-NIH committee, operating under the Office of Research on Women's Health, that received an NIH Merit Award "For exceptional accomplishments in revising NIH policies and procedures for monitoring inclusion and facilitating gender analysis in biomedical research," at the Office of the Director Honor Awards Ceremony 2002 held August 14, at NIH.

STAFF ACTIVITIES

Mr. O'Fallon, OPD/CEMBB, collaborated with Drs. T. Nastoff and D. Drew from the Agency for Toxic Substances and Disease Registry (ATSDR) and Dr. J. Phillips from the National Institute for Nursing Research to organize and convene a roundtable meeting on nursing and environmental health on August 26-27. The three agencies jointly sponsored the meeting which focused on the following themes: Research, Education and Translation to Practice. The interagency planning committee invited experts from around the country to participate in the roundtable meeting with the anticipated outcome of identifying gaps in these three areas and recommending next steps to address the identified needs. A final meeting report will be available in November.

Dr. Srinivasan, OPD/CEMBB, chaired a plenary track thematic session at the American Sociological Association national meeting in Chicago, Illinois, on August 18 entitled "Profiling in Health," which examined the affects of social environment in prevention of disease and access to care and the growing health disparities among low income minority populations.

Mr. Hughes, OD/WETP, and staff hosted the NIEHS/Worker Education and Training Program Training Skilled Support Personnel meeting in Research Triangle Park, North Carolina on August 15. The focus of the meeting was on to discuss future safety and health training program initiatives regarding weapons of mass destruction incident response with a particular focus on what training is appropriate for skilled support personnel. The meeting also focused on the feasibility of establishing a national registry of trained personnel to respond to future terrorist actions. Staff attending the meeting and participating in various activities included *Ms. Beard, Mr. Outwater, Ms. Thompson, and Ms. Chaney, OD/WETP.*

Dr. Collman, OPD/CEMBB, organized and chaired two sessions on Children's Environmental Health at the International Society of Environmental Epidemiology and International Society of Exposure Analysis joint meeting in Vancouver, BC, Canada on August 12. The topics for the sessions were exposures assessment in large scale epidemiologic studies of children's environmental health and using biomarkers to translate exposure to health effects. Representatives from all of the Centers of Children's Environmental Health and Disease Prevention Program gave presentations on new findings from their research.

Dr. Shreffler, OPD/OSTB, participated in a panel discussion on Postdoctoral Fellowships at the Workshop on Grant Writing for Success sponsored by the University of North Carolina Postdoc Association in Chapel Hill, NC, on July 31. The Workshop was designed to assist individuals in postdoctoral positions in uncovering funding opportunities, understanding types of granting mechanisms, and getting started in the grant-writing process.

Ms. Anderson, OPD, served as the moderator for the session "Biological Activity and Potential Toxicity of the Products of Biodegradation" at the Bioremediation and Biodegradation: Current Advances in Reducing Toxicity, Exposure and Environmental Consequences meeting, which was held July 9-12, in Pacific Grove, California.

Dr. Van Houten, OPD/PAB, and *Dr. Weis, OPD/OSTB*, participated in a Working Group on Ethical, Legal, Social and Policy Issues in Toxicogenomics held at the Woodrow Wilson International Center for Scholars Washington, D.C., July 18-19. Dr. Van Houten gave a lecture entitled, Establishing Toxicogenomics: Necessary Steps and New Initiatives.

Mr. Hughes, OD/WETP, attended the International Association of Fire Fighters Instructor Development Conference in Las Vegas, Nevada on July 17-19. He presented on the Weapons of Mass Destruction Report to the National Response Team Subcommittee.

Dr. Srinivasan, Mr. O'Fallon and Dr. Tyson, OPD/CEMBB, organized a meeting entitled "Built Environment - Healthy Communities, Healthy Homes, Healthy People: Multilevel, Interdisciplinary Research Approaches," which was held on July 15-16, in Research Triangle Park, North Carolina in conjunction with the Health Disparities grantee meeting. The meeting was co-sponsored by the Office of Behavioral and Social Science Research. The Office of Rare Diseases provided additional funding. The purpose of this conference was to focus on the state of the science and explore future directions in conducting research on built environment and health.

Dr. Mastin, OPD/OSTB, was appointed to the Steering Committee of the National Asthma Education and Prevention Program, and was invited to make a brief presentation on NIEHS-funded asthma research at the annual meeting of the Steering Committee, June 10, in Arlington, Virginia.

Dr. Heindel, OSTB/OPD, gave a presentation, "Endocrine Disruptors: A View from NIEHS " at the Endocrine Disruptors Gordon Conference at Mt. Holyoke College, Massachusetts, July 9.

Dr. McClure, OPD/OSTB, has been appointed to a three-year term on the Hitchings and Elion Award Advisory Committee of the Triangle Community Foundation. Drs. Hitchings and Elion shared the 1998 Nobel Prize in Physiology or Medicine for a series of scientific breakthroughs in pharmaceutical chemistry that revolutionized the world of drug design. Founded with Nobel Prize funds, the Triangle Community Foundation, in partnership with the Burroughs Wellcome Fund, supports awards to outstanding young investigators in the Triangle region of Wake, Durham, Orange and Chatham Counties. The George H.

Hitchings New Investigator Award in Biomedical Research and the Gertrude B. Elion Mentored Medical Student Award is an annual national competition that honors with remembrance the strong belief of the two Nobel Laureates in the high value of supporting the training and mentoring of the next generation of young scientists.

Dr. McClure, OPD/OSTB, accepted an invitation to serve on the faculty of the American Association for the Advancement of Science sponsored 2002 Science Laboratory Management Course for Young Investigators funded by the Howard Hughes Medical Institute and Burroughs Wellcome Fund. The course was attended by 245 M.D., M.D./Ph.D., or Ph.D. young scholars. His presentation, entitled: "Science Fair to Science Fare: A Darwinian Budgetary View of the Scientist Species" presented an overview of the changing budget needs of and strategies for fueling and refueling an independent research laboratory from start-up onward. His presentation was invited to be published in *Science* in an upcoming 2002 issue. The HHMI plans to publish the course as a multimedia manual.

Dr. McClure, OPD/OSTB, represented the NIH at the Appalachian College Association's Federal Agency and Foundation Briefing for Research Program Faculty Development held June 10-11, in Asheville, North Carolina. Dr. McClure presented an overview of the Extramural Research Grant Programs and Opportunities entitled: "The National Institutes of Health: Encouraging the Biomedical Research that improves Public Health." Attendees of the meeting were college presidents, senior administrators, and research faculty from 33 private sector colleges from Kentucky, Tennessee, North Carolina, Virginia and West Virginia. He also participated in small group and individual conferences on NIH funding mechanisms and opportunities.

Dr. McClure, OPD/OSTB, presented the Legacy Lecture "Population and the Environment" at the opening session of the Frontiers in Reproduction (FIR) Research Training Course at the Marine Biological Laboratory (MBL), Woods Hole Massachusetts on May 19th. The course is a research concepts, strategies and technology applications "wet" lab career development experience mentored (1:2) and instructed by an international faculty of nearly 40 world-class scientists. Sixteen outstanding young scholars, selected by an annual international, peer-reviewed competition, were awarded scholarships to attend the FIR course.

Ms. Beard, OD/WETP, attended and presented at the Brownfields Interagency Taskforce Meeting in Washington, DC on May 30.

Mr. Hughes and Mr. Outwater, OD/WETP, presented at the 12th Annual Construction Safety and Health Conference and Exposition on May 21-23 in Chicago, Illinois. The Worker Education and Training Program along with Center to Protect Workers' Rights, NIOSH, the Construction Safety Council and many other organizations sponsored this conference. The conference shared information and ideas about effective safety and health interventions and how to move "best practices" from inception to practical implementation.

UPCOMING MEETINGS and WORKSHOPS

NIEHS and ATSDR are jointly sponsoring a meeting, "Thyroid Hormone & Brain Function: Translating Molecular Mechanisms to Population Risk," September 23-25. The purpose of this conference is to bring together a multidisciplinary group of research scientists (epidemiologists, clinicians, basic and molecular biologists, developmental biologists and toxicologists, and endocrinologists) in a joint forum to discuss the current state of emerging, multidisciplinary knowledge relevant to the role of thyroid hormones in brain development and the effects of exposures to environmental agents on this system. Focus will be on maternal thyroid status and neurological function of the offspring; basic studies on brain development; the role of thyroid hormones in brain development; the effects of environmental agents on thyroid hormone action during brain development; and future directions for research with emphasis on the use of genomics, genetically modified animals, imaging and translation of basic and toxicological research into public health benefit. The information gained on the state of the science and data gaps will be used to direct future collaborations between the two agencies.

The annual Center Directors' meeting will be held October 21-22, in Seattle, Washington. It will be hosted by the Environmental Health Sciences Center at the University of Washington.

NIEHS (through the Worker Education and Training Program), National Institute for Occupational Safety and Health (NIOSH), Johns Hopkins Education and Research Center for Occupational Safety and Health, and MidAtlantic Public Health Training Center are co-sponsoring the Technical Workshop on the Worker Training in a New Era: Responding to New Threats. This conference will draw upon lessons learned from recent terrorist attacks to help attendees better understand and anticipate the safety and health-training needs of workers who would be required to respond to terrorist incidents in the future. The conference will be held in Baltimore, Maryland on October 26-27. On October 25, the semi-annual WETP Awardee Meeting will be held.

Tthe Brownfields 2002 Conference will be held in Charlotte, North Carolina on November 13-15. This national conference will showcase brownfields cleanup, redevelopment and policy issues. Included will be a meeting of the awardees of the Brownfields Minority Worker Training Program to discuss progress in this new training program and promote the model of community based environmental job training program. Representative from local, state, and federal Brownfields programs will

also be invited. The meeting will provide an excellent setting to promote the WETP Minority Worker Training Program and Brownfields Minority Worker Training Program.

The University of Arizona will host the 2002 annual national meeting of the Superfund Basic Research Program (SBRP), November 3-6 in Tucson, Arizona. The intent of this year's meeting, "Transitioning Basic Science into Practical Applications to Meet Environmental and Public Health Challenges," is to highlight technology transfer activities that have evolved from basic laboratory research to practical applications, with discussion on the pathways that investigators have taken to achieve this. An important aspect of this goal is to present "emerging technologies" that have the potential to enhance the capacity of basic research to address Superfund hazardous waste issues. In addition to the scientific sessions, running concurrently will be an administrators' meeting, designed to enhance the sharing of ideas among the administrators, and an outreach workshop, highlighting the major themes of the outreach cores.

STAFF CHANGES

Recruitments:
Dr. Janice Allen joins *OPO/SRB* as a Scientific Review Administrator. After working as a research technician and chemist at the University of North Carolina at Chapel Hill and the National Institutes of Health in Bethesda, respectively, studying the cell-cell and molecular interactions of pro- and anti-inflammatory mediators in arthritis, AIDS, and hepatic granulomas, Dr. Allen received her Ph.D. from North Carolina State University College of Veterinary Medicine in Cell Biology and Biotechnology where she subsequently joined the faculty and investigated the role of transforming growth factor beta and nitric oxide in endotoxin-induced uveitis.

Dr. Mike Humble joined *OPD/OSTB* on August 12 as a Health Sciences Analyst. Dr. Humble will work with the Toxicogenomics Research Consortium and the Collaborative Centers for Parkinson's Research Consortium. He is a native of Minnesota, and received his BA from St Olaf College and his MS from the Univ of Minnesota, both in Chemistry. The MS research and training focused on studies of nicotinic acetylcholine receptor protein binding sites for its ligand. Dr. Humble was a high school department chairman and chemistry teacher prior to earning his PhD in Toxicology from UNC-CH. His dissertation and post-doctoral research were performed in the laboratory of Dr. Ray Tennant, NIEHS, where he conducted research using transgenic mice to explore promoter regulation of transcriptional control of the carcinogenic process leading to a unique, unexpected form of skin tumor .

Dr. Brenda K. Weis joined *OPD/OSTB* as a Health Scientist Administrator in May 2002. She serves as the Extramural Toxicogenomics Research Coordinator and the Program Administrator for Metabonomics. Prior to joining OSTB, Dr. Weis served as a Scientific Review Administrator for SRB. She came to NIEHS from Agency for Toxic Substances and Disease Registry (ATSDR) in Atlanta, where she served as the Acting Deputy of the Office of the Associate Administrator for Science. During her 10-year employment at the ATSDR, Dr. Weis served as project leader for numerous multidisciplinary health investigations and was instrumental in developing science policy and setting environmental health research priorities for ATSDR and the Centers for Disease Control.

Mr. Larry Reed, has joined *OPD* as a Guest Researcher where he will be working on the Superfund Basic Research Program. He comes from EPA, where he has worked for the past 28 years, most recently as the Deputy Director (and Acting Director) of the Office of Emergency and Remedial Response. Among his positions, Mr. Reed has served previously as the Director of the Hazardous Sites Evaluation Division, Chief of the Compliance Information Branch (Office of Water Enforcement and Permits), Deputy Director of the Chicago Regional Planning and Management Division, and Chief of the Toxics Integration Branch (Office of Pesticides and Toxic Substances). He has an MPA from Harvard University.

Mr. Benigno Encarnacion has joined *OPO/GMB* as a Grants Financial Analyst.
Ms. Elizabeth McNair has joined *OPD/OSTB* as a secretary.
Ms. Anne Thompson has joined *OPD/PAB* as a secretary.
Mr. David Sedgley has joined *OPO/RCB* as a procurement technician.

Departures:
Dr. Jose Velazquez, OPD/CEMBB, is leaving DERT on September 20 to become Chief, Genomics and Proteomics Branch, in the Division of Basic Sciences at the National Institute of Alcohol Abuse and Alcoholism.