Issue 1, 2009

NEWSLETTER

EMS SHINES IN MENTORING

As a fourth year undergraduate student, attending my first Scientific Society Conference was not only very exciting, but also very intimidating. I was looking forward to participating in my first conference and to meeting other scientists from my area of research. However, I was also very nervous because I was preparing my first poster presentation. Nevertheless, this nervousness and anxiety dissipated immediately upon my first interaction with an EMS member. Also, the conference check-in staff were so friendly, helpful and supportive, which was an indicator to me and my fellow student that everything was going to be okay. Moreover, we felt that we would definitely fit in with all conference attendees. At this point, all of my insecurities were transformed into eager anticipation for the next five days.

As my colleague and I began perusing the conference program, we could not believe the myriad of events that were scheduled for the duration of the conference. We were especially impressed with the amount of "New-Investigator" or "Young Scientist" workshops and lectures. This made us feel as though every conference participant (some of which seemed quite intimidating due to the number of times I have referenced his or her work in mine…) genuinely cared about our work and participation. All of the mentoring workshops were very informative and inspiring; the workshops influenced my confidence on my ability to understand other attendee's research and boosted the transformation of my scientific career from imagination to reality. The many keynote speakers, poster sessions, lecture series, and breakfast workshops were dynamic and informative presentations. These experiences made my research much more exciting considering that some presentations were gatherings of specialists in my specific area of research. This made me feel a unique exhilaration that I continue to speak highly of to this day.

My poster presentation was a very positive experience. I was impressed with the turnout of attendees at the event and their engagement and participation with the poster presenters. I sensed that this was a rare and true exchange of knowledge and information; the poster gala was an enlightening experience as I witnessed the passion of every poster presenter and participant for their specific topics and interest. Conversations I had with EMS members transitioned from a discussion of research to life experiences, which only "fine-tuned" the society's remarkable focus on mentoring.

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Editors' Entry

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The Environmental Mutagen Society was founded in 1969 and incorporated under the laws of the District of Columbia. It is operated to encourage the study of mutagens in the human environment (particularly as they may affect public health) and to engage in and sponsor research, study and dissemination of information related to this problem. Membership is open to all interested scientists.

Dear EMS Members,

Welcome to our newsletter devoted to The 2008 EMS Annual Meeting in San Juan Puerto Rico.

In our previous newsletter, Carlos A. Torres-Ramos, Ph.D. prepared a creative and informative welcome to this fantastic meeting locale and our readers commented on not only how helpful that article was but how San Juan exceeded expectations!

Thank you to Pam and Sandy for an extensive photo library that allows us to share the sun, sand and smiles with our readers. Their clever and creative photos captured with our EMS members thoroughly enjoying both scenery and sicence. Many thanks to Cathy Klein for not only reviewing and formatting all submissions but also assembling our photo collage. Thank you Tonia and Becca for all of your efforts and attention to detail in proof reading versions of the newsletter.

This is the first EMS Annual Meeting that I (Kathleen) missed since 1995 and am delighted that the EMS newsletter can share the sights and science with those of us who missed the meeting. It is so great that the pages of an EMS newsletter bring us together once more during the year and set our minds to thinking of our next meeting. Thank you to all of our contributors and we invite all readers to send us your news, comments and suggestions for our next issue.

Kathleen and Cathy

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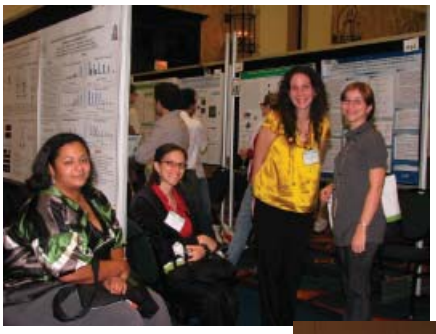

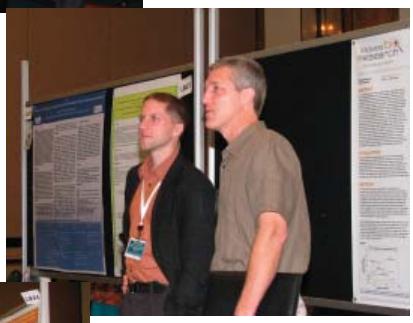

EMS Student REPORT

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The sharing of knowledge and information were topped off with “fun” events. Meal times and the occasional evening beverage only enabled conversations to continue that may have been cut short earlier and further promoted the development of friendship. Other events, such as the “BioBay” kayak trip, also fostered a sense of camaraderie which I just realized a few days before.

Overall, I had a fantastic and inspiring week. This conference presented me with the opportunity to broaden my knowledge, to network with some inspiring individuals, and to further grow and mature as a young scientist.

Thank you EMS!

Submitted by: Skylar Van Osch H.BSc. Genetics
The University of Western Ontario, London, Ontario
CANADA

PRESIDENTIAL POSES

San Juan Meeting

Congratulations to our Award Recipients

2008 EMS Student Education Award

Brinda Mahadevan
Schering-Plough Research Institute

2008 Annual Meeting Travel Awards

Rajalakshmi Asur
Wayne State University
Meredith E. Crosby, Ph.D.
Yale University School of Medicine
Cory S. Gresham
University of Georgia
Kimberly D. Jacob
Penn State College of Medicine
Kaarthik John, Ph.D.
National Cancer Institute
Saravanan Kaliyaperumal
University of Toledo
Igor Koturbash, Ph.D.
University of Lethbridge
Rohan Kulkarni
Wayne State University
Kristy R. Kutanzi
University of Lethbridge
Michele La Merrill, Ph.D.
Mt. Sinai School of Medicine
Maurizio Mauro
NYU-School of Medicine
Jennifer McAllister
Health Canada
Jennifer Rahn, Ph.D., M.D.
Anderson Cancer Center
Sangeetha Rajagopalan
University of Tennessee
Lindsey J. Stallons
University of Louisville
Eric Thompson
University of Arizona
Elisia D. Tichy
University of Cincinnati
Salina M. Torres
Lovelace Respiratory Research Institute
Benedicte Trouiller, Ph.D.
University of California, Los Angeles

Best Student Poster

Cory S. Gresham, University of Georgia
Runner up: **Salina M. Torres**

Best New Investigator Poster

Jessy Abraham, Ph.D., National Institute on Alcohol Abuse and Alcoholism, NIH

San Juan Meeting

2008 Alexander Hollaender Travel Award
Elias A. Rahal, University of Arizona

Poster/Oral Presenter (FASEB MARC Program)
Homero Camacho, University of California, Riverside

Faculty/Mentor & Students/Mentees (FASEB MARC Program)
Amal M. Abu-Shakra, Ph.D.,
North Carolina Central University Lakeshia Copeland, North Carolina Central University Hatajai Lassiter, North Carolina Central University

Thank you to our 2008 Exhibitors

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FASEB
Helix3, Inc.
Huntingdon Life Sciences
IIT Research Institute
MetaSystems Group
Midwest BioResearch
MOLTOX (Molecular Toxicology Inc.)
Perceptive Instruments, Ltd.

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Ronald Snyder, Ph.D.

The Procter & Gamble Company
Marilyn J. Aardema, Ph.D.

Thank you to our 2008 Meeting Sponsors

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Society of Toxicology

Thank you to our 401 meeting registrants!

San Jaun Meeting Workshops

OMICS – WORKSHOP

Application and Impacts on Genotoxicity Assessment

Chairs: **Brinda Mahadevan** (Schering-Plough Research Institute) and **Matthew A. Coleman** (Lawrence Livermore National Laboratory)

This workshop was organized as part of the New Technologies Special Interest Group to help introduce the society to current and rapidly developing technologies. The session was very well attended. The six speakers were selected to provide a broad level of scientific experience in the areas of Genomics, Proteomics and Metabolomics. At the end of the session a brief discussion was held to allow the participants to debate the pros and cons of varying scientific approaches for addressing genotoxicity assessments. A brief synopsis of each of the talks follows.

Paul Van Hummelen (Microarray Facility, VIB, Belgium) opened the session with a presentation titled "Current State-of-the-Art of Genomics in Toxicology". This talk addressed the utility of transcriptomics in toxicology. The transcript analysis presented illustrated that toxicogenomics could provide additional information on the mode of action for drug testing.

Brent W. Segelke (Lawrence Livermore National Laboratory) spoke on "Practical Applications of Structural Genomics Technologies". Dr. Segelke introduced the concept of structural genomics followed by a description of current approaches for solving new structures based on linkage to DNA sequence information. The presentation further highlighted the growth in informatics tools for structural biologist to help identify the function of genes annotated with unknown function and to suggest their role in larger proteins networks and pathways.

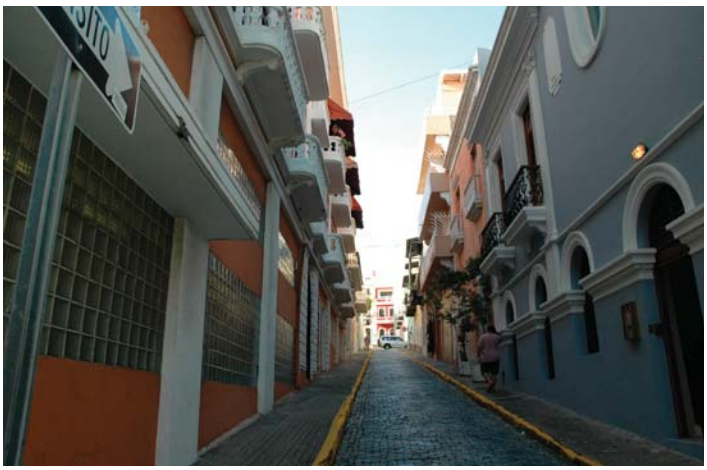
Olga Kovalchuk (University of Lethbridge, Canada) discussed "Understanding epigenetics using high-throughput technologies". This talk highlighted recent advances in understanding the role of chromatin structure using novel technologies to decipher DNA methylation, histone modification and cellular regulatory control using micro-RNA. Importantly, many of these techniques are readily available to bench-scientists.

Christopher Becker (PPD Biomarker Discovery Sciences, LLC) presented on the "Recent Advances in Proteomics and Metabolomics". Dr. Becker gave an overview of how current measurements for proteomics and metabolomics are made, and highlighted some particular new advances. He discussed the basics for use of liquid-chromatography-mass spectrometry (LC-MS) on high resolution equipment with label-free quantification for profiling studies for discovery.

Michael Milburn (Metabolon) gave a talk entitled "Metabolomics and its Applications to Improve Drug Safety". Dr. Milburn began his presentation with a brief introduction on the handling of samples and proprietary software used for global and whole pathway biochemical analysis. Dr. Milburn addressed the adoption and applications of metabolomics as a technology and presented a few case studies as examples.

Suri Vulimiri (A.W. Spears Research Center, Lorillard Tobacco Co.), the final speaker in the workshop, presented the results obtained from an *in vitro* study where-in a metabolomic approach was used as a screening tool to evaluate conventional and novel tobacco products. Dr. Vulimiri's presentation was titled "A Metabolomic Approach to Study the Effects of Cigarette Smoke in Human Lung Epithelial Cells". Dr. Vulimiri concluded his presentation offering future directions of metabolomics and its use as a technology in advancing the science of the assessment of environmental risks to human health.

San Juan Meeting Workshops



Rapid *In Vivo* Mutation Analysis Using The Endogenous PIG-A Gene

Chairs: **Richard Albertini** (University of Vermont) and **James MacGregor** (Toxicology Consulting Services)

The first EMS workshop on *in vivo* mutation assays based on the Phosphatidylinositol Glycan Class A (PIG-A) gene occurred at the Puerto Rico meeting. Sponsors and speakers were pleased with the high attendance. James MacGregor (Toxicology Consulting Services), provided background information on the PIG-A locus, including the important features of the gene which make it well-suited for mutation assays, including: X-chromosome location, conserved across species, and cell surface mutant phenotype that can be rapidly scored by flow cytometry. Furthermore, Dr. MacGregor introduced paroxysmal nocturnal hemoglobinuria (PNH), a human disease caused by PIG-A mutation which provides much insight into the types of lesions and cell types that can be studied with assays developed around this locus.

Stephen Dertinger (Litron Laboratories) illustrated the cross-species potential of Pig-a mutation assays by sharing data from two species, mouse and rat. These data demonstrated the feasibility of conducting *in vivo* experiments using erythrocytes. The analytical approach involved flow cytometry in conjunction with fluorescent antibodies against glycosylphosphatidylinositol (GPI) anchored proteins in order to differentially label and enumerate wild-type and mutant phenotype cells. Through the use of a nucleic acid dye, two populations of peripheral blood erythrocytes were considered: the total pool of erythrocytes (RBCs), and the immature fraction (reticulocytes, RETs). The rationale for choosing RBCs is that low blood volumes would be able to provide millions of cells for analysis. Thus, mutation frequency measurements would not require animal sacrifice, a feature that increases the potential for the assay to be integrated into on-going toxicology studies. The decision to evaluate RETs in addition to total RBCs was based on the hypothesis that there may be advantages to studying this subpopulation: induced mutation would be expressed sooner in these cells compared to RBCs, and if Pig-a mutation causes a shortened lifespan in circulation, this measurement may represent a more accurate representation of mutation frequency. Time course studies from ENU- and DMBA-treated rodents were presented that support these assumptions. Stephen concluded his talk by speaking of the large amount of work that is still needed, including the identification of adequate and preferred dosing/harvesting schedules, analysis of additional genotoxicants and non-genotoxicants, and the assessment of intra-laboratory reproducibility as well as inter-laboratory portability.

Daishiro Miura (Teijin Pharma Limited) talked about molecular aspects and potential advantages of Pig-a based mutation assays using rat peripheral blood RBCs. Data from a mutant manifestation study showed persistence of an ENU-induced increase in Pig-A mutant frequency for at least 6 months after a single dose. In addition, splitting the dose of ENU produced essentially the same mutant frequency as the single dose, indicating that, at least for ENU, Pig-a mutants accumulate in an additive fashion; this could be an important advantage of Pig-a-based assays in detecting weak mutagens by repeated/subchronic dosing protocols. Another study demonstrated the molecular basis of the assay. The approach used here was to develop a Pig-a mutant assay for rat spleen T-cells, which have nuclei, and demonstrate that results in RBCs and T-cells are essentially equivalent. Presumptive Pig-a T-cell mutants were selected and expanded for molecular analysis using proaerolysin (ProAER). The data indicated a good correlation between the frequencies of Pig-a mutants in RBCs and T-cells. In addition, ProAER mutant selection of T-cells, flow cytometric analysis of the selected clones, and sequencing of the Pig-a gene in the selected clones clearly demonstrated the mutational basis of the assay; the GPI-anchored protein-deficient cells, which are assessed in the assay with flow cytometry, were actually Pig-a gene mutants, and the GPI-anchored protein-deficient phenotype was actually increased by treatment with ENU. Daishiro indicated that the T-cell mutation assay may be useful as a follow-up assay for the rapid RBC screening assay, when it is important to confirm the response in the RBC assay and establish the mutational basis of the response.

Richard Albertini (University of Vermont) reported on the development of an assay for PIG-A mutations in lymphoyctyes and granulocytes as reporters for somatic mutations in humans. Initial validation studies in normal volunteers compared PIG-A with HPRT lymphocyte mutant frequencies (MF) as determined by cloning, finding comparable results in the same individuals (i.e. 10^{-5} to 10^{-6} range). Using these PIG-A MF values as the expected, lymphocyte PIG-A variant frequencies (VF) determined by cytometry were compared with the cloning values. Finally, granulocyte PIG-A VF as determined by cytometry were compared with lymphocyte

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VF values. In all cases, values for the same individuals were comparable and in the10^{-5} – 10^{-6} range, indicating that background PIG-A MF/VF in both cell types are similar to HPRT values in lymphocytes. Initial cytometric studies in cancer patients receiving mutagenic chemotherapy showed gross elevations of lymphocyte VF without substantial change in granulocyte MF when blood samples were obtained three weeks after the agents were administered. This demonstrated that the mutagenicity of chemotherapy is reflected in lymphocytes but not granulocytes at this specific time after treatment. Presumably this is due to the long term presence of lymphocytes in the peripheral blood but the extremely short time granulocytes spend in this compartment, with the lymphocyte mutants induced by the chemotherapy still present but the granulocyte mutants no longer present at the time of blood sampling. Projected studies will continue investigations in cancer patients and begin field studies of individuals exposed to environmental mutagens. Also, the PIG-A background molecular mutational spectrum is being defined using lymphocyte isolates from cloning assays.

Radim Sram (Czech Institute of Experimental Medicine) presented his plans to incorporate PIG-A mutation measurements into epidemiological work. The relative ease by which flow cytometric human blood-based mutation assessment can be accomplished should add great value to these studies for low additional cost.

Audience participation was encouraged, and questions arose regarding: the possibility of obtaining DNA sequence information from reticulocyte-derived mRNA transcripts; the extent to which clonal expansion might impact PIG-A data; and the direction of future studies. The success of this session has stimulated efforts to organize another PIG-A lunchtime workshop at the 2009 meeting.

Mentoring Workshop

Co-Chairs: **Catherine B. Klein** (NYU)
and **Ofelia A. Olivero** (NCI)

The Environmental Mutagen Society launched its new Mentoring Program during the annual meeting in Puerto Rico at an opening day Workshop attended by about 80 participants.

Cathy Klein opened the workshop with an introduction outlining the history of mentoring, the goals of the new EMS program, and briefly summarized the process that will be used to generate a cadre of EMS mentors and the establishment of EMS mentee/mentor partnerships.

Dr. John Mulvihill (University of Oklahoma), the keynote speaker, presented a wonderful overview of mentoring in a talk entitled “Double Happiness: Mendel and Life-Long Mentoring.” Dr. Mulvihill provided the audience with an in depth historical perspective on mentoring, then brought the topic to life by describing his own enlightening and rewarding experiences as both a mentee and a mentor at many different levels throughout his career.

The workshop continued with a panel discussion among participants facilitated by Ofelia Olivero with Katherine S. Squibb (University of Maryland School of Medicine), Miriam C. Poirier (NCI) and John Mulvihill as panelists. Many different aspects of mentoring relationships and benefits were discussed. The processes that will be put into action for the EMS Mentoring Program was reviewed and sign-up forms for prospective mentors and mentees were circulated, with good return throughout the duration of the Puerto Rico meeting.

The Mentoring Workshop was immediately followed by the Saturday evening Student and New Investigator Poster Session and Welcome Reception. This lively gathering provided an opportunity for students and new investigators to meet each other, as well as to meet EMS members and other meeting attendees. Everyone enjoyed the social gathering and the opportunity to make new acquaintances, catch up with old acquaintances, and to discuss science or review posters in a relaxing setting.

Further opportunities for mentoring discussions took place a few days later during the well attended Student and New Investigator Breakfast meeting. There, Dr. Mulvihill presented a synopsis of his workshop talk for the benefit of those who could not attend the Saturday workshop. The presentation was followed by lively discussion among the breakfast attendees.

Women of EMS Symposium

Chair: Glenda Gentile
Director of Undergraduate Research,
University of Arizona

Glenda Gentile, opened the session by recognizing that this was the second event sponsored by Women in EMS and thanked the NIH Office of Research on Women's Health for their generous support of this event. Over 50 individuals had registered for the session.

Dr. Katherine Squibb (University of Maryland School of Medicine) provided a keynote address entitled "Are Science Based Regulations Putting Science at Risk?" She emphasized the challenges faced by scientists who are engaged in "high profile" research in areas of interest important to public health and of equal regulatory importance to industry. Of particular importance were the difficulties that ensued when the data from individual college or university laboratories were challenged by much larger laboratory coalitions supported by industrial dollars. Dr. Squibb provided some specific examples of such situations and also provided suggestions as to how individual laboratory groups, when under such challenges, could respond in a professional, ethical and appropriate manner. Special concerns were noted for early-career researchers (those individuals who had yet to accrue a national reputation in their field). The research results from such researchers were noted as being particularly more at risk for strong challenges compared to data produced from laboratories of senior researchers with long publication histories.

There were considerable questions from the audience, and the ensuing discussions were thoughtful and lively. An overwhelming theme from both the presentation and the conversations was that a professional group such as the Environmental Mutagen Society, which boasts a membership comprised equally of academic, industrial and federal researchers, as well as members of the regulatory agency communities, provides a remarkable environment for the presentation of potentially controversial findings as well as a forum for members of the broad scientific communities of the society to provide thoughtful insight and perspective to the researchers and all other interested parties.

Immediately following the WEMS event, box lunches were provided. Participants interested in further discussion of the topic with the speaker met in a small round table discussions over lunch. This provided time for interchange among all participants, as well as with Dr. Squibb.

Overall the session was an unqualified success. Women of EMS will continue to have a key placement in the program for the 2009 annual EMS meeting. The WEMS group is rapidly evolving into the culture of the EMS in both its contributions to science and science policy discussion, as well as for the mentoring of women in science. A special thank you to everyone who made this event a success!

Transcription Meets DNA Damage

Chairs: **Phil Hanawalt** (Stanford University) and **Mats Ljungman** (University of Michigan)

This symposium focused upon events occurring when translocating RNA polymerase (RNAP) encounters DNA alterations that can arrest transcription or cause transcriptional mutagenesis. RNAP is an extremely sensitive lesion detector, to initiate the pathway of transcription-coupled DNA repair (TCR). Cells deficient in TCR are unusually sensitive to some DNA damaging agents and exhibit high levels of apoptosis. It is important to appreciate the remarkable resistance to carcinogenesis associated with genetic diseases in which only TCR is deficient. The presentations in this session provided complementary perspectives on the processing of different obstructions encountered by RNAPs. There was good attendance and excellent audience participation, with challenging questions and elaboration of the topic in discussion after each of the presentations.

Phil Hanawalt (Stanford University), in his introductory remarks, outlined potential consequences of arrested transcription and the compounded threat of blocked replication at a stalled RNAP. He suggested that the primary purpose of TCR is to remove potential blocks to replication, rather then to repair active genes more rapidly. He reviewed current models for TCR and the correlations of TCR *in vivo* with RNAP arrest at various lesions *in vitro*. He reviewed examples of genomic instability at naturally occurring non-canonical DNA structures and raised the possibility that futile cycles of "gratuitous" TCR near such structures might be mutagenic. He pointed out that better understanding of TCR might lead to novel therapeutic regimens.

Mats Ljungman (University of Michigan Medical School) described his work showing that stalled RNAP triggers activation of ATR kinase leading to phosphorylation of p53. Using microinjection of antibodies to block transcription, he showed that an effect of RNA synthesis inhibition is loss of mRNA export, causing nuclear entrapment of proteins such as p53. Thus, RPA and ATR link stalled RNAPII complexes to p53. He suggested that whereas apoptosis in stationary phase cells may be induced by accumulated p53, in proliferating cells apoptosis might be a consequence of a "tug of war" between replication and stalled transcription complexes. Noting recent reports that most of the genome is transcribed, Mats raised the implications for a role of RNAP in global clearing of damage at some low level even in relatively silent genomic domains.

Leon Mullenders (Leiden University Medical Center) described his chromatin immunoprecipitation approach to learn the assembly of factors required for TCR, detailing the differential roles of CSB and CSA, and noting the need for the CSB ATPase activity for the recruitment of the XPG endonuclease. While CSB is the primary coupling factor and recruits CSA as well as ERCC1, XAB2, XPA and RPA; CSA (part of an E3 ubiquitin ligase complex) is required for recruitment of chromatin remodeling factors, most notably HMGN1; both CSB and CSA have roles in recruitment of TFIIS and the P300 chromatin remodeling factor. HMGN1, which acetylates histone H3, may serve to "relax" nucleosomes behind the RNAP to facilitate backtracking from the obstruction; some experimental support for this model was presented. Knock down of TFIIS affected recovery of RNA synthesis but did not affect TCR.

Altaf Sarker (Lawrence Berkeley National Laboratory) detailed functional interactions of NEIL2 (glycosylase that can remove oxidized bases from bubble substrates) with XPG (which stimulates NEIL2 incision activity) and CSB (for which NEIL2 stimulates ATPase activity). Association of NEIL2 with elongating RNAPII was documented in a complex also containing XPG, CSB, and TFIIH, leading to the suggestion that this complex could be involved in the initiation of TCR of oxidative DNA damage. The association of NEIL2 with RNAPII in the cell required both XPG and active transcription.

Kelly Trego (Lawrence Berkeley National Laboratory) reported that XPG is heavily phosphorylated and interacts with DNA-PKcs and Ku, but this does not appear to reflect a role for XPG in double-strand break repair. However, inhibition of DNA-PKcs resulted in defective recovery of RNA synthesis after UV irradiation, implicating TCR. Since TCR was shown not to be affected by this inhibition, the results provide evidence for an independent role of DNA-PKcs in RNA synthesis recovery following UV. DNA-Pkcs was required for transcription after UV even on an undamaged template, further suggesting a role of DNA-PKcs in controlling recovery of RNA synthesis after UV damage in a process that is separable from repair.

John Tainer (Lawrence Berkeley National Laboratory, Skaggs Institute for Chemical Biology and the Scripps Research Institute) focused upon insights obtained from his analysis of the detailed molecular structure of the TFIIH component XPD, a DNA-dependent ATPS with helicase activity, which is required for both transcription initiation and for the pathways of excision repair. With this detailed molecular model, it becomes possible to understand why mutations in specific domains of the protein might selectively affect the helicase activity or more generally the structural integrity of the TFIIH complex, giving rise to different genetic diseases and the endpoint phenotypes of cancer and/or aging.

PJ Brooks (NIAA, NIH) reviewed his studies with several unique DNA adducts and their effects upon transcription, the oxidative lesion 8,5'-cyclo-deoxyadenosine (cyclo-dA) and

N2-ethyl-2'-deoxyguanosine (N2-Et-dG), a stable analog of the main DNA adduct of acetaldehyde. While cyclo-dA is a strong block it also leads to wild-type and mutant transcripts *in vivo*, but only UTP incorporation by RNAPII or T7 RNAP *in vitro*, but with no reduction in rate of incorporation by the latter. Molecular modeling was presented to predict that T7 RNAP should incorporate U opposite cyclo-dA, and to generally yield insights into the differential behavior of the single and multisubunit RNAPs. CSB was shown to modulate the frequency of deletions, but neither CSB nor XPD were required for transcriptional mutagenesis *in vivo*. N2-Et-dG blocks E.coli RNAP as well as both mammalian and yeast RNAPII. While RNAPII incorporates CTP opposite N2-Et-dG and then stalls, the T7 RNAP cannot incorporate anything opposite this lesion. The yeast RNAPII appears better able to incorporate nucleotides opposite lesions than the mammalian RNAPII. No stimulation of bypass by TFIIS was noted.

Systems Toxicology: An Emerging Approach for Investigating Mechanisms of Toxicity and Risk Assessment

Chairs: **Jiri Aubrecht** (Pfizer, Groton, CT) and **Martyn Smith** (University of California, Berkeley, CA)

Testing for genetic toxicity and carcinogenicity has become an essential component of safety assessment paradigm for drugs and chemicals. However, the low throughput, limited understanding of the underlying carcinogenic mechanisms and high cost are incompatible with modern drug discovery and demands for the testing of large numbers of industrial and environmental chemicals. Therefore, the development of broad mechanism-based approaches is important. The cellular response to chemicals triggers a complex web of molecular pathways involved in cell survival and/or cell death. The emerging field of systems toxicology utilizes genomic science and technologies to gain insights into these pathways and mechanisms of toxicty. Recently, systems toxicology approaches has been used for development biomarkers and/or applied to risk assessment. Several international consortia in the US (Critical Path, HESI) and EU (Carcinogenomics, IMI) have been pursuing the development of biomarkers of carcinogenic mechanisms. The System Toxicology Symposium provided an overview of state-of-the-art systems toxicology approaches that are applicable for risk assessment.

Al Fornace (Georgetown University) introduced a systems approach based on transcriptomics and phosphoproteomic analysis of the cellular stress response that enables interrogation of molecular pathways and networks and provides insights into molecular mechanisms of toxicants. Jiri Aubrecht (Pfizer) introduced a case study that indicated the potential of toxicogenomics analysis and resulting genomic biomarkers to provide a key mechanistic context to current genetic toxicity testing and thus facilitate risk assessment.

Heidrun Elllinger Ziegebauer (Bayer) presented results of a toxicogenomic analysis of the livers of rats treated with a broad range of carcinogens showing the potential of toxicogenomics and pathway analysis to provide insights into carcinogenic mechanisms *in vivo*.

Martyn Smith (University of California at Berkeley) provided an example of applying toxicogenomics in molecular epidemiology studies in human populations in order to develop novel biomarkers.

Ivan Rusyn (University of North Carolina) offered a pharmacogenetic approach capable of providing insights into sensitivity of populations treated with model agents was presented by.

Kate Guyton (US EPA) concluded the symposium. In her presentation she provided examples of applying systems-based analysis of available data to provide insights into toxic mechanisms and applicability of such approaches in risk assessment.

Mode-of-Action Cancer Research & Risk Assessment: A Case Study Using Propylene Oxide

Chairs: **Lynn H. Pottenger** (The Dow Chemical Company) and **Rita Schoeny** (USEPA)

The 2008 EMS Symposium 'Mode-of-Action Cancer Research & Risk Assessment: A Case Study Using Propylene Oxide' (PO), was sponsored by the Global PO Toxicology Research Group (American Chemistry Council PO/PG Panel and Cefic PO & Glycols Sector Group). The symposium reviewed results from the extensive PO Industry research programme to support understanding of the cancer mode-of-action (MoA). The symposium also described how a complex MoA with a practical threshold could be incorporated into quantitative risk assessment.

Lynn Pottenger (The Dow Chemical Company) reviewed background information on PO, which is a high production volume intermediate and monomer. Inhalation cancer bioassays demonstrate that chronic exposures to high concentrations of PO induce significant nasal histopathology

and site-of-contact tumors in nasal mucosa in rodents; the highest no effect level for tumors is 200 ppm. She described a targeted research strategy that evolved over 10 years to elucidate the MoA for nasal tumors induced by inhaled PO; this included studies to support identification of key events in the cancer process as well as development of a physiologically-based pharmacokinetic (PBPK) model. The research programme was a co-ordinated, multi-laboratory, internationally co-operative effort that culminated in a MoA-based quantitative risk assessment for PO.

Johannes Filser (Helmholtz Zentrum München) provided an overview of the PBPK model he developed to describe the metabolism and fate of PO. Substantial data were collected on input parameters, and there was extensive model validation using both rodent and human data. A nose compartment was included to address target tissue internal dose. The validated model was then used to predict concentrations of PO in venous blood and respiratory nasal mucosa under both inhalation bioassay conditions and occupational exposure scenarios.

James Swenberg (University of North Carolina) reviewed the research results from the PO programme. These data demonstrated disparate dose-response curves for tumors compared with both DNA adducts (N7Hydroxypropylguanine, N7HPG), and hemoglobin adducts, (Hydroxypropylvaline, HPVal). He described the cross-laboratory methodology validations for the adduct assays, and the extensive datasets on induction of cell proliferation in nasal mucosa. The latter effect demonstrated a threshold; exposures up to 200 ppm PO for 4 weeks did not induce the cell proliferation response in target tissues, but 300 ppm PO for 3 days did. These exposure levels are similar to no effect levels for rodent tumors; neither 100 ppm nor 200 ppm PO lifetime exposures resulted in tumors, whereas both 300 ppm and 400 ppm PO were tumorigenic. These data supported the hypothesis that induction of cell proliferation in target tissue is a key event in the PO MoA. More recent data demonstrated that induction of severe, sustained depletion of nasal glutathione (GSH), either by PO or by known depleting agents, was linked to induction of cell proliferation in the tumor region of the rat nasal mucosa.

Dick Albertini (University of Vermont, Emeritus) presented a comprehensive overview of the genotoxicity profile of PO. The genetic toxicity measures were categorized as either “mutations” or “non-mutational genotoxic events” (non-mutations). The latter are endpoints that demonstrate interaction with DNA but do not change genetic information content, per se (e.g., sister chromatid exchanges and DNA adducts). The non-mutations may be potential precursor events, but are not surrogates for mutational events. Mutational events, which result in heritable changes in genetic information, include gene mutations, chromosomal aberrations, and micronucleus formation. Dr. Albertini summarized the rich PO database on *in vitro* and *in vivo* systems as follows. PO reacts directly with DNA and causes mutagenicity in all *in vitro* systems investigated. By contrast, *in vivo* PO exposure results in mutagenicity only when administered in high doses via a non-physiological route (i.p.). Several studies using inhalation exposure were not able to demonstrate any *in vivo* mutagenicity, including a 2-yr exposure to 300 ppm in monkeys. He described the rate-limiting key event in the cancer MoA as the induction of cell proliferation in the target tissue preceded by severe, sustained GSH depletion; both of these endpoints have identified practical thresholds. Thus, although DNA reactivity may be a necessary component of PO’s overall genotoxicity profile and rodent carcinogenicity, it is likely not sufficient to cause the observed rodent tumors. Rather the associated, rate-limiting tissue toxicities appear to be required for tumor development. This complex mode of action with a practical threshold has implications for PO cancer risk assessment, especially at low exposure concentrations.

Vicki Dellarco (US EPA) presented an overview on application of the MoA and Human Relevance Framework (HRF) to risk assessment (developed under the auspices of the International Programme for Chemical Safety and the International Life Sciences Institute), using cacodylic acid as a data-rich example. This Framework is an analytical tool that enables greater transparency, identification of key missing data, and helps ensure consistency in MoA evaluations. She described a set of well-supported, quantifiable key events leading to tumors of the urinary bladder in mice, which demonstrated consistency across doses and over time. She noted that the proposed MoA for PO is biologically plausible and consistent with what is known about carcinogenic processes. Dr. Dellarco further discussed importance of applying a biologically-based pharmacokinetic model in demonstrating the relevance to human risk assessment of identified key events.

Mike Gargas (Sapphire) built on the previous presentations by reviewing the key events in the complex PO cancer MoA with a practical threshold, and presented a quantitative risk assessment based on the MoA. Evaluation of MoA data followed the framework presented in the U.S. EPA 2005 Guidelines for Cancer Risk Assessment; this framework applies modified Hill criteria to determine the weight-of-evidence for a MoA. Applying the HRF questions supported the relevance of the MoA to humans. Quantitation included determining appropriate dose metrics using the PB-PK model for the rat and external dose for the mouse. This was followed by analysis showing that a practical threshold was best supported by the data. He also described the results from a biologically-based, 2-stage clonal growth (CG) model that incorporated the cell proliferation data into the risk assessment, which accurately predicted the dose-response curves for the actual tumor data. Human equivalent concentrations were calculated, and the selection

of uncertainty factors was explained. The resulting reference concentration (RfC) values were presented: mouse, 0.5 ppm; rat, 0.7 ppm; and CG model-based, 0.4-0.7 ppm PO. The conclusion of the quantitative risk assessment was that, based upon a consideration of the MoA for nasal tumors produced by PO in rodents, PO is not likely to be carcinogenic to humans at inhaled concentrations less than 0.5 ppm PO (continuous exposure).

Lively discussion occurred throughout the session, beginning with the program overview. Some key questions centered on the relevance of the negative *in vivo* chromosomal effects data for a site-of-contact carcinogen. One approach to better understand could be to use the PB-PK model to predict equivalent site-of-contract exposures. It was also noted that there is a lack of *in vivo* site-of-contact mutagenicity data; however, there are data from the target tissue showing no increases in apurinic sites despite the very heavy adduction (N7HPG) of rat nasal mucosa.

Consequences of Genotoxic Damage to Mitochondrial DNA

Chair: **Bennett Van Houten** (University of Pittsburgh)

This symposium, sponsored by the Ellison Medical Foundation, focused on understanding the formation, repair and consequences of mitochondrial DNA damage, including subsequent mutations, alterations in bioenergetics and cell death.

Bennett Van Houten (University of Pittsburgh) introduced the symposium with a brief overview of mitochondrial physiology and DNA repair. Human mitochondria contain a small genome of 16.5 kbp that encoded 22 tRNA, 2 ribosomal RNA and 13 polypeptides, the latter of which are essential for electron transport (ET) and synthesis of ATP during oxidative phosphorylation (OXPHOS). During ET, there is leakage of electrons to oxygen creating superoxide anion radicals which can form other reactive oxygen species (ROS). It is believed that defective mitochondria produce increased amounts of ROS. Mitochondrial have efficient base excision repair (BER), but are apparently deficient in other repair pathways, making them prone to endogenous and exogenous damaging agents.

William Copeland (National Institute of Environmental Health, Sciences NIH) discussed how specific mutations in the gene encoding the human DNA polymerase gamma are associated with several human diseases, including: Alpers syndrome, progressive external opthalmoplegia, and ataxic neuropathy. Copeland and coworkers have mapped over 150 different human mutations to specific sites in the polymerase. Structure-function of several of these mutations has helped to delineate the polymerase defects associated with the disease. Copeland described how specific mutations can decrease pol gamma's interaction with DNA and cause decrease catalytic efficiency while increasing mis-incorporation causing mutations and subsequent disease phenotypes.

Lawrence A. Loeb (University of Washington) described an incredibly sensitive, Random Mutation Capture assay, for non-selected mitochondrial DNA mutations based on amplification of single mitochondrial DNA molecules. Dr. Loeb showed that in mice, mitochondrial mutations accumulate with age. He also presented data that mice deficient in pol gamma exonuclease prematurely age. However, and surprisingly, heterozygous mice with one good copy of pol gamma, while having a large increase in mutations, but do not apparently suffer any premature aging defects. These results argue against the idea that mitochondrial mutations significantly contribute to the aging phenotype.

Bruce Demple (Harvard University) provided compelling evidence that Fen1 is found in the mitochondria and participates in long patch BER in the mitochondria. Demple also described how DNA2 a member of the helicase/nuclease family, localizes to the mitochondrial where it plays an important role in long patch base excision repair. He further showed that knockdown of DNA2 with siRNA in HeLa cells causes defective removal of hydrogen peroxide induced mitochondrial DNA damage, whereas repair in the nucleus was unaffected. These exciting results suggest that both Fen-1 and DNA2 work together to remove 5' flaps associated with replication and repair intermediates in the mitochondria.

Sylvette Ayala-Torres (University of Puerto Rico) showed using a gene specific QPCR assay that there is an accumulation of mitochondrial DNA damage in the affected brain regions of both a chemical model and genetic model of Huntington's disease (HD) in mice. Dr. Ayala-Tores also showed an age-dependent accumulation of mtDNA damage. She further presented evidence that antioxidant treatment can reduce mtDNA damage, and lessen the neurological effects in a genetic mouse model of HD. These data support the idea that mitochondrial dysfunction and subsequent mitochondrial DNA damage are important steps in the genesis of HD, which is exacerbated by age associated increases in ROS.

Joel N. Meyer (Duke University) discussed the fate of bulky DNA damage in mitochondria in the nematode, C. elegans. Since mitochondria do not possess nucleotide excision repair, there is no known method of removal of these lesions from mitochondria. Using a gene-specific QPCR that Meyer adapted to worms, he presented evidence that chronic

UV damage causes an elevation of mitochondrial but not nuclear damage. These chronically damaged developing worms showed larval development delays and neurological problems. Surprisingly this damage is slowly lost from mtDNA. Meyer presented evidence that genes controlling mitochondrial dynamics help contribute to the removal of this mtDNA damage.

Bennett Van Houten (University of Pittsburgh Cancer Institute) presented evidence that Friedreich's Ataxia (FRDA) patients have altered gene expression that is consistent with chronic DNA damage. FRDA is caused by a GAA triplet repeat expansion in the first intron of the gene frataxin. This nuclear gene encodes a mitochondrial protein that is essential for the formation of FeS centers in key proteins involved in various enzymatic processes including electron transport and DNA repair. Analysis of DNA damage in the nucleus and mitochondria in peripheral blood cells from these patients indicated a significantly elevated level of damage in both genomes as compared to a control group.

This symposium was well attended and generated good discussion after each presentation indicating that, while the mitochondrial genome is small, it has huge importance in genotoxicty associated with a large number of degenerative diseases.

Global Warming and Environmental Health

Chairs: **Nina T. Holland** (University of California, Berkeley) and **Christopher J. Portier** (National Institute of Health Sciences, NIH)

This is the first time that the topic of Global Warming and Climate Change has been addressed in a special EMS session. Four speakers covered a broad spectrum of issues including an overview of the challenges that global warming will present to countries around the world, innovative climate models, specific predictors of pollution factors (such as O3 and methane), biomarker studies and new technologies that can improve monitoring of exposure, prevention and intervention, and most importantly how climate change will affect public health. Just a few days before presenting at the EMS meeting, two of the session's speakers (K.Smith and C.Portier) participated in the WHO Consultation on a Global Research Agenda for Health and Climate Change, October 5-9, 2008 in Madrid.

Christopher Portier (Associate Director, National Institute of the Environmental Health Sciences), in his talk "Research Gaps in Environmental Health: Global Climate Change", recounted historical and dramatic recent changes of air pollutants such as CO_2, global temperature, and glacier melting. He outlined direct and indirect routes by which energy sources may affect human health, and identified the range of health outcomes likely to be affected by climate change. He further emphasized that to improve prediction it will be necessary to characterize and quantify exposures, define susceptible populations, improve climate modeling, improve surveillance and collection of climate data, as well as enhance health modeling. Climate change and health impacts/risks are becoming important research foci on the NIEHS agenda.

Kirk Smith (University of California, Berkeley) and 2007 Nobel Laureate for his work on climate change (along with Al Gore and a large group of scientists), focused on "Mitigating, Adapting and Suffering: Health Effects of each on Vulnerable Populations". Current estimates indicate that the largest health burden (88% of the global total) of global warming will actually be borne by children in developing countries because they are most vulnerable to current environmental risks; risks that are increasingly being exacerbated by climate change. Several relationships between consequences of climate change and adverse health outcomes have been explored. Malaria and diarrhea, two of the major causes of child mortality, are thought to be directly influenced by climate change. Malnutrition, the single most important risk factor for child mortality, may be increased by both climate change itself and, potentially, by efforts to combat climate change through biofuel expansion, etc. Yet some methods for dealing with climate change offer co-benefits in the form of both substantial reduction in carbon emissions as well as lower child mortality.

Jason West (University of North Carolina, Chapel Hill) in his talk "Assessing Links between Air Pollution, Climate Change, and Public Health using Atmospheric Chemical Transport Models " highlighted the use of a global chemical transport models (CTM) for evaluation effects of air pollution on mortality. Analysis of the global burden of outdoor air pollution on human mortality should include consideration of anthropogenic fine particulate matter (PM2.5) and ozone (O_3). First, using the CTM, it is possible to assess global changes in surface PM2.5 and O_3 concentrations since pre-industrial levels. His estimates of human mortality are significantly higher than those of the WHO that focused solely on urban areas. Second, he discussed the benefits of methane emission reductions. Methane is a greenhouse gas that also reacts in the atmosphere to contribute to global background concentrations of O_3. He has shown that a 20% reduction in global anthropogenic methane emissions reduces O_3 globally by ~1 ppb and causes about 30,000 avoided premature mortalities in 2030. These benefits are shown to exceed the costs of the 20% methane reduction, apart from other benefits for slowing global climate change. Finally, this presentation has demonstrated the broad links between air pollution and climate change, including the effects of climate change on future air quality.

Nina Holland (University of California, Berkeley) focused her talk on "Biomarkers of Air Pollution in the Age of Global Warming". She emphasized that air pollution will be a major challenge of the 21st century, as its impact will increase with global warming and industrial development in South Asia and other parts of the world. Biomarkers can be effectively used as indicators of exposure; cellular and molecular changes that fall along the pathway of disease and can be employed as predictors of disease. This presentation primarily focused on O3 as a marker of ambient pollution that, based on exposure models, may disproportionably increase in different areas of the world with climate change. Molecular, cytogenetic and "omics" biomarkers as well as emerging nanotechnology can be used to project and prevent adverse changes in environmental health in the age of global garming.

Global Health in the Americas: the impact of the Environment

A symposium showcasing Latin American scientists, sponsored by The March of Dimes, National Cancer Institute, and NASA Space Radiation Program, Johnson Space Center.

Chairs: **Graciela Spivak** (Stanford University) and **Ofelia Olivero** (National Institutes of Health, USA)

This symposium was a special feature of this year's meeting, in recognition of the unique opportunities presented by the Puerto Rican venue.

The chairs of this symposium, both originally from Argentina and featured speakers, work in or were trained in Latin American countries in a variety of scientific specialties related to DNA repair and genome stability, genetic diversity and environmental contaminants. The symposium was well attended and elicited active participation from the audience during the question periods, which were led by Helena Groot (University of Los Andes, Colombia).

Andrés Ruiz-Linares (University College London, UK), originally from Colombia,, set up the tone of the symposium with his talk on genetic diversity of Latin American populations. He described the use of genetic markers for studies of native and mestizo populations, particularly in the Antioquía province of Colombia with a focus on the inheritance of neuropsychiatric disorders.

Patricia Ostrosky Wegman (Universidad Nacional Autónoma de México) spoke about the effects of arsenic contamination in drinking water on the incidence of diabetes type 2. Studies from her group have shown that subtoxic arsenite concentrations inhibit insulin-stimulated glucose secretion in pancreatic cells, resulting from decreased activity of calpain-10.

Gustavo Folle (Instituto de Investigaciones Biológicas Clemente Estable, Uruguay) presented work on the organization of chromatin within the nuclei of vertebrates. Depending on the cell type and other variables, chromosome territories are highly organized withgene-rich and smaller chromosomes toward the center, and gene-poor and larger chromosomes around the periphery of the nucleus. In addition, active chromatin appears more prone to damage and rearrangements.

Ofelia Olivero (NIH) presented her work on centrosomal amplification induced by anti-AIDS drugs. Special attention was placed on the impact of the nucleoside analogs in pregnancy and the genotoxicity of the compounds in the fetus.

Carlos Menck (University of Sao Paulo, Brazil) described the identification of DNA repair genes in the bacterium Caulobacter crescentus. The SOS regulon was characterized, and a separate operon was found to control genes related to translesion synthesis and targeted mutagenesis. Transposon-directed mutagenesis allowed the isolation of UV- and MMS-sensitive mutations affecting DNA repair, membrane transport, signal transduction and energy metabolism.

Marcelo Larramendy (Universidad Nacional de La Plata, Argentina) reported on the effects of pesticides as pure compounds, or as commercially applied formulations, on markers of clastogenicity such as sister chromatid exchanges, chromosomal aberrations, cell cycle progression, induction of DNA breaks, and other assays. The studies show that commercial formulations cause more damage than that induced by pure pesticides.

Jaime L. Matta (Ponce School of Medicine, Puerto Rico) was the final speaker who described a host cell reactivation assay to measure DNA repair in lymphocytes from healthy individuals and from breast cancer patients. The study showed a nearly 50% decrease in DNA repair capacity in cancer patients, and suggests that repair capacity can be a useful biomarker for prediction of breast cancer risk.

The speakers enjoyed the congenial atmosphere at the conference and the many opportunities for networking and development of collaborations between U.S., Canadian, and Latin American scientists working in environmental genetic toxicology and related areas.

Formaldehyde and Leukemia: Epidemiology, Potential Mechanisms, and Implications for Risk Assessment

Chairs: **Luoping Zhang** (University of California at Berkeley) and **Babasaheb Sonawane** (US EPA)

IARC recently classified formaldehyde, an economically important industrial chemical, as a human carcinogen that causes nasopharyngeal cancer, a relatively rare cancer in the U.S. The impact of formaldehyde on the human cancer burden would increase considerably if it were found to cause leukemia for which IARC stated there was "strong but not sufficient evidence". In order to address the potential association of formaldehyde and leukemia, this symposium was organized with sponsorship from the US EPA. Four speakers from academic institutions and government agencies were invited to present current findings on formaldehyde exposure, epidemiology, potential mechanisms, and implications for risk assessment. At the symposium, Dr. Sonawane briefly summarized epidemiological, toxicological and biological information regarding formaldehyde and leukemia and introduced each of the speakers.

Laura Beane-Freeman (National Cancer Institute) reviewed the epidemiological data on formaldehyde and leukemia obtained from case-control studies in the general population, proportionate mortality studies of professionals (e.g., funeral industry workers and pathologists), and cohort studies of industrial workers. Overall, it was concluded that for the majority of studies, the epidemiological evidence supports an association between occupational formaldehyde exposure and leukemia, particularly myeloid leukemia.

Luoping Zhang (University of California at Berkeley) presented an overview of formaldehyde exposure and regulation in the U.S. and worldwide, showing that China currently has the highest levels of formaldehyde production and exposure, and that the U.S. has weak regulation compared with that of many other nations. She also shared recently published results from a meta-analysis of formaldehyde and leukemia, and provided three hypothetical mechanisms by which this association could be biologically plausible.

Jun Nakamura (University of North Carolina) showed that DNA damage response pathways are essential for cells to counteract the genotoxic effects (e.g. DNA-protein crosslinks) of formaldehyde. Recently published work by his group demonstrated that chicken cells deficient in the BRCA/FANC pathway and human cells deficient in FANCC and FANCG, were hypersensitive to plasma levels of formaldehyde.

Stephen Hecht (University of Minnesota) summarized his group's recent research on formaldehyde-DNA adducts, which, if un-repaired, lead to mutations in important growth control genes, ultimately resulting in genomic instability and carcinogenesis. The Hecht group developed mass spectrometric methods for the analysis of formaldehyde-DNA adducts and was the first to demonstrate such adducts in the tissues of laboratory animals.

John Vandenberg (US EPA) discussed uncertainties and challenges in the health risk evaluation of formaldehyde, which arise from data in both experimental animal and human epidemiological studies. Despite these issues, he concluded that recent developments, not only in hazard identification but also in the mode and mechanism of action of formaldehyde on hematopoietic and immune cells, provide an increasingly informed basis on which to evaluate the utility of epidemiological studies for the human health risk assessment of formaldehyde exposure.

Martyn Smith (University of California, Berkeley) concluded the session with a discussion on future directions, in which about 50 attendees participated. Suggestions from the floor for future work focused on: the development of chemical-specific measurements of formaldehyde and formaldehyde addition products (adducts) in blood, bone marrow and other target tissues; the need for biomarker studies in human populations; further exploration of the epigenetic effects of formaldehyde; and, the potential application of Pig-A mutation assays and/or a knock-out mouse models to clarify the mechanisms of formaldehyde-induced leukemogenesis. The interactions between speakers and participants were very active and positive and, as a result additional pertinent suggestions were made and future collaborations among different institutions were discussed. Further, it was agreed that a symposium report based on contributions from each speaker and led by Dr. Luoping Zhang, would be generated and submitted to EMM as a joint paper.

Methylphenidate Treatment of ADHD: Are Pediatric Patients at Risk of Induced Genetic Damage?

Organizers: **Kristine L. Witt** (NIEHS) and **Suzanne M. Morris** (NCTR, U.S. FDA)

Chairs: **Donald R. Mattison** (National Institute of Child Health and Human Development) and **Michael D. Shelby** (NIEHS)

Speakers: **Donald R. Mattison** (National Institute of Child Health and Human Development), **Michael D. Shelby** (NIEHS), Benedetto Vitiello (National Institute of Mental Health), Kristine L. Witt (NIEHS), **James D. Tucker** (Wayne State University), **Suzanne M. Morris** (NCTR, U.S. FDA), **Mugimane Manjanatha**, (NCTR, U.S. FDA), **William J. Rodriguez** (U.S.FDA), **William Slikker, Jr**., (NCTR, U.S. FDA)

Methylphenidate (MPH) is commonly used to treat attention deficit hyperactivity disorder (ADHD) and attention deficit disorder (ADD) in children. It is estimated that 5% of children are treated with this drug. Until 2004 it was thought that the risk of treatment with MPH was outweighed by therapeutic benefits. Since that time, however, several safety signals concerning its use emerged, including; genetic toxicity, cardiovascular effects and growth impairment. Given the widespread use of this drug, and potential for adverse public health effects, HHS under the Best Pharmaceuticals for Children Act (BPCA) convened a group of clinicians and scientists to explore and respond to concerns of genetic toxicity. Initially visits were made to the laboratory to review methodology and data from the initial publication. Subsequently this team designed studies to explore the concerns using experiments in rodents, nonhuman primates and humans. This summarizes the EMS symposium which reviewed research conducted by HHS in response to concerns of genetic toxicity.

ADHD is a disorder, apparent before age 7, which includes: inattention, hyperactivity, and impulsivity. These features are developmentally abnormal, persistent and pervasive, and impair school performance and inter-personal relationships. There are currently no laboratory tests which make the diagnosis of ADHD; it is made clinically based on symptoms and impairment in performance. Treatment of ADHD includes psychosocial and/or pharmacological approaches. Among the pharmacological approaches, stimulants, including MPH are commonly used. Treatments with MPH have been demonstrated by many studies to be effective at reducing the symptoms of ADHD. These studies have demonstrated improvement in attention, behavior and interpersonal relationships as judged by both parents and teachers, which persist as long as the MPH treatment is continued. Treatment however is not without side effects, which necessitates further study of treated children.

In 2005 the NIEHS National Toxicology Program (NTP) Center for Evaluation of Risks to Human Reproduction (CERHR) conducted a peer-review of the published literature concerning the reproductive and developmental toxicology of MPH. Based on its review of the animal literature the committee concluded that there is limited evidence of developmental toxicity, based on growth restriction. Based on review of human data the committee concluded that there limited evidence of no adverse developmental effects. In summarizing the data reviewed however, the committee noted that there was insufficient hazard or exposure data, despite long-term and common use of this medication.

Two clinical studies were reported which replicated the initial research suggesting that MPH produced genetic toxicity. These new studies were designed with greater power and also added another commonly used stimulant drug; the mixed amphetamine salts (MAS) or behavioral therapy for comparison. One study randomized 60 children following their initial diagnosis of ADHD to one of two treatment arms, MPH or MAS, obtained a pretreatment blood sample for baseline measures of gene toxicity then after 3 months of continuous treatment obtained another blood sample for comparison. All samples were coded so evaluations were blinded to treatment group and time of sample draw. At the 3-month time point 22 children had been treated continuously with MAS and 25 with MPH. No treatment related changes were observed in chromosomal aberrations, sister chromatid exchange or micronuclei. Another clinical study replicated the initial research using Ritalin LA and/or behavioral therapy in treatment naïve children. Children with the diagnosis of ADHD were randomized to one of two treatment arms; behavioral therapy or MPH and behavioral therapy with bloods taken before and after 3 months of therapy, and following a 6-week washout period. All samples were coded so evaluations were performed blind to treatment or time of sampling. None of the 3 cytogenetic endpoints; chromosome aberrations, sister chromatid exchange or micronuclei were altered by either treatment, nor did they differ from pre- or post- treatment measures.

Another study reported the pharmacokinetics and genetic toxicology of MPH in non-human primates (NHP). These studies were conducted because higher doses of MPH could be utilized than were used in children. Animals were divided into three dose groups; control, low, or high dose based on blood concentrations so the low dose group approximated

human therapeutic concentrations and the high dose group approximated 10x human therapeutic concentrations. Cytogenetic endpoints were evaluated monthly, beginning prior to treatment and extending 19 months after initiation of treatment. Chronic treatment with MPH produced no cytogenetic changes at any time-point, either mutations or chromosomal aberrations in NHP.

Rodent studies were designed to evaluate genetic toxicity of MPH in chronically treated mice following evaluation of MPH metabolism and pharmacokinetics. Similar to humans and NHP ritalinic acid is the major metabolite of MPH in mice. The Big Blue mouse was chosen for these experiments because there are multiple copies of λ shuttle vector (bacteriophages) with cII integrated into the genome of every cell. Consequently, λ shuttle vectors with cII as a reporter gene can be retrieved for mutational analysis from any tissue. Animals were treated with MPH (0 to 4000 ppm in feed) and cII mutant frequency determined in the liver, MN frequency determined in peripheral blood and Hprt mutant frequency measured in spleen lymphocytes. There was no treatment related alteration in any of the cytogenetic endpoints evaluated.

All of these diverse evaluations were negative suggesting that there is little public health risk from genetic toxicology however other endpoints of safety concern need further evaluation. As a result of recent congressional legislation the FDA, NIH and Industry have substantially increased activity in pediatric drug development. These activities have resulted in increased information in drug labels concerning appropriate use, safety and efficacy in pediatrics. Additionally, there has been increased attention to safety signals for children treated with drugs. Examples include the use of Ketamine for conscious sedation and potential neurotoxicity, the putative cytogenetic toxicity of MPH and liver toxicity of propylthiouracil. These evaluations of emerging safety signals illustrate how BPCA coupled with diverse resources of HHS can respond to pediatric drug toxicity, rapidly developing teams with appropriate competence to thoughtfully probe issues of pediatric drug toxicity.

DNA Damage in Neurodegeneration, Aging, and Cancer

Chairs: **Laura Niedernhofer** (University of Pittsburgh Cancer Institute) and **Patricia Opresko** (University of Pittsburgh)

The goal of this symposium was to stimulate a multi-disciplinary discussion of how environmental factors interact with genetics to influence the rate of aging and associated diseases, in particular neurodegeneration and cancer. The session was well attended and all the speakers fielded questions after their presentation, indicating that the audience was engaged and participated in the session.

Laura Niedernhofer (University of Pittsburgh Cancer Institute) presented an introductionon the contributory role of DNA damage to neurodegeneration, aging and cancer. The causal role of DNA damage in human disease is dramatically illustrated by observing patients with inherited defects in genome maintenance mechanisms. Failure to repair DNA damage leads to developmental abnormalities, as seen in Fanconi anemia and Cockayne syndrome, neurodegeneration as in ataxia telangiectasia, accelerated aging seen in Werner syndrome and XFE progeroid syndrome, and cancer for example in Bloom syndrome and xeroderma pigmentosum. A common feature of all of these genome instability disorders is decreased growth hormone and/or insulin signaling, which accounts for growth defects and diabetes seen in many of these patients. Dr. Niedernhofer presented data illustrating that this was recapitulated in several mouse models of genome instability syndromes. Genome-wide expression analysis in multiple tissues of mice mimicking XFE progeroid syndrome and Cockayne syndrome reveal a highly significant correlation with the expression pattern in calorically restricted mice or long-lived mouse strains. These data support the hypothesis that the endocrinopathies seen in genome instability disorders represent a highly conserved protective response triggered by stress, including genotoxic stress.

Sandy Chang (MD Anderson Cancer Center) discussed aging and cancer in mouse models of telomere dysfunction. The Protection of Telomeres 1 (POT1) protein is a single-stranded telomere binding protein that is essential for proper maintenance of telomere length. Disruption of POT1 function leads to chromosome instability and loss of cellular viability. Dr. Chang presented data showing that targeted deletion of the mouse Pot1b gene results in increased apoptosis in highly proliferative tissues. In the setting of telomerase haploinsufficiency, loss of Pot1b results in depletion of germ cells and complete bone marrow failure due to increased apoptosis, culminating in premature death. Pot1b-/- mTR+/- hematopoietic progenitor and stem cells display markedly

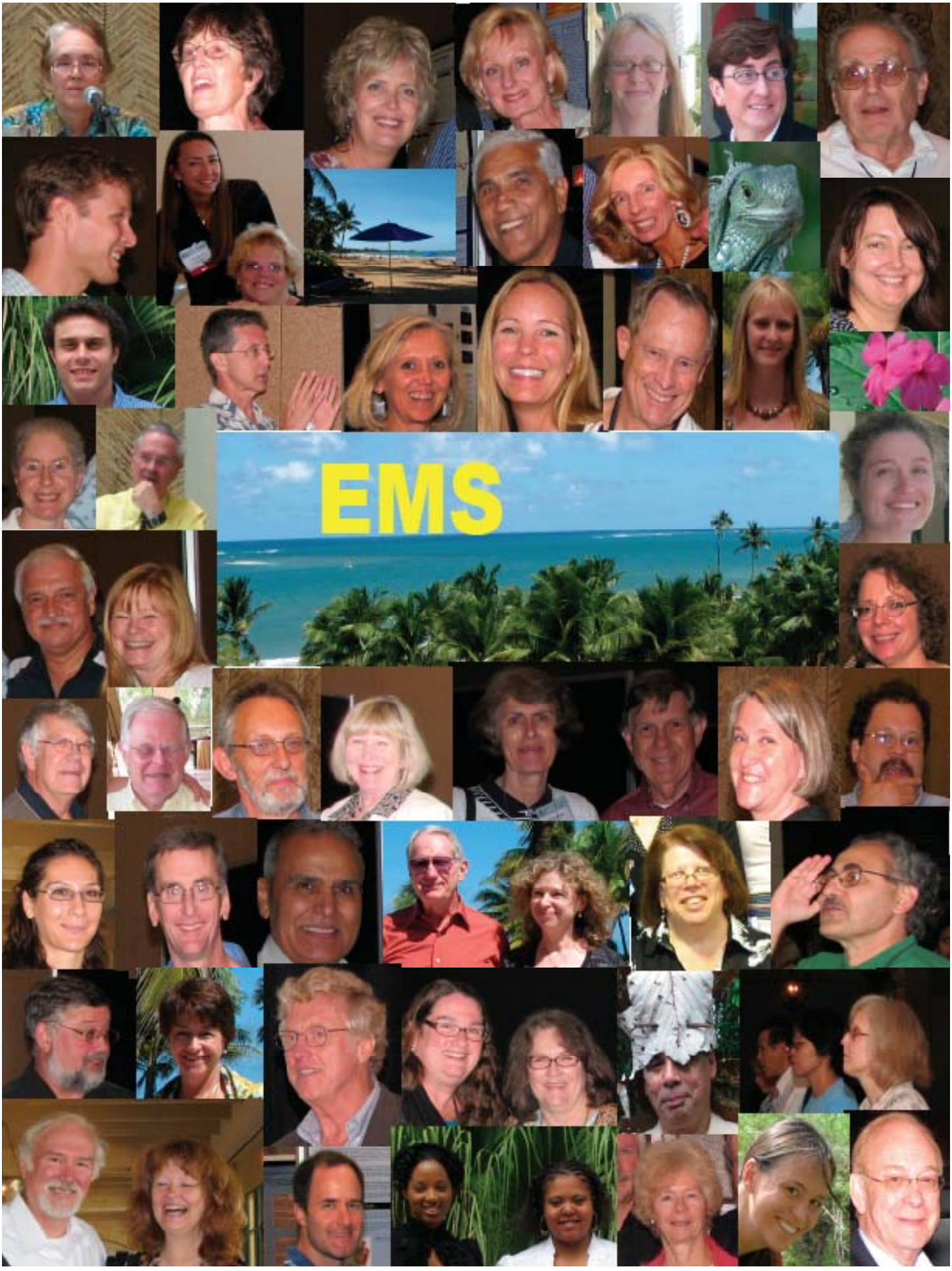
reduced survival potential *in vitro*. Accelerated telomere shortening, increased G-overhang length and elevated number of chromosome end-to-end fusions that initiates an ATR-dependent DNA damage response were also observed. These results indicate an essential role for POT1b in the maintenance of genome integrity and the long-term viability of proliferative tissues. Interestingly, these phenotypes closely resemble those found in the human disease dyskeratosis congenita (DC), an inherited syndrome characterized by bone marrow failure, hyperpigmentation and nail dystrophy. Dr. Chang noted that they anticipate this mouse will serve as a useful model to further understand the pathophysiology of DC.

Patricia Opresko (University of Pittsburgh) presented on the Werner Syndrome protein in telomere preservation and repair. Loss of the RecQ helicase WRN protein causes the cancer-prone progeroid disorder Werner Syndrome (WS). WS cells exhibit defects in DNA replication, repair and telomere preservation. Dr. Opresko discussed her biochemical studies investigating roles for the WRN helicase/exonuclease in replication and repair at telomeres. She also discussed the important role of POT1 in preserving telomeres, and roles for both WRN and POT1 in preventing aberrant recombination at the telomeres. POT1 stimulates WRN unwinding of forked telomeric duplexes and D-loops that mimic intermediates in replication and repair. Her lab constructed plasmid-based physiologically relevant telomeric D-loops containing POT1 binding sequences both in the duplex region (84 bp) of the invading strand and the displaced ssDNA region. The plasmid-based D-loops were superior substrates for the WRN helicase, compared to previously tested oligonucleotide-based constructs. POT1 did not alter the total percent of D-loops unwound by WRN, but did increase the percent of displaced full-length strands by stimulating the WRN helicase and inhibiting the WRN exonuclease. Replication protein A (RPA) both increased the percent of D-loops unwound by WRN and the amount of released full length strands. Their data suggest POT1 stimulates WRN helicase primarily by maintaining partially unwound strands in a melted state, whereas RPA may also recruit or retain WRN on the substrates during unwinding. While POT1 and RPA are both ssDNA binding proteins that interact with WRN, they differentially regulate WRN activity on telomeric substrates.

Mitch McVey (Tufts Univrsity) spoke on DNA double strand break repair in drosophila focusing on alternative pathways and aging. To determine whether DNA double-strand break repair pathways promote longevity in a model eukaryote, Dr. McVey's lab measured the lifespan of Drosophila melanogaster lacking either DmBlm or DNA ligase IV, which are involved in homologous recombination and non-homologous end joining, respectively. DmBlm mutants had a significantly shortened lifespan, while deletion of ligase IV had no effect on longevity. Using a somatic mutation reporter system described by Garcia et al. (2007), they found that DmBlm mutants also have an increased somatic mutation rate relative to either wild-type or ligase IV mutants. These data suggest a model whereby homologous recombination is important for promoting longevity in Drosophila and in its absence an alternative end-joining repair pathway is utilized, resulting in shortened lifespan. Dr. McVey preliminarily identified DNA polymerase theta, encoded by mus308 in Drosophila, as an important protein in alternative double-strand break repair.

David Wilson (National Institute on Aging, NIH) discussed base excision repair in cancer susceptibility and neurodegeneration. DNA repair systems represent a major protective mechanism against the cytotoxic effects of clinical DNA-interactive drugs. A primary goal of current investigations is to devise combinatorial treatment methods that (a) protect normal cells from and (b) enhance the sensitivity of tumor cells to the toxicity of anti-cancer agents. In Part I of his talk, Dr. Wilson presented studies aimed at assessing the role of base excision repair (BER) in mediating cellular resistance to clinically relevant alkylating drugs and antimetabolites using a catalytically-inactive, dominant negative protein form of human APE1, termed ED which blocks repair steps. ED enhanced cellular sensitivity to streptozotocin and temozolomide, and increased the cell killing effect of 5-fluorouracil and 5-fluorodeoxyuridine. The data suggest that APE1, and BER more broadly, is a potential target for inactivation in anti-cancer treatment paradigms that involve alkylating agents and antimetabolites. In Part II, Dr. Wilson presented studies investigating the contribution of single-strand break repair (SSBR) to oxidative stress resistance in both dividing and non-dividing neural cell populations. Recently XRCC1, a global participant in SSBR processing, was reported to associate with proteins causally linked to human spinocerebellar ataxias. Dr. Wilson reported that XRCC1 knockdown in human SH-SY5Y neuroblastoma cells results in increased sensitivity of the terminally differentiated cells to redox-cycling agents. Using hypoxanthine-xanthine oxidase as the oxidizing method, XRCC1 deficiency affected both dividing and non-dividing SH-SY5Y cells. Primary Xrcc1+/- mouse cerebellar granule cells showed increased SSBs and increased apoptosis following menadione treatment. Moreover, knockdown of XRCC1 in primary human fetal brain neurons lead to enhanced sensitivity to menadione, as indicated by increased SSBs relative to controls. The cumulative results implicate XRCC1, and more broadly SSBR, in the protection of non-dividing neuronal cells from the genotoxic consequences of oxidative stress.

Cynthia McMurray (Mayo Clinic) spoke about a toxic oxidation cycle related to aging in Huntington's Disease. Huntington's disease is a progressive neurodegenerative disorder caused by the expansion of a simple trinucleotide sequence in the HD gene that occurs with age. The result is the production of a toxic protein that contains an abnormal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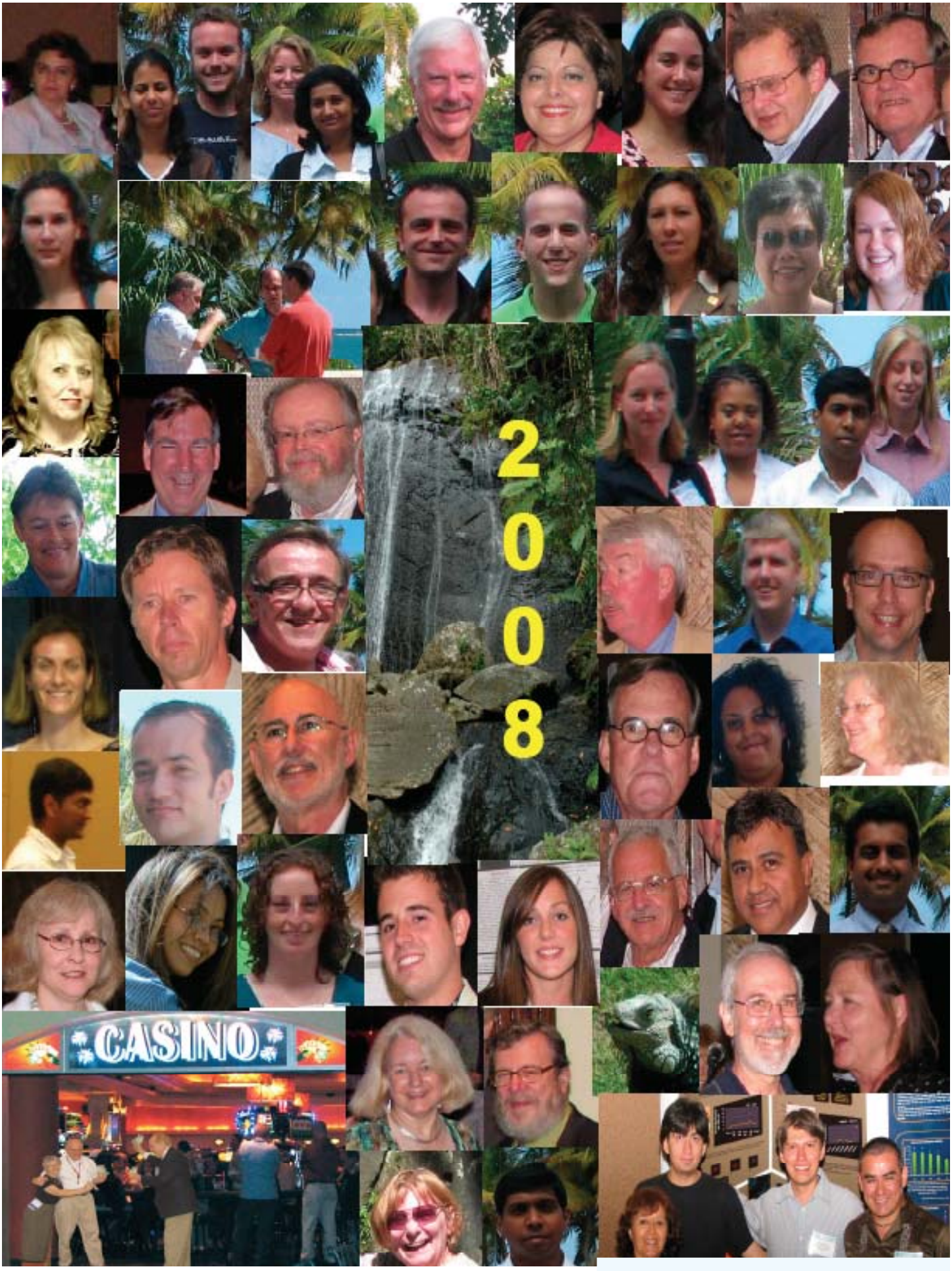
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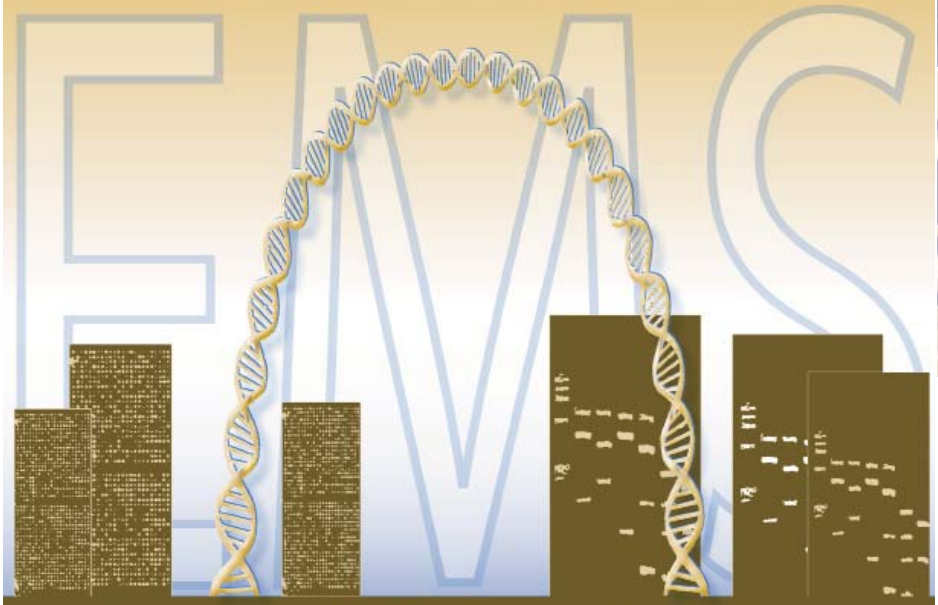


Genomics in the Environmental Century
St. Louis, Missouri

Environmental Mutagen Society

EMS President:
Priscilla K. Cooper

Program Chair:
Michael J. Plewa

Marriott St. Louis Union Station

tract of glutamines. This toxic protein eventually leads to death of neurons controlling movement. Dr. McMurray discussed her *in vivo* work examining the cause of CAG repeat expansion in HD. She shared evidence that DNA replication may cause deletion of the repeats, but DNA repair causes their expansion. Mice lacking the mismatch repair (MMR) proteins MSH2 or MSH3, or the DNA glycosylase OGG1, which removes oxidized DNA bases, did not exhibit expansion of the CAG repeats in the HD gene, implicating these repair proteins in repeat expansion. CAG repeats can form hairpin structures that are stabilized by MSH2 and MSH3 if a single-strand break occurs in HD or an oxidative lesion is incised by OGG1. Binding to the hairpin inhibits the ATP hydrolysis activity of MSH2:MSH3 that is essential for dissociation of the proteins from DNA. She proposed a model in which mismatch repair complexes are "hijacked" by CAG repeat hairpins causing aborted MMR leading to cell death.

New Perspectives and Issues Emerging from 30 Years of Research on Drinking Water Disinfection By-products

Chairs: **David M. DeMarini** (US EPA) and **Michael J. Plewa** (University of Illinois)

This symposium was an overview of research that has occurred during the past 30 years on the epidemiology of drinking water and cancer, as well as on the occurrence, genotoxicity, and carcinogenicity of disinfection by-products. The symposium derived largely from the first review article written on this subject (Richardson et al., Mutat. Res. 636:178, 2007) and was aimed at providing not only an historical look at the toxicology of drinking water but also to indicate new areas of research that are needed to better understand the potential health risks from drinking water.

Kenneth Cantor (NIH/NCI, Bethesda, MD) reviewed the cancer epidemiology of drinking water, noting that bladder cancer is the primary cancer associated with drinking water, and colorectal cancer is also weakly associated. He reported recent results from a case-control study of bladder cancer and drinking water conducted by him and colleagues in Barcelona, Spain, which is the first epidemiological study of drinking water and cancer in which the route of exposure was differentiated (drinking vs. bathing/showering/swimming). The study indicates that dermal/inhalation exposure to drinking water and its associated trihalomethanes, rather than oral exposure, is most strongly associated with risk for bladder cancer, and that subjects who had one or two copies of the GSTT1-1 gene and/or a genetic variant of GSTZ1 were at greater risk than those who were GTT1-1-null or were wild type for GSTZ1.

Susan Richardson (U.S. EPA/Athens, GA) reviewed the results of a recent survey of a large number of U.S. water treatment plants. Although more than 600 disinfection by-products have been identified, half of the organic halide fraction of drinking water is yet to be chemically characterized. Recent changes from chlorination to ozonation/chloramination or just chloramination have resulted in the formation of new disinfection by-products, such as halonitromethanes, iodo-acids, iodo-trihalomethanes, halofuranones, haloamides, haloacetonitriles, and nitrosamines. Most of these occur at low ng/L levels to low µg/L levels; however, several have been found to be highly mutagenic and carcinogenic. Strategies for reducing the formation of some of these compounds include filtration methods and other disinfection procedures.

Michael Plewa (U. Illinois, Urbana, IL) presented the results of his systematic study of >70 disinfection by-products for their cytotoxicity and DNA-damaging ability (comet assay) in CHO cells. For many of these compounds, these data are the only genotoxicity data that have been generated thus far, even though most of these compounds are present in most of the drinking water in the U.S. Many of the disinfection by-products formed from disinfection methods other than chlorination, such as chloramination or ozonation/chloramination, are more cytotoxic and genotoxic than the regulated, well-characterized disinfection by-products. The data generated by a systematic approach in the same assay in the same lab has permitted a clear demonstration that most iodo compounds are more genotoxic than brominated compounds, which are more genotoxic than chlorinated compounds. Nitrogen-containing compounds are more cytotoxic and genotoxic in general than carbonaceous compounds. Only a systematic approach such as this provides a comparative assessment of the toxicological properties of disinfection by-products.

David DeMarini (U.S. EPA/RTP, NC) reviewed the genotoxicity and carcinogenicity of the 11 disinfection by-products currently regulated by the U.S. EPA, as well as an additional 76 unregulated compounds for which there is occurrence data available. Among the 11 regulated compounds, 5 exhibit the toxicological properties of human carcinogens (i.e., they are trans-species carcinogens). However, 2 (chloroacetic acid and chlorite) are not carcinogenic in 2 species, and 1 (bromoacetic acid) has never been tested for carcinogenicity. Among the unregulated compounds, 8 are carcinogenic, 2 are being tested for carcinogenicity, and 5 exhibit some of the toxicological features of human carcinogens. Among the 76 unregulated disinfection by-products, 29 are genotoxic, and 14 have not yet been tested for genotoxicity. No dermal exposures in rodents have been performed with any disinfection by-products, and this appears to be an important area of study considering the new epidemiology described above by Dr. Cantor. Much more work is required to explore

the toxicology of drinking water per se as a complex mixture as opposed to the individual disinfection by-products.
Rita Schoeny (U.S. EPA/Washington, DC) noted the remarkable success that drinking water disinfection has achieved in reducing water-borne disease, and she reviewed the regulations for trihalomethanes and haloacids, which were viewed initially as surrogates for other disinfection by-products. However, recent analyses have shown that this is generally not the case, especially for the newly emerging disinfection by-products. Current approaches to assessing risk involve determining a toxicant's mode of action, relevance of animal data to humans, conditions under which a compound is toxic, whether to use a linear or non-linear low-dose extrapolation, and whether there are special risks to children. Although alternative disinfection methods have been shown to produce new disinfection by-products that are generally more genotoxic than those that are regulated, data generated more than a decade ago showed that organic extracts of drinking water prepared by chloramination or ozone/chloramation were less mutagenic than those from chlorinated water.

All of the speakers identified areas for future research and unresolved questions regarding the toxicology of drinking water. Audience members expressed interest in the use of filters of various sorts for their homes and the safety of bottled water. People also wondered about the use of filters on showers and baths as opposed to drinking water. Swimming was also raised as an exposure of potential concern, given the emerging epidemiology indicating that there is an increased risk for bladder cancer from swimming. Some audience members expressed surprise that there are both regulated and unregulated human carcinogens in drinking water. Overall, the symposium communicated to the audience that drinking water is relatively safe, but that it is not just H_2O and may present some low-level risk for some adverse health effects.

Low Dose Radiation-induce Genome and Epigenome Instability

Chairs: **William F. Morgan** (Pacific Northwest National Laboratory) and **Matthew Coleman** (Lawrence Livermore National Laboratory)

This symposium was sponsored by the National Institute for Alcohol and Infectious Diseases, and their support was acknowledged and greatly appreciated.

William Morgan (Pacific Northwest National Laboratory) opened the session with introductory remarks and presented the Pacific Northwest National Laboratories new integrated programmatic approach to studying low dose radiation effects using systems biology. His hypothesis was that radiation risk extrapolations assume that the deposition of energy by radiation is likely related linearly related to dose but the cellular/tissue responses to exposure might not be. How cells in tissues interact to modulate radiation-induced changes is poorly understood, and this is a multi-scale, systems-level challenge. Dr. Morgan speculated that addressing this problem will require knowing the pathways involved in radiation responses and developing the computational modeling approaches to organize complex biological data in a predictive, multi-cellular framework.

Olga Kovalchuk (University of Lethbridge, Alberta, Canada) followed with an elegant synopsis on epigenetic deregulation of targeted and non-targeted effects of ionizing radiation. Dr. Kovalchuk summarized work from her laboratory analyzing epigenetic changes in bystander and transgenerational radiation effects using animal models. Interestingly her data suggested that epigenetic changes (DNA methylation histone modifications and microRNAome changes) were important in a plethora of non-targeted effects of radiation exposures.

Dana Dolinoy (University of Michigan) discussed how maternal exposures to low doses of ionizing radiation might alter the fetal epigenome. She presented evidence that epigenetic dysregulation during early development is mechanistically linked to the pathogenesis of adult-onset disease. Her data indicated that the protective effect of low doses of radiation may be mediated by epigenetic mechanisms that lead to increased DNA methylation at critical sites in the genome.

Yuri Debrova (University of Leicester, UK) offered the keynote presentation in the symposium. Dr. Dubrova described his exciting research on the impact of in utero irradiation on mutation rates at expanded simple tandem repeat DNA loci in exposed mice and their first generation offspring. Data from his laboratory implied that the passive erasure of epigenetic marker(s) in the maternal genome could

diminish the transgenerational effects of fetal irradiation. This interesting observation implicates important clues as to the mechanisms of radiation-induced genomic instability. Matthew Coleman (Lawrence Livermore National Laboratory) concluded the symposium with his discussion of low dose radiation induced genomic regulation of pathways critical to altering chromatin conformation. His new data indicated that a number of previously unsuspected cellular pathways are involved in responses to low doses of ionizing radiation and may modulate specific cellular and tissue responses associated with such low dose exposures, particularly radiation induced adaptive responses.

The session was particularly well attended and there was a stimulating question and answer period reflecting the burgeoning interest in effects of exposures to low doses of ionizing radiation and potential genetic and epigenetic responses.

Spontaneous and Oxidative Mutagenesis *In Vivo*

Chairperson: **Takehiko Nohmi** (National Institute of Health Sciences, Tokyo, Japan)

Each human cell metabolizes approximately 1012 molecules of oxygen per day to generate ATP. However, about 1% of oxygen metabolism results in the production of reactive oxygen species (ROS) by a sequence of one electron reduction. ROS is also generated by exposure of cells to radiation and chemical carcinogens. Because ROS damages nearby cellular components such as DNA, proteins and lipids in membrane, it has been implicated in the etiology of human degenerative diseases, aging and cancer. In this session, five speakers presented their latest results with rodent models to analyze mutations and discuss the roles of ROS in spontaneous and induced mutagenesis *in vivo*.

Takehiko Nohmi (National Institute of Health Sciences, Tokyo, Japan) introduced basic features of gpt delta mice/rats and showed evidence that Spi- selection is useful to identify deletion mutations induced by oxidizing agents such as potassium bromate. He also showed the results of Nrf2 knockout mice where mutation frequencies are elevated in the liver and lung even without treatments to damage DNA. The results suggest that endogenous genotoxic agents are actively detoxified by phase II enzymes whose expressions are regulated by Nrf2.

Steve Sommer (City of Hope) reported that spontaneous “mutation showers”, clusters of spontaneous mutations, occur in mice at an estimated frequency of about 1% or more of spontaneous mutations. The event may be caused by transient relax of DNA replication and have implications in oncogenesis and evolution.

TeruhisaTsuzuki (Kyushu, University, Japan)) introduced his work on Mutyh-deficient mice, which exhibit a marked predisposition to spontaneous tumorigenesis in various tissues, especially in the small intestines. The high susceptibility to intestinal tumor-development is well correlated with the symptom of MUTYH-associated polyposis patients. G:C to T:A transversions are further induced in the small intestines of Muthy-deficient mice, resulting in elevated incidence of intestinal tumors when treated with potassium bromate in drinking water. He suggested the abnormality in Wnt signaling pathway may be causatively associated with oxidative stress induced tumorigenesis in the small intestines of the Mutyh-deficient mice.

Robert Schiestl (University of California, Los Angeles) reported that treatments of dam of Atm-/- mice with N-acetyl-cysteine (NAC), an antioxidant, reduced the levels of DNA damage and deletion mutations, which occur in somatic cells in pun/pun mouse embryos by deletion of a 70kb DNA fragment. The results suggest that nutritional intake of antioxidants may counteract DNA damage, DNA instability and cancer in Ataxia Telangiectasia (AT) patients.

Carrie Valentine (National Center for Toxicological Research, US FDA) introduced unique features of the phiX174 Malling mouse model and compared the responses to other mouse models such as lacI (Big Blue mouse) or gpt delta mice. She concluded that the lacI, gpt and phiX174 target genes have spontaneous mutant frequencies in spleen as low as endogenous genes.

This symposium focused on oxidative and spontaneous mutations *in vivo*. Since each speaker used distinct rodent models for mutagenesis, i.e., gpt delta (Nohmi), lacI Big Blue (Sommer), rpsL high tech (Tsuzuki), pink eye unstable (Schiestl) and phiX174 (Valentine), it might be beneficial and convenient for the audience to directly compare the merits and limitations of each model. These rodent models for mutagenesis may be useful not only for research purposes but also for administrative regulation of chemicals such as pharmaceuticals, pesticides, food additives or veterinary medicines. The chairperson received multiple positive responses from the audience after the session. We believe this session shed new light on the mechanisms of oxidative and spontaneous mutations in mice and perhaps in humans.

Mutagenic and Carcinogenic Mechanisms

Chairs: **Jason Bielas** (Fred Hutchinson Cancer Research Center) and **David Schild** (Lawrence Berkeley National Laboratory)

There were eight excellent presentations covered diverse topics interrelated by mutagenesis and carcinogenesis chosen from the submitted abstracts. The session was well attended and the talks elicited several pertinent questions and discussions.

Manel Camps (UC Santa Cruz) discussed 'Mutagenesis by Error-Prone DNA Polymerase I: Optimization and Applications'. He summarized a powerful system that employs an error-prone DNA polymerase I to generate random mutant libraries, which can be subsequently selected or screened for enhanced and/or novel function. The polymerase is expressed in a DNA Pol I-deficient strain, and the library generation occurs *in vivo* in E. coli. Dr. Camps presented the different model systems of protein evolution used in his laboratory. He also described recent advances to increase the efficiency of mutagenesis, including iterative transformation, increasing the growth time, and use of super-rich media. Finally, he showed that the frequency of mutagenesis is independent of distance from origin of replication, suggesting that error-prone polI can replicate the entire plasmids.

Jeffrey Schwartz (Univ. of Washington, Seattle) gave a talk titled 'Effect of p53 Codon-72 Polymorphisms on Radiation Responses *In Vitro* (Apoptosis, Cell Cycle Progression, Chromosome Damage), and *In Vivo* (Cancer Risk, Graft-Versus-Host Disease)'. Other investigators have reported that the codon 72R (arg) polymorphism of p53 (p53-72R) is more sensitive to p53-dependent apoptosis while p53-72P (pro) leads to strong p21 induction, and p53-72R is associated with increased risk of basal cell carcinoma. Schwartz et al. examined p53-72R in both *in vitro* and *in vivo* responses to radiation. p53-72R acted dominantly to confer susceptibility to apoptosis and resistance to G2 delay. In contrast, it was p53-72P that acted dominantly to confer resistance to chromosome aberration induction. In a preliminary study of patients who developed a second cancer following irradiation prior to hematopoietic cell transplant, genotype frequencies were similar to those reported for non-cancer populations.

Guliang Wang (MD Anderson) spoke on 'Non-B DNA Structure-Induced Genetic Instability in Mammalian Cells'. DNA repeat sequences can form non-canonical DNA structures, and Dr. Wang reported that these H-DNA and Z-DNA structures are intrinsically mutagenic in mammalian cells. They found that the endogenous H-DNA induced mutation frequencies ~20-fold, largely as double-strand breaks (DSBs), and Z-DNA forming CG(14) repeats also lead to DSBs, suggesting that both structures are sources of genetic instability. They have constructed mice containing defined H-DNA or Z-DNA-forming sequences, and have detected increased genetic instability in many offspring.

David Ferguson (Univ. of Michigan) gave a talk entitled 'Mrell Nuclease Activity has Essential Roles in DNA Repair and Genomic Stability Distinct from ATM Activation'. MRN (Mre11/Rad50/NBS1) bridges DNA ends and also activates the ATM kinase, and Mre11 possesses DNA nuclease activities. Targeted mouse alleles were engineered that either eliminate nuclease activities or inactivate the entire MRN complex. Mre11 nuclease deficiency causes a phenotype indistinguishable from the absence of MRN. They reported a crucial role for the nuclease activities in homology-directed repair and a contributing role in ATR kinase activation, but not in ATM activation. They concluded that 'nucleolytic processing by Mre11 is an essential function of fundamental importance in DNA repair, distinct from MRN control of ATM signaling.'

Joann Sweasy (Yale) spoke on 'The D160N Gastric Cancer-Associated Variant of DNA Polymerase Beta Induces Cellular Transformation and Genomic Instability'. DNA polymerase beta (Pol ß) is altered in many human tumors and half of these alterations result from a single amino acid substitution. Dr. Sweasy reported that the D160N Pol ß variant induces cellular transformation in mouse cells and induces elevated levels of base substitution mutations, likely arising by DNA misalignment. Their results suggest that D160N induces cellular transformation by a mutational mechanism.

Anthony Berdis (Case Western Reserve) gave two back-to-back talks titled 'Replication of Cisplatin-Induced DNA Lesions: A Mutagenic Event' and 'Enhancing the Effectiveness of Chemotherapeutic Agents by Inhibiting Pro-Mutagenic DNA Synthesis'. Cisplatin, a widely used chemotherapeutic agent, shows cytotoxicity due to DNA crosslinks, and can cause misreplication and the generation of mutations. A hypothesis to explain the pro-mutagenic behavior was described and tested, using the incorporation of natural and non-natural nucleotides opposite a particular lesion. The results of their study were discussed with respect to the dynamics of misreplicating other cross-linked DNA lesions such as the thymine dimer. The second talk centered on evaluating the misreplication of O6-methylguanine (O6-MeG), a miscoding DNA lesion formed by various alkylating agents. Using *in vitro* techniques, they demonstrate that dTTP is incorporated opposite O6-MeG five-fold more efficiently than dCTP. They hypothesized and determined that isosteric analogs of dTTP could be preferentially incorporated opposite the miscoding lesion. As a result, they conclude that non-natural nucleotides have the potential to inhibit promutagenic DNA replication and could thus enhance the effectiveness of current chemotherapeutic treatments.

L. J. Stallons (Univ. of Louisville) discussed 'Cell Death and Cell Cycle Pathways as Potential Targets for Tumor Suppression by Pol Iota: A Systems Biology Approach'. Y family DNA polymerases function in replication of damaged DNA, including error-prone replication. From experiments in mice, the authors hypothesized that pol iota functions as a tumor suppressor through a mechanism other than its catalytic activity. To investigate this function, they treated various polymerase deficient fibroblasts with UV and collected total RNA for global mRNA and miRNA analyses. They found many genes and miRNAs whose expression was differentially altered by UV between the cell lines. Their results indicated that pol iota may act as a tumor suppressor by affecting cell death and/or cell cycle after UV irradiation.

Epigenetic Mechanisms: Damage-Induced Epigenome Changes

Chairs: W. **David Sedwick** (Case Western Reserve University) and **Dana C. Dolinoy** (University of Michigan)

Sabine Lange (University of Texas MD Anderson Cancer Center) presented on "High Mobility Group Protein B1 Enhances DNA Repair and Chromatin Modification Following DNA Damage." High mobility group protein B1 (HMGB1) is a multifunctional protein with roles in chromatin structure, transcriptional regulation, V(D)J recombination, and inflammation. HMGB1 also binds to and bends damaged DNA, but the biological consequence of this interaction is not clearly understood. Lange demonstrated cells lacking HMGB1 showed no histone acetylation upon DNA damage. Additionally, purified HMGB1 protein enhanced chromatin formation in an *in vitro* chromatin assembly system. These results reveal a role for HMGB1 in the error-free repair of DNA lesions. Because strategies targeting HMGB1 are currently in development for treatment of sepsis and rheumatoid arthritis, these findings draw attention to potential adverse side effects of anti-HMGB1 therapy in patients with inflammatory diseases.

Umut Aypar (University of Maryland) next presented on "Epigenetic Alterations in High and Low LET Radiation Induced Genomic Instability." Radiation induced genomic instability (RIGI) results in an increased frequency of genetic alterations in the progeny of irradiated cells. Aypar and colleagues tested the intriguing hypothesis that RIGI is initiated and supported by altered DNA methylation by measuring global DNA methylation of LINE-1 and Alu repeat elements in two cell lines. Data indicated methylation differences based on cell line and radiation exposure.

Kristy Kutanzi (University of Lethbridge) presented on "Epigenetic Mechanisms Underlie Estrogen- and Radiation-Induced Mammary Carcinogenesis." This talk discussed the role of epigenetics in ionizing radiation (IR) and estrogen (E2) induced malignant transformation of normal breast epithelial cells. Using a rodent model, Kutanzi and colleagues show that combined E2 and IR exposure induces morphological changes as well as DNA hypomethylation; aberrant expression of methyltransferases DNMT1, DNMT3a, and MeCp2; altered expression of chromatin modifying enzymes; and hyperacetylation of histone residues. Furthermore, E2, IR, and E2+IR significantly altered known oncogenic microRNAs, which are emerging epigenetic marks associated with disease risk.

Lucy Anderson (National Cancer Institute) presented the fourth talk in this session on "Chemical Stressors in Fathers Alter Allele-Associated DNA Methylation of 45S RRNA in Progency." Anderson et al. investigated the role paternal chromium III exposure on offspring ribosomal RNA methylation and SNPs and reported that SNPs in regulatory regions of the rRNA gene influence gene methylation, and (2) this relationship can be perturbed by exposure of fathers to Cr. For example, ACC alleles tended to be hypomethylated in treated versus control group in the lung. However, the CCA allele was associated with hypermethylation in exposed offspring.

Malathi Banda (Wayne State University) discussed "Bax Splice Variant Expression Following Radiation Exposure in Human and Lymphoblastoid Cells." Using two normal lymphoblastoid cell lines and one T-leukemic cell line, Banda et al examined radiation dose- and time- dependent expression levels of BAX splice variants. Presented results indicated differential expression of variants with time and to some extent with dose. BAX variants α, β, σ, and δ showed elevated expression following radiation while BAX ε variant exhibited decreased expression following radiation with dose and time. The BAX ε contains 41 amino acids which are different from BAX α, the full length variant. These 41 amino acids show similarity to a zinc finger domain suggesting a regulatory role for this variant.

Steve Ayers (Life Sciences Division, Lawrence Berkeley National Laboratory) continued this session with a presentation of "Genome Organizer SATB1 in DNA Repair." SATB1 serves as a nuclear architectural protein in thymocyte nuclei, recruiting DNA damage sites marked by γ-H2AX to the SATB1 regulatory network. In response to DNA damage, SATB1 recruit DNA repair proteins. Dr. Ayers's data demonstrates that SATB1-null thymocytes are deficient in DNA repair. Dr. Ayers and his colleagues have further identified DNA repair proteins in the SATB1-containing protein complex, after purification using DNA affinity chromatography.

Bo Hang (Life Sciences Division, Lawrence Berkeley National Laboratory) presented on "Functional Interaction of Human TDG with FMRP and XPG in Base Excision Repair of T/G

Mismatches." Fragile X syndrome (FXS) is characterized by promoter hypermethylation of the FMR1 gene, leading to its silencing. Hang and colleagues investigated the role of the base excision repair enzyme thymine-DNA glycosylase (TDG) in mediating DNA demethylation. Hang reported that FMRP, the protein that is missing in FXS, physically interacts with TDG and significantly stimulates *in vitro* TDG activity against a T/G mismatch, suggesting a link between a disrupted demethylating mechanism and the hypermethylated status of the FMR promoter in this disorder.

Sundaresan Venkatachalam (University of Tennessee, Knoxville) concluded this platform session with a discussion of the "Role of Chromatin Remodeling in DNA Repair, Cell Cycle, and Tumor Suppression." Using a Chd2 mutant mouse model, Venkatachalam et al report that lower levels of Chd2 affect multiple biological systems including hematopoietic, vascular, and lymphoid development. Chd2 heterozygous mice display susceptibility to spontaneous lymphomas. Furthermore, Chd2 mutant cells exhibit defects in DNA damage induced transcriptional activation responses and accumulate higher levels of the chromatin associated DNA damage response mediator, γH2AX, after DNA damage induction by X-rays.

Approximately 55 EMS participates attended this session and audience participation was lively.

New Technologies Special Interest Group

Leaders: **Brinda Mahadevan** (Oregan State University) and **Patricia Escobar** (University of Pittsburgh)

The New Technologies SIG breakfast meeting, "New Tools to Detect Mutagenicity/Immunotoxicity" was held on Monday, October 20, 2008 and over 30 participants from academia, government and industry attended. The meeting was opened by the SIG chairs Brinda Mahadevan and Patricia Escobar with an update on activities of the SIG and its critical components for the new EMS mission statement. Input on the mission statement and other activities of the SIG from the SIG breakfast attendees was also captured through a survery. The survey was a success as the feedback allowed the chairs to provide the SIG mission statement, contact volunteers to work on the SIG web page and organize future SIG related activities. The survey and discussion was followed by two very interesting scientific presentations.

The first speaker was Dr. Andrew J. Olaharski, from the Discovery and Investigative Safety, Roche, Palo Alto, CA, who presented on "Utilizing kinase inhibitory profiles to predict *in vitro* micronucleus assay results". This was followed by a presentation by Dr. Paurene Duramad, from the Toxicology & Safety Assessment, Genentech, South San Francisco, CA, titled "Developing Biomarkers of Immunotoxicity".

In addition to the breakfast meeting, the New Technologies SIG organized a workshop titled "Omics – Applications and Impacts on Genotoxicity Assessment" on Saturday, October 18, 2008. There were over 50 registered participants and a special issue in the journal Mutation Research on topics covered at the workshop is in the works.

We have worked with the Program Committee for the active representation of this SIG in scientific symposia/workshops for the 40th Annual Meeting of EMS in St. Louis, Missouri, October 24-28, 2009 with a great outcome. We are proud to present the following as organized/endorsed events by the New Technologies SIG at the EMS Annual Meeting in October 2009.

1. Symposium session titled " Genetic Toxicology in the 21st Century" chaired by Drs. Raymond Tice and Brinda Mahadevan, 1-3:30 p.m. on Monday, October 26, 2009.

2. Symposium session titled "Nanotechnology: An Insight on the Toxicology of Nanoparticles" chaired by Drs. Patricia Escobar and David DeMarini, 3:15-5:15 p.m., Wednesday, October 28, 2009.

3. Interactive workshop titled "Bacterial Mutagenicity Screening for Discovery Support and Drug Candidate Selection" 8:30-12 noon, Saturday, October 24, 2009.

This workshop will:
- Facilitate the discussion on several bacterial mutagenicity screening platforms and strategies followed across the pharmaceutical industry.

-Focus on the different assays used in early drug development to understand the value of each individual assay and its advantages.

-Provide an opportunity to share and learn about the sensitivity/specificity of the mutagenictiy screening assays with reference to proprietary/internal compounds and their concordance /predictivity in GLP Ames.

Ultimately, how the data from the screening assays are being utilized and the recommendations of assays for early and late discovery genetic toxicology screens will also be presented. Look for the details of the workshop in the preliminary program and we strongly encourage you to register for the workshop and obtain a wealth of unpublished information on screening strategies followed across pharmaceutical companies in early drug development and lead optimization processes.

We are preparing the agenda for the New Technologies SIG breakfast meeting at the EMS meeting in St. Louis Missouri. So, those interested in assisting with this and other activities

of the SIG including suggestions for speakers, please contact Brinda Mahadevan (brinda.mahadevan@spcorp.com) or Patricia Escobar (patricia.escobar@boehringer-ingelheim.com).

Epigenetics Special Interest Group Breakfast

Co-Leaders: **David Sedwick** *(Case Western Reserve) and* **Randy Jirtle** *(Duke)*

The breakfast gathering of the Epigenetics SIG in Puerto Rico drew a large, lively crowd despite the early hour. Only three years old, the Epigenetics Special Interest Group has grown rapidly to become the second largest SIG within the EMS, and it is continuing to attract the interest of current and new EMS members. David Sedwick (Case Western Reserve) opened the breakfast meeting with a brief slide presentation of a coordinated vision to expand the contributions of the Epigenetics SIG to future EMS meetings. He briefly outlined scientific areas in which epigenetics plays an increasingly recognized role. Going beyond the epigenome as a now well-established complex regulator of gene expression, the presentation further identified the epigenome as a sensitive target of toxic environmental chemical agents, physical stressors (e.g. ionizing radiation) and nutritional compounds, and ended with the epigenome as a ripe source of promising biomarkers of human disease and a novel target for disease prevention and therapeutics. The group discussed future plans for developing epigenetics symposia at upcoming EMS meetings, and in particular sought speakers for the next EMS meeting in St. Louis in 2010. They briefly discussed the new web EMS web site under development, and noted potential Epigenetics SIG content for those web pages. New leadership was discussed, with the reigns of the SIG management being turned over to Cathy Klein (NYU) and Janet Baulch (University of Maryland), and Olga Kovalchuk (University of Lethbridge, CA) taking the lead as programming coordinator for the SIG, as well as serving as the SIG liaison with the EMS annual meeting program committee.

Following the organizational portion of the breakfast, the program was turned over to three speakers who were invited by Olga Kovalchuk and Cathy Klein to briefly present their recent work. The diverse scientific presentations by Janet Baulch (University of Maryland) on global LINE-1 methylation changes in irradiated human cell cultures, by Igor Kovalchuk (University of Lethbridge, CA) on the role of epigenetic changes in plant adaptation to environmental stressors, and by Hilda Van Gijssel (Valley City State University, ND) on the potential epigenetics effect of alcohol exposure in a Drosophila model were all extremely interesting, and very well received.

The Epigenetics SIG looks forward to increasing its involvement in future EMS meetings, and seeks the active participation, input and suggestions of all of its current and potential new members.

Transgenic and *In Vivo* Mutagenesis Special Interest Group

Outgoing Leader: **Carrie Valentine** (National Center for Toxicological Research, US Food and Drug Administration; Retired 6/30/08)

Incoming & Existing Leaders: **Manju Manjanatha** (National Center for Toxicological Research, US Food and Drug Administration) & **Kathleen Hill** (The University of Western Ontario)

The TIVM interest group convened over breakfast on Sunday, October 19, 2008 at the annual meeting in Puerto Rico. There were about 35 attendees registered to the event and as the first item of business, new nominations were solicited for the election of new Leader and Co-Leader. However, the attendees, instead of submitting new nominations overwhelmingly voted for the current officers to continue serving the SIG for 2009-2010.

The breakfast meeting kicked off with four oral presentations all of which were highly informative and interesting. The first speaker for the morning session was Dr. Cheryl Hobbs of the Integrated Laboratory Systems, Research Triangle Park, NC, and her talk entitled “Time Course of Chemical-Induced *In Vivo* Genotoxicity Evaluated Using a Combined Protocol for Micronucleus and Comet Analyses”. In order to minimize the use of animals and maximize information gained from an *in vivo* genotoxicity assays, Cheryl and her co-workers are developing protocols to measure simultaneously micronucleated reticulocyte (MN-RET) frequencies by flow cytometry, and primary DNA damage by the Comet assay. She presented several nice studies in B6C3F1 mice treated with ethyl methanesulfonate (EMS) acutely and chronically to demonstrate that a combined 4-day protocol for parallel evaluation of MN-RET and DNA damage in the same set of experimental animals is not only feasible but also practical. Finally, she indicated that when all the studies are done, these protocols for simultaneously measuring MN-RET frequencies and primary DNA damage would be proposed to National Toxicology Program (NTP) for conducting *in vivo* genotoxicity assays.

Dr. Jason Bielas from the Fred Hutchinson Cancer Research Center, University of Washington WA, presented an excellent study on nuclear and mitochondrial mutations in cancer. They noted that the large numbers of somatic mutations seen in human cancers could not be explained by

the low rate of mutation in normal cells. They investigated this hypothesis by using a recently developed sensitive assay to measure spontaneous frequency of nuclear and mitochondrial mutations in human tissues. Their study showed that in normal tissues, the frequency of spontaneous nuclear somatic mutations was very low (less than 10-8 per basepair) whereas in tumors, it was greater than 210-fold higher. Thus, the genomes of human cancer cells display greatly elevated frequencies of point mutation instability (PIN). They have extended similar studies to evaluate the frequencies of mitochondrial mutations in normal and diseased cells. The results from these studies suggested that cancer cells express a mutator phenotype at the single-nucleotide level and the increased mutagenesis persisted during tumor progression. Based on this observation, they recommend using PIN as a novel prognostic indicator of tumor progression.

The third speaker was Dr. George Douglas from Health Canada, Ottawa, ON, Canada, who reported on validation of transgenic rodent gene mutation (TGR) assays using DNA sequence data. He made a nice argument that although the mutagenicity is a primary event in the etiology of most cancers and therefore the predictivity of genotoxicity tests for carcinogenicity is an important consideration in the regulatory arena, the association between the two end points is imperfect. He reasoned that there are non-carcinogens that are genotoxic presumably because mutagenicity per se is insufficient for the development of tumors in such cases as well as there are carcinogens that are non-genotoxic mainly due to mechanisms that do not involve genotoxicity as primary event in the development of cancer. Based on this premise, he suggested that instead of using carcinogenicity as an endpoint against which to validate TGR assay, DNA sequence data from the DNA isolated from mutant phenotypes can be used effectively to estimate the Positive Predictive Value (PPV) of these presumptive mutant genotypes. He showed some review data from over 140 studies in which a total of 32,751 mutant phenotypes were sequenced yielding 31,659 mutant genotypes with a high PPV of 0.967. Therefore, he indicated that for validation of the predictivity of TGR assays, the DNA sequence data might be more useful than carcinogenesis as an end point.

The last speaker for the morning breakfast session was Dr. Tim Singer from Health Canada, Ottawa, ON, Canada. His topic was entitled “Detection of Weak Mutagens in Transgenic Rodent Mutation Assays: Challenging the International Workshops on Genotoxicity Testing Protocol Recommendations”. His work emphasized the applicability of the International Workshops on Genotoxicity Testing (IWGT) expert panel recommendation that treatment duration of 28 days and sampling/manifestation time of three days (28+3) would be optimal for the detection of broad spectrum of mutagens with varying potency. Tim’s group evaluated the IWGT recommendation using lacZ mice and a weak mutagen, ethyl carbamate. They administered different doses of the test chemical and followed experimental protocols using the treatment duration + sampling time such as 7+3, 7+28, 28+3, 28+28, and 56+3 for detecting lacZ mutations in the bone marrow and liver of lacZ mice. The results on mutant frequencies studies suggested that 28+3 IWGT experimental protocol was sufficiently robust to detect a mutagenic response induced by the weak mutagen in the two tissues with varying rates of cell proliferation. Based on this, Tim proposed that 28+3 protocol should become the initial basis for the development of an OECD test guideline.

Germ Cell, Stem Cell and Human Diseases

Co-Leaders: **Carole Yauk** (Health Canada) and **Steve Sommer** (City of Hope)

The GeSteHug special interest group had a busy and informative meeting on Monday October 21, 2008 in Puerto Rico. A number of presentations were delivered spanning different model organisms, types of mutations and processes including: (a) how transcription may influence rates of base pair substitution in yeast (Mac Lippert), (b) methods for detecting and quantifying homologous recombination hotspots (Norm Arnheim), (c) the role of DMN in sex deviation and in male-mediated trans-generational effects in Drosophila (Patricial Ramos Morales) and (d) targeted and non-targeted tandem repeat mutations in male and female mice exposed in utero to diesel exhaust particles (Lynn Berndt-Weis, Carole Yauk). An overview of new massively parallel sequencing approaches was delivered by Steve Sommer. The presentations were followed by a stimulating discussion led by Dr. John Mulvihill on a proposal to study germline mutation rates in childhood cancer survivors. Discussions centered on the most appropriate tools available for studying inherited mutations, and what are the advantages and disadvantages of these approaches.

Introducing the Heritable Mutation and Disease Special Interest Group

The GeSteHug SIG has been actively engaged in discussions on the future of the group and developing EMS web site content. During these discussions, the group voted to change the name of the SIG to something more concise that captures the importance of this research field. Thus, the GeSteHug SIG will now be known as the Heritable Mutation and Disease SIG. Thanks to everyone for the stimulating discussions and for the numerous new name suggestions.

Carole Yauk (Health Canada)

Letter from the President-Elect

Dear Colleagues:

Meet me in St. Louis to celebrate our 40th anniversary of EMS! Our meeting will be held from October 24-28, 2009 at the Marriott Hotel at St. Louis Union Station. This is a great venue with its adjacent mall and food courts, shops and attractions. In celebration we will review the past accomplishments of our Society, focus on the future and together, will generate an outstanding scientific and social gathering for all our members.

The focus of the 40th Annual Meeting is on the EMS members. The 2009 EMS meeting Working Group and the Program Committee have worked hard to plan a meeting of high science quality and value for money. We endeavor to have a meeting in which all EMS members play an important role in the evolution of their Society. To be honest, I believe that we have lost too many members to other scientific societies. For the 2009 EMS meeting I will continue the attention of our past Program Chair, Priscilla Cooper, to integrate basic research with translational research, risk assessment and regulation as in our 2008 meeting in Puerto Rico. Thus, the science content of our 40th anniversary meeting will continue the demanding levels of excellence that we have experienced in the past. We have a 2009 program that is balanced and yet inclusive of the scientific issues expressed by the EMS interest groups. We offer young and mid-career members greater roles in presenting their research to their peers. As always our Society will continue to welcome students and young professionals into our ranks.

In this anniversary year we appreciate the past, and yet our gaze is firmly locked toward the future of Genomics in the Environmental Century. The 2009 meeting will have celebratory sessions to mark our 40th anniversary; I appointed Jim Gentile as the Chair of the EMS Anniversary Subcommittee. We will have events to review where EMS excelled in the past and our dynamic efforts to forge a path to a bright future. Our welcome session will include a presentation on U.S. national science education policy and the role EMS can play in enhancing the understanding and appreciation of science in our Society. Jim Gentile has committed support from Research Corporation for this endeavor. Throughout the meeting we will have an exhibition booth with the history of EMS. At the start of our program a plenary symposium will be held with past EMS presidents who reflect our scientific diversity and demonstrate the impact of EMS on research, industry and regulation over the past 40 years. This will be a great kick-off for our 40th anniversary.

To have a lasting impact of this 40th anniversary celebration we will host a special issue of our Society Journal, Environmental and Molecular Mutagenesis. I am very happy to announce that Suzanne Morris, Manju Manjanatha, Vasily Dobrovolsky, Barbara Parsons and Bob Heflich (National Center for Toxicological Research) will serve as co-editors for the special issue.

Our scientific program will have sessions on DNA damage and repair, epigenetics, genetic instability and disease, risk assessment and regulation, epidemiology, toxicogenomics, genetic toxicology and environmental mutagenesis and women's health issues. We will introduce new research areas such as the evolution and application of DNA aptamers to detect environmental toxins. I will work with the symposia chairs to select speakers from relevant abstracts to deliver 15-minute talks at the end of the symposia. This approach will incorporate into the symposia selected new and exciting information. In addition, I have asked the chairs for each symposium to limit their speaker selection to one non-member. In our Society we have the expertise of excellent scientists and orators to inspire, participate and lead. I hope that this arrangement will be an inducement for more members (especially young and mid-career scientists) to attend the meeting and present their results. I appreciate the many favorable comments that I have received regarding this. We will also have enhanced opportunities for mentorship, networking, planning and group meetings by having an open networking room throughout the meeting. Workshops are planned for the first day to encourage progress in specific scientific topics. The 2009 EMS meeting will have a balance of non-member and member plenary and symposia speakers to provide a scientific dynamic within the topical content of the 2009 meeting.

During this economic recession I know that members have limited travel resources. With all frankness our Society, the mother of all Environmental Mutagen Societies, will suffer if we have diminished attendance at the 40th anniversary meeting. I urge all members to attend the St. Louis meeting. If a principal investigator cannot attend, then send your staff, post-doctoral fellows and students. Working with our Executive Director, Tonia Masson, and our management team (AIM) we will make the St. Louis meeting especially attractive scientifically and with outstanding value. I ask all EMS members to invest in their Society by attending the 40th anniversary meeting. The quality and success of our meeting and the future of EMS rests with you.

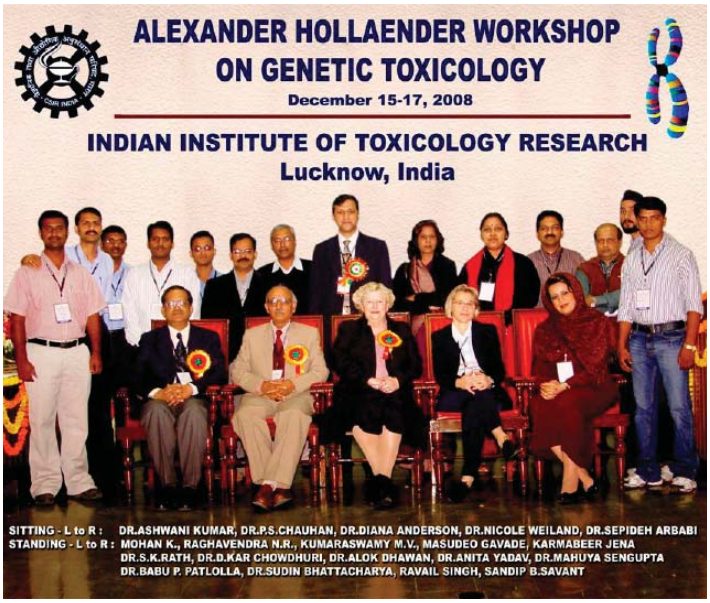
Meet me in St. Louis.

Sincerely,
Michael J. Plewa
President-Elect and 2009 EMS Program Chair

Alexander Hollaender Workshop

Alexander Hollaender Workshop on Genetic Toxicology

December 15-17, 2008 Indian Institute of Toxicology Research, Lucknow, India



A three-day "Alexander Hollaender Workshop on Genetic Toxicology" was organized at the Indian Institute of Toxicology Research (IITR), Lucknow, India from December 15-17, 2008 as a satellite event to the 14th Alexander Hollaender Course organized at IICB, Kolkata. The workshop brought together experts working in the field of genetic toxicology from India as well as abroad. The goal of the workshop was to impart hands-on training to researchers on different techniques used in genetic toxicology. During the inaugural session, Dr. Ashwani Kumar, Director IITR, highlighted the scientific contributions of Alexander Hollaender and welcomed all the participants. The Workshop was inaugurated by Prof. Diana Anderson, Established Chair, University of Bradford, U.K. During her inaugural speech, she stressed the importance of such workshops, especially with respect to creating trained manpower in the area of genetic toxicology in India. Prof. Anderson also released the "Protocol Manual" at this occasion. Dr. P.S. Chauhan, Chairman, Research Council, IITR, while addressing the gathering, lauded the effort of IITR in holding the prestigious Alexander Hollaender Workshop for the young scientists and researchers in India. He emphasized that such workshops shall not only fulfill the dream of Prof. Hollaender but also help the Indian scientists and research students to learn firsthand different techniques in genetic toxicology and their implementation to understand the mechanism of DNA damage. He also shared his scientific experience and told about the history of genetic toxicology. Prof. Alok Dhawan, Co-Director, AHW, in his speech thanked Dr. David DeMarini, Prof. William Au, and all those who helped in organizing the Workshop at IITR, India.

After the inauguration, the first lecture was delivered by Dr. Nicole Weiland, Switzerland on the Ames test. She discussed the conventional Salmonella (Ames) test as well as a microplate format. The mutagenicity of compound in the new liquid microplate format was measured by adding a pH indicator medium to the treated bacteria, which showed a change in color after 48 hours of incubation. All the participants were then brought to the laboratory where they conducted the Ames test on their own and got an opportunity to see their results on the last day.

The second lecture was delivered by Dr. S. K. Rath, Central Drug Research Institute, Lucknow, India, on the micronucleus (MN) assay. He discussed both the in vivo and in vitro assay after a brief introduction of how micronuclei are formed. This was followed by a laboratory exercise where the students conducted the MN assay themselves in the presence of Dr. Rath and Prof. Alok Dhawan, IITR, Lucknow. The models chosen were mouse bone marrow for in vivo and human lymphocytes for in vitro. Dr. Sepideh Arbabi, Tehran, Iran gave a lecture on the role of Biomarkers in Cancer and presented data on breast cancer patients from Iran.

On the second day, Dr. S. K. Rath, CDRI, Lucknow, India gave a lecture on the chromosomal aberration (CA) assay and its role in regulatory toxicology. He dwelled in detail of how CAs are formed and the different diseases and exposure scenarios in which CAs have been monitored and were found to be linked to the exposure. After the lecture session, participants performed the in vivo and in vitro CA test in mouse and human lymphocytes.

Prof. Alok Dhawan, IITR, Lucknow, India then gave a lecture on the in vivo and in vitro comet assay in which he highlighted the technique and how it has been used worldwide in different models, including human monitoring. He also addressed the technical points, advantages and disadvantages of the assay, and its regulatory status. Prof. Diana Anderson, University of Bradford, UK in her lecture discussed the application of the comet assay, especially the sperm comet assay. Prof. Keshav Singh, Roswell Park Cancer Institute, USA spoke on the significance of mitochondrial DNA in toxicological studies and presented his data on how mitochondrial DNA could reveal much more than nuclear DNA both in terms of sex-liked diseases as well as toxicity of chemicals. The participants subsequently performed the comet assay in different tissues of mouse as well as in human lymphocytes. The participants spent the third day in the lab discussing their experimental results and seeking clarifications from the faculty.

The Chief Guest of the Valedictory session was Prof. Hasan Mukhtar, University of Wisconsin, USA. Dr. Ashwani Kumar, Prof. Alok Dhawan, Dr. Mukul Das, Dr. Rishi Shankar, Dr. S.K. Rath were also present. Dr. Ashwani Kumar thanked

the committee for organizing a laboratory workshop in the important area of genetic toxicology. Prof. Hasan Mukhtar also shared his experience and emphasized the importance of such workshops in creating a trained human resource in genetic toxicology. He then distributed the certificates of participation. Prof. Alok Dhawan thanked all the faculty members as well as the participants for their cooperation in making this workshop a success. He also expressed his gratitude to IAEMS for its support and assured the participants that more such workshops shall be organized in the future.

Alok Dhawan
Indian Institute of Toxicology Research, Lucknow India

Report of the 14th Alexander Hollaender Course held in Kolkata and Lucknow, India

The Alexander Hollaender course (AHC) is organized every year in countries where environmental mutagenesis and health issues are of major concerns. This time, the "14th Alexander Hollaender Course on Genetic Toxicology: Genomic and Proteomic Approaches" and a Special Workshop on "Arsenic Exposure Assessment" was held on 10 – 12th December, 2008 under the auspices of the Indian Institute of Chemical, Biology, Kolkata, India. This is the first occasion on which this prestigious event has been hosted in India. The course and workshop were intended to review the advances in environmental mutagenesis and health.

The lecture course in Kolkata was well represented by scientists in the relevant fields and generated much interest in academic circles all over India. Invited speakers from different corners of the globe attended this conference, including Dr. David DeMarini (U.S. EPA, USA), Dr. Stefano Bonassi (NCRI, Italy), Dr. Christopher States (University of Louisville, USA), and Dr. Brenda Eskenazi (UC-Berkeley, USA). There were 29 invited speakers in all, from India, Japan, Germany, UK, USA, Thailand, etc., and 38 participants took part in the course and were both from India as well as abroad. The program got off to a flying start through a colorful inauguration program held in Meghnad Saha Auditorium in Central Glass and Ceramic Research Institute that was chaired by Dr. Siddhartha Roy (Director, IICB), Dr. H. S. Maiti (Director, CGCRI), Dr. David DeMarini (President, IAEMS), Dr. Ashok K. Giri (Organizing-Secretary, 14th AHC), and Dr. A. B. Prasad (President, EMSI).

There were 9 sessions in all, including 7 lecture sessions, 1 poster session, and the concluding session. Eminent scientists addressed key issues in the field of genetic toxicology, such as "Population Monitoring for Health Hazards," "Mutagenic Hazards of Environmental Toxic Substances," "Current and Novel Mutagenic Assays," "Mechanisms of Mutagenesis and Carcinogenesis," "Genomic and Proteomic Approaches in Genetic Toxicology", "Special Workshop on Arsenic Exposure Assessment," and "Environmental Mutagens and Health Research."

A poster session was held in this course depicting the ongoing work in the field of genetic toxicology in the present day, and 27 out of the 38 participants presented their posters. A great variety in the study topics, novelty in the methodologies, as well as striking results could be seen. The entire session was highly appreciated by the judges, invited speakers, and participants. Several posters were of a very high quality and there was a keen contest for the best posters awards, which were financially support by Dr. A. (Ben) Aidoo at NCTR, Jefferson, AR, USA. Four posters were given awards: Dr. Prtiha Ghosh and Dr. Sarmishtha Chanda were awarded $150 each, whereas Mr. Mayukh Banerjee and Dr. Sepideh Arbabi Bidgoli were awarded $100 each for their presentations along with certificates. Awards were presented to the winners by Dr. David DeMarini during the concluding session.

From the discussions ensuing during the concluding session, the following recommendations were made for future research:

- Development of a reliable field test kit for determining arsenic concentration in water and other samples is urgently required.
- It would be very important and interesting to see if arsenic has the potential to be a germ-cell mutagen.
- Nanomaterial toxicology presents unique problems and needs to be addressed immediately with new strategies and novel methodologies need to be developed towards that end.
- "omics" technologies are very powerful tools for new-age toxicologists and provides the worker with the unique opportunities to seek out specific transcriptomic or proteomic signatures for any particular toxic material in real time
- Methods for the assessment of genotoxic damage beyond the comet assay need to be developed.

After the academic session was over, all the participants enjoyed a city tour and a grand banquet party on the river Ganges on the evening of 12th December. All in all, the 14th Alexander Hollaender Course was a great success from every conceivable point of view.

6th Pan African Environmental Mutagen Society Conference

The 6th Pan African Environmental Mutagen Society Conference (PAEMS 2008) was held in Cape Town, South Africa at the Cape Town International Convention Centre from November 3-5, 2008. The PAEMS 2008 conference follows on successful scientific meetings that were held in different African countries since 1993.

Meetings have taken place every3-4 years, in Egypt (1993 and 2003), South Africa (1996), Zimbabwe (1999) and Morocco (2005). The Organizing Committee of the PAEMS 2008 conference consisted of 14 members, mainly scientists from South Africa, with Dr. Hester Vismer as the Chairperson.

More than one hundred delegates from 21 countries attended PAEMS 2008. Ten countries from Africa were represented and included Egypt, Tunisia, Cameroon, Nigeria, Morocco, Zimbabwe, Lesotho, Botswana, South Africa and Mauritius. The other countries that were represented were Croatia, Slovakia, Poland, Russia, Switzerland, India, France, Germany, Italy, United Kingdom and USA.

Participants could participate in two satellite courses, one on Scientific Writing presented by Prof. John Leslie from Kansas State University, Kansas, USA, held prior to the conference on November 2, 2008, and the other on Current Trends in Genetic Toxicity Assessment following the conference on November 6, 2008. Dr. Jeanine Marnewick from South Africa coordinated the latter workshop and the presenters for this course were: Dr. David DeMarini (USA), Prof. Volker Mersch-Sundermann (Germany) and Prof. Wagida Anwar (Egypt). Each course was attended by thirty delegates, many of whom were sponsored.

Eminent scientists from all over the world were invited as plenary and invited speakers, including Prof. Wagida Anwar (Department of Community, Environmental and Occupational Medicine, Ain Shams University, Egypt); Prof. Theeshan Bahorun (Department of Biosciences, University of Mauritius, Republic of Mauritius); Dr. David DeMarini (US Environmental Protection Agency and President of the International Association of Environmental Mutagen Societies [IAEMS]); Prof. Wentzel Gelderblom (Director of the PROMEC Unit, Medical Research Council, South Africa); Prof. Mary Gulumian (National Institute for Occupational Health, South Africa); Prof. Michael Kew (Department ofMedicine, University of Cape Town, South Africa); Prof. Volker Mersch-Sundermann (Director of the Department of Environmental Health Sciences, University Medical Centre Freiburg, Freiburg, Germany); Prof. Iqbal Parker (Director of the International Centre for Genetic Engineering and Biotechnology, University of Cape Town, South Africa) and Prof. Chris Wild (Director-elect of the International Agency for Research on Cancer). These and other speakers set the scene for the topics that were discussed during nine sessions of the conference, i.e. Air-, Water-, Food-borne and Occupational Mutagens and Carcinogens; Antimutagenesis; Chemoprevention; Cancer; DNA Repair; Genomics; Genotoxic Risk Factors; Micro-organisms as Mutagens and Carcinogens; Molecular Mechanisms of Mutagenesis and Carcinogenesis; Radiation as a Mutagen and Carcinogen; Risk Assessment and Intervention Strategies. Sessions were well attended. The full program and book of abstracts can be viewed at: http://www.mrc.ac.za/promec/paems/.

Attended poster sessions formed an important part of the program. More than 50 posters were presented on the topics mentioned above. Three poster prizes, gold, silver and bronze, for scientists younger than 35 years, were awarded. Four independent poster judges, three international and one local judge, selected the winning posters. Poster prizes were sponsored by the PROMEC Unit of the Medical Research Council of South Africa. The gold poster award was won by Ms. Lorraine Moses for her work on “Real-time PCR analyses of fumonisin biosynthetic fum genes in Fusarium verticillioides MRC 826 subcultures”, the silver award by Ms. Hester-Marie Burger for her work on “The importance of using validated dietary assessment tools in human exposure studies: A case for mycotoxin exposure in the former Transkei region, South Africa” and the bronze award by Ms. Ivonne Schuhr “The antioxidant potential of oleic acid and effect on cell survival in carcinogenesis”, all three PhD students in South Africa.

The main goals of the PAEMS meetings are to spread new information and technologies concerning Environmental Mutagens and Carcinogens, to stimulate cooperation and to offer training programs with countries in Africa and countries aboard. For these reasons the organizers sincerely thanked the sponsors for their financial support, which enabled the Organizing Committee to present sponsorships to 38 participants, mainly to young scientists from Africa. Eighteen sponsorships were for the waiver of conference registration fees, 11 for registration and accommodations and nine were full sponsorships (conference registration, accommodation, air tickets and registration for satellite courses). The main sponsors for the conference were the Medical Research Council (Corporate, Diabetes Discovery Platform and Technology and Innovation Directorate), Southern African Regional Co-operation in Biochemistry, Molecular Biology and Biotechnology (SARBIO) in collaboration with the International Program in the Chemical Sciences (IPICS), the International Association of Environmental Mutagen Societies (IAEMS), the United Nations Educational, Scientific and Cultural Organization (UNESCO), the Cancer Association of South Africa (CANSA) and the Water Research Commission (South Africa). Sponsors were acknowledged during the conference, as well as in the book of abstracts and in the final program of the conference.

The General Assembly of the PAEMS took place during the conference and it is clear that the society faces many

challenges. The main concerns are the financial constraints and limitations of resources, difficulties in collecting membership fees from delegates in African countries, and the inconsistency of representation from different African countries, amongst other issues. The revised PAEMS constitution and membership forms were distributed amongst the members present at the General Assembly, as well to delegates attending the conference, to facilitate the sustainability of the PAEMS activities and to recruit new members.

The current Officers of the PAEMS are: Dr. Hester Vismer (President, South Africa), Prof. Wagida Anwar (President-Elect, Egypt), Prof. Fatima-Zahra Squali (Former President, Morocco), Prof. Alaoui Abdelilah (First Vice-President, Morocco), Prof. Roland Ndip (Second Vice-President, South Africa), Prof. Wentzel Gelderblom (Secretary General, South Africa), Dr. Gordon Shephard (Treasurer, South Africa).

For the first time in the history of the PAEMS, honorary membership was awarded to one of it's members. Prof. Wagida Anwar from Egypt received this distinction for her unsurpassed dedication to the Society since 1992.

Sincere thanks were given to the PAEMS 2008 Organizing Committee for the time and commitment that they have put into organizing this conference, and the sponsors for their financial support.

Dr. Hester F Vismer
Chairperson PAEMS 2008
Email: hester.vismer@mrc.ac.za

Prof. Wentzel Gelderblom (left) handing the Gold Award for the best poster to Ms Lorraine Moses (right).

Prof. Wagida Anwar (right) receiving honorary membership of the PAEMS during an award ceremony at the conference from Prof. Walter Marasas (left), a former President of the PAEMS.

14th Alexander Hollaender Course held in Kolkata and Lucknow, India

The inaugural session [From Left: Dr. Siddhartha Roy (Director, IICB), Dr. A. K. Giri (Organizing-Secretary, 14th AHC), Dr. H. S. Maiti (Director, CGCRI), Dr. David DeMarini (President, IAEMS) and Dr. A. B. Prasad (President, EMSI)

Faculty and Participants of 14th Alexander Hollaender Course

Future Course

15th Alexander Hollaender Course

Genomic-Environment Interactions and Genetic Toxicology

September 23–26/27, 2009, Astana, Kazakhstan

In Memorium

Barry Margolin passed away on the evening of January 28 after many years of illness. He grew up in the Bronx, New York, graduated from the City College of NY, and got his PhD in statistics from Harvard University. After a number of years on the faculty at Yale University, he was recruited in 1977 to NIEHS to work on the statistics of genetic toxicology tests. Genetic toxicology testing was a relatively new field at the time, and Barry knew almost nothing about biology. That didn't stop him, or even slow him down, and with his curiosity and brilliant mind he quickly learned about bacterial plate tests and fluctuation tests, Drosophila tests, in vitro chromosome aberration and SCE tests, bone marrow MN tests, transgenic mouse tests, etc.

He left NIEHS in 1987 to become Professor and Chairman of the Department of Biostatistics at UNC-Chapel Hill, where he became involved in statistical issues relating to epidemiology studies and clinical trials, but his main research interests were still with genetic toxicology.

Based on his publications, presentations, and comments at meetings he rapidly became the premier world expert in the statistics of genetic toxicology tests, and was much sought after for his comments and guidance on the design of genetic toxicity tests and inter-laboratory validation programs, and approaches to analyzing new types of data. Barry was not the type of person you went to if you wanted to find out if a particular response was statistically significant, or whether the p value was .05, .01, or .001. In fact, he would be the first to say that if the response was obvious, you do not need statistics. He was the person to consult if you wanted to learn about the parameters of a test procedure, such as the sources of error and contributions of each of those sources to the test variability, or about minimum or optimum sample sizes, or how to model a dose-response.

Barry had an always-present smile and easygoing manner, and a sense of humor, and it was a pleasure to collaborate with him. He was open about what he knew, and even more open about what he didn't know, and had no hesitation to inviting colleagues to participate in projects if he felt that they would bring a strength or a skill that he didn't have. He was an excellent teacher, and had the patience to explain statistical issues so that someone with no statistical training would easily understand. However, he had no patience for people who didn't know as much they insisted they knew, but was always even tempered, even when disagreeing with someone. At meetings, he always had the softest voice, yet, like in the commercials, when Barry started to make a comment or ask a question, everybody listened and heard what he had to say.

It was a unique experience to collaborate with Barry on a manuscript. He was not the fastest writer in the world, but he made up for it by writing exceptionally well. He would write the statistical aspects of the manuscript and I would write the biological. Because we were both wordsmiths, the manuscript was constantly in flux. When we got to the point where we felt it was finally finished, we would sit down at the table together and go over the text, sentence by sentence – does it say what we want it to say? Did we use the best words? Would it read better this way or that? And after we finished with the sentences, we did the same with the paragraphs – does it say what we want it to say? Will it fit better here, or there? It slowed things down, but he wouldn't submit a manuscript without first going through that process.

Barry was a fellow of the American Statistical Association, a member of the Institute of Mathematical Statistics, the International Statistical Institute, the Environmental Mutagen Society, and the Genetics and Environmental Mutagen Society. He served for many years as the statistical editor for *Environmental and Molecular Mutagenesis*, and was the recipient of best paper of the year awards from the American Statistical Association, in 1981 and 1983, for publications on statistical parameters and sources of variability of the Salmonella mutagenicity assay.

Barry stepped down from the Chair at UNC in 1987 and retired a few months later because of his health issues. However, for the next few years he still received invitations to speak at conferences, and kept his interests in genetic toxicology. Despite all his problems, he never lost his wonderful sense of humor. However, he was also fully aware of his problems and limitations, and there was always the hint of regret for the things, both physical and mental, that he no longer could do.

Barry was a vital member of the genetic toxicology community during its most active period and made major contributions to its growth and maturation. For those of us who knew and worked with him, he will be fondly remembered and sorely missed.

Errol Zeiger

EMS Announcements

FACE TO FACE

Upcoming Meetings

For Continuous updates on additional Meetings See our EMS Web site at
http://www.ems-us.org/meetings/other_meetings/index.asp.

40th Annual Meeting
EMS Society
Genomics in the Environmental Century

October 24 to 28, 2009

St Louis Missouri

GORDON RESEARCH CONFERENCE

Mutagenesis

August 1-6, 2010

Colby College

Waterville, ME

Chair:
Nancy Maizels

Vice Chair:
Bruce Demple

EMS Election News

One of the many benefits of being an EMS member is electing the leadership for the Society. I am pleased to present to you the elected leaders who will assume their office after the 2009 meeting in St. Louis.

President-elect, **Jeffrey Schwartz, Ph.D,**
University of Washington

The following members will serve as ***Councilors***:

Andrew B. Buermeyer, Ph.D.,
Oregon State University

Krista L. Dobo,
Pfizer Global R&D

Patricia A. Escobar, Ph.D.,
Boehringer Ingelheim Pharmaceuticals

Francesco Marchetti, Ph.D.,
Lawrence Berkeley National Laboratory

Thomas E. Wilson, M.D., Ph.D.,
University of Michigan

EMS appreciates the willingness of all candidates to serve

Martina Veigl, Ph.D.
Nominating Committee Chairperson

EMS Officers:

President: Priscilla Cooper
President-Elect: Michael Plewa
Past President: Andrew Wyrobek
Secretary: Suzanne Morris
Treasurer: Barbara Shane
Past Past President: Martina Veigl

EMS Councilors:

Marilyn Aardema
P.J. Brooks
Bevin Engelward
Olga Kovalchuk
Patricia Ostrosky-Wegman
John Tainer
Jonathan Ward
Carole Yauk
William Baird
James Carney
Randy Jirtle
Ofelia Olivero
Miriam Poirier
Karen Vasquez
Paul White (EMM Editor)

2009-2010 ENVIRONMENTAL MUTAGEN SOCIETY COMMITTEES

Awards & Honors: A. Buermeyer, P.J. Brooks, B. Engelward, R. Elespuru, T. Nohmi, J. Sasaki
Education, Student and New Investigator Affairs: O. Kovalchuk, C. Klein, A. Abu-Shakra, M. Crosby, D. DeMarini, G. Gentile, J. Gentile, R. Heflich, K. Hill, S. Morris, J. Mulvihill, O. Olivero, J. Pluth, A. Polyzos, P. Stambrook, E. Tiedtke H. van Gijssel, J. Ward, P. White, R. Young
Executive Board: PJ Brooks, P. Cooper, S. Morris, M. Plewa, B. Shane, M. Veigl, J. Ward, A. Wyrobek
FASEB Representatives: Board Representative: L. Niedernhofer; Peer Review Subcommittee: K. Vasquez; Publications and Communications Committee: R. Heflich,
Finance: R. Snyder, A. Kligerman, D. Eastmond, J. Fuscoe, B. Bhaskar Gollapudi, B. Parsons, M. Plewa, B. Shane
Hollaender: M. Ljungman, A. Abu-Shakra, B. Aidoo, W. Au, W. Baird, P. Cooper, M. Crosby, D. DeMarini, J. Gentile, H. Groot de Restrepo, P. Hanawalt, N. Holland, G. Krishna, M. Manjanatha, J. Nath, P. Ostrosky-Wegman, L. Ribeiro, D. Shankel, P. Stambrook, M. Waters, P. White
2009 Program: M. Plewa, D. Anderson, W. Au, J. Bishop, M. Coleman, D. Eastmond, R. Elespuru, B. Engelward, P. Escobar, G. Gentile, J. Gentile, H. Groot de Restrepo, P. Hanawalt, K. Hill, R. Jirtle, N. Keshava, M. Ljungman, B. Mahadevan, M. Manjanatha, T. Masson, L. Niedernhofer, O. Olivero, P. Opresko, J. Pluth, D. Sedwick, B. Shane, M. Smith, R. Snyder, S. Sommer, R. Sram, J. Sweasy, K. Vasquez, M. Veigl, E. Wagner, J. Ward, R. Wood, C. Yauk, R. Young
Membership & Professional Development: K. Williams, O. Olivero, S. Aaron, D. Benz, S. Bonassi, J. Chen, M. Donner, M. Edelbrock, B. Ford, D. Kirkland, C. Klein, S. Morris, R. von Borstel, E. Von Halle, J. Ward, A. Wyrobek,
Nominating: P. J. Stambrook, P.J. Brooks, S. Galloway, J. Sweasy, M. Veigl, A. Wyrobek, C. Yauk
Parliamentarian: G. Erexson
Publication Policy: M. Manjanatha, P. Lee, R. D. Benz, B. Ford, K. Hill, C. Klein, S. Morris, J. Mulvihill, L. Niedernhofer, P. Ostrosky-Wegman, B. Parsons, J. Schwartz, C. Torres-Ramos, Public Relations: M. Lippert, S. Dertinger, P. Arenaz, A. Brooks, L. Claxton, S. Galloway, R. Heflich, G. Hoffmann, S. Majumdar, M. Shelby, P. Stambrook, V. Wilson, E. Zeiger

Special Interest Group Leaders: DNA Repair & Mutagenic Mechanisms: M. Ljungman, J. Sweasy; Epigenetics: C. Klein, O. Kovalchuk; Heritable Mutations and Disease: S. Sommer, C. Yauk; Molecular Epidemiology: M. Poirier, R. Sram; New Technologies: B. Mahadevan, P. Escobar; Risk Assessment: D. Eastmond, N. Keshava; Transgenics & In Vivo Mutagenesis: K. Hill, M. Manjanatha; Women in the EMS: G. Gentile, J. Pluth

Make a difference in EMS! Find a committee, sign up, and be active!

EMS Newsletter
Environmental Mutagen Society
1821 Michael Faraday Drive, Suite 300
Reston, VA 20190

Thanks to everyone who made a submission