GREG STUART

GEMS

Genetics and Environmental Mutagenesis Society

23rd Annual Fall Meeting

"Genetics of Aging"

Wednesday, October 26th, 2005

UNC Friday Center
Chapel Hill, NC

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ANNUAL FALL MEETING

October 26th, 2005

Friday Center of the University of North Carolina

"Genetics of Aging"

8:00 Registration with Continental Breakfast

8:30 Welcome: Dr. Barbara Shane, GEMS President
Speaker Introduction: Dr. Les Recio, GEMS President-Elect

8:45 **Dr.Olga Sedelnikova**, National Cancer Institute
"Aging, genomic Integrity, and H2AX"

9:30 **Dr. Christopher Corton**, U.S. Environmental Protection Agency
"Nuclear Receptors and Longevity"

10:15 Break/Refreshments/Visit Sponsor Booths

10:45 Poster presentations

11:30 Talks by student/postdoc/technician
"Tumor Promotion as a Target for Chemoprevention of Lung Cancer" S. Dance
"Biomarkers of Oxidative Damage due to PCB Exposure in Corbicula Clams in the Lab and the Field (Brier Creek, NC)" D.W. Lehmann

12:00 Lunch
1:00 Business Meeting

1:30 Talks by student/postdoc/technician
"Medaka Fish as a Model for Developmental Ethanol Toxicity: Investigation of Windows of Heightened Sensitivity" S. Oxendine
"A New Model for DNA Methylation-Dependent Epigenetic Regulation of Gene Expression" A.G. Rivenbark
"General Enhancement of Mutagenic Potency of Various Mutagens Due to Deleted Genes in the UVRB Strains TA98 and TA100 of Salmonella Compared with Strains Containing Only a Point Mutation in UVRB" C. Swartz
"Mutagenesis by Exocyclic Alkylamino Purine Adducts in Escherichia Coli and HEK293 Cells" D. Upton

2:30 **Dr. Norman Sharpless**, UNC-CH
"INK4a/ARF, Senescence, Cancer and Aging"
3:30 Awards
3:45 Reception

Dear GEMS Members,

Welcome to our 23nd Annual Fall Meeting. The key person responsible for organizing the meetings is our current President-Elect, Leslie Recio, who has done a great job in putting together an outstanding group of speakers on a very interesting and timely topic "Genetics of Aging". I can't think of a better individual to take over as president in the coming year.

I would like to extend a warm thank you and appreciation to our corporate sponsors. We could not hold these meetings without the very generous financial assistance of our corporate sponsors, many of whom have been long time supporters of our organization. Our sponsors will have tables set up to exhibit their most up-to date products and laboratory equipment at the meeting. We have set aside a special time slot during the meeting for you to interact with our corporate sponsors and we encourage each of you to show your appreciation for their continued support of GEMS by speaking with company representatives. Please discuss with them your laboratory needs and products of interest, and thank them for their continuing support.

Organizing the Fall meeting requires the help of a dedicated cadre of volunteers. One of the exciting aspects of being a member of GEMS and serving on the Board is the great group of people with whom you interact. I encourage each of you to get involved and volunteer. Having served on the Board as a Councilor for 3 years and then as President-elect and President, I can say that I have very much enjoyed working with this great group of people. I especially want to thank Susan Ross, our Treasurer, and Gloria Jahnke, our Secretary, who have done an outstanding job throughout the year, and Errol Zeiger for his work on the GEMS By-Laws. GEMS thanks NIEHS for their continuing support of our meetings. We also acknowledge the support of CIIT Centers for Health Research for hosting our bimonthly Board meetings and supplying the meeting badges and registration materials for a number of years. I also want to express my appreciation to our other current Board members, Mary Smith, Jayne Boyer, Janice Allen and Alan Townsend, as well as our outgoing Board members, Stephen Little, Greg Stuart and Witold Winnik. We especially appreciate the time and effort that Stephen Little, has devoted to GEMS. He has been the newsletter editor for more than five years and helped in the compilation of the program booklet for many of the Fall meetings.

There are a number of other people who deserve special recognition for their constant efforts on behalf of GEMS. These include Carolyn Harris, who has maintained our membership list for several years, Frank Stack, our dedicated Webmaster who has spent many hours setting up our blog and on-line voting capabilities, Kristine Witt, who arranges for the award plaques and coffee mugs for both the Fall and Spring meetings, and Rob Blackman, who has taken over as our Corporate Sponsor Representative. Rob has done an enormous amount to work encouraging our cooperate sponsors to exhibit at the meeting despite the many cutbacks that companies have adopted in recent years. This is another reason for you to visit their booths.

One of the most important activities of GEMS is to encourage the growth and development of our young scientists. The GEMS meetings offer them the opportunity to learn something new about a scientific area in which they may not be very familiar and permit their interaction with colleagues and other mentors, and I would encourage our junior colleagues to take full advantage of this opportunity. As usual, we will have the Best Student Talk and the Best Poster awards. This year, we have divided the poster awards into 3 categories: student, postdoctoral, and technician, to acknowledge their separate, but important, contributions to our society. The winner of the Best Student Talk serves on the Board as the Student Representative. Last year we had two students, Beth Van Emburgh from Wake Forest University and Roberta Clewell from CIIT Centers for Health Research, on the Board as they shared the "Best Student Talk" award at the last fall meeting. We appreciate their attendance and input at our Board meetings.

I have very much enjoyed my 5-year tenure as a member of the GEMS Board. It has been an honor and privilege to serve as your President

Barbara S. Shane
GEMS President

A special thanks to NIEHS and CIIT Centers for Health Research for their financial support

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INVITED SPEAKER PRESENTATIONS

S1

AGING, GENOMIC INTEGRITY, AND H2AX

O. A. Sedelnikova, I. Horikawa, A. Nakamura, D. B. Zimonjic, N. C. Popescu, J. C Barrett and W. M. Bonner. Laboratory of Molecular Pharmacology, Laboratory of Biosystems and Cancer, Laboratory of Experimental Carcinogenesis, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20892

The DNA double-strand break (DSB) plays dual role in genome integrity. On the one hand DSB formation is essential in several normal cellular processes, but on the other, accidental DSB formation may lead to permanent genomic damage, to cancer, and to death. A universal cellular response to a DSB is the phosphorylation of several thousand molecules of a histone protein H2AX to form γ-H2AX in the chromatin flanking the break site. Immunocytochemical analysis with anti-γ-H2AX reveals the number and position of each nuclear DSB as a focus of γ-H2AX (γ- focus). Most cells of tumor lines generally exhibit >3 foci per nucleus, but some tumor lines exhibit many fold more. Unstressed normal human cells exhibit increasing numbers of γ-foci as they senescence and similar increases in the incidence of γ-foci occur in both the somatic and germline tissues of mice as they age. The senescence-related γ-foci have multiple origins, telomeric and non-telomeric, which may result from oxidative damage. DNA DSB repair proteins accumulate at these foci, indicating that they are sites of DSBs and repair. When DSBs are generated in human cell cultures with ionizing radiation, their numbers are similar in cells at different stages of senescence but the rates of dimensional focal growth and accumulation of DSB-repair proteins are substantially slower in late passage cells and even slower in cells taken from Werner syndrome patients, who exhibit premature aging and genome instability. Thus, the ability of cells to repair DNA damage may decrease with age. Radiation-induced DSBs are repaired in young and old cultures, but age-related DSBs are not. The results demonstrate that mammalian cells aging in vitro and in vivo accumulate unrepairable DSB-containing lesions that may play a causal role in aging.

S2

NUCLEAR RECEPTORS AND LONGEVITY

J.C. Corton, Toxicogenomics Program, NHEERL, US Environmental Protection Agency, RTP, NC 27711.

Dietary restriction of calories (caloric restriction [CR]) increases longevity in phylogenetically diverse species. CR retards or prevents age-dependent deterioration of tissues and an array of spontaneous and chemically induced diseases including cardiovascular disease, diabetes, and cancer. An understanding of the molecular mechanisms that underlie the beneficial effects of CR will help identify novel dietary, pharmacological, and lifestyle strategies for slowing the rate of aging and preventing these diseases as well as identify factors which modulate chemical toxicity. There is increasing evidence that transcriptional co-activator proteins, peroxisome proliferator-activated receptor (PPAR) γ coactivator 1 (PGC-1) α and β, and regulated nuclear receptors (NR) mediate the phenotypic changes found in models of longevity which include rodent CR models and mouse mutants in which insulin and/or insulin-like growth factor-I signaling is attenuated. PGC-1α and PGC-1β regulate the ligand-dependent and -independent activation of a large number of NR including PPARα and constitutive activated receptor (CAR). These NR regulate genes involved in nutrient and xenobiotic transport and metabolism as well as resistance to stress. CR reverses age-dependent decreases in PGC-1α, PPARα, and regulated genes. Strategies that target one or multiple PGC-1-regulated NR could be used to mimic the beneficial health effects found in models of longevity.

S3

***INK4A/ARF* EXPRESSION, CANCER AND AGING**

Norman E. Sharpless, Departments of Medicine and Genetics, The Lineberger Cancer Center, The University of North Carolina School of Medicine, Chapel Hill, NC 27599

The *Ink4a/Arf* locus encodes two tumor suppressor molecules, $p16^{INK4a}$ and ARF, which are principal mediators of cellular senescence. To study the links between senescence and aging *in vivo*, we examined *Ink4a/Arf* expression in rodent models of aging. We show that expression of $p16^{INK4a}$ and ARF markedly increases in almost all rodent tissues with advancing age, while there is little or no change in the expression of other related cell cycle inhibitors. The increase in expression is restricted to well-defined compartments within each organ studied and occurs in both epithelial and stromal cells of diverse lineages. The age-associated increase in expression of $p16^{INK4a}$ and ARF is attenuated in the kidney, ovary and heart by caloric restriction, and this decrease correlates with diminished expression of an *in vivo* marker of senescence, as well as decreased pathology of those organs. Moreover, we show that germline $p16^{INK4a}$ deficiency in mice is associated with enhanced proliferation and function of certain long-term renewing tissue compartments such as the pancreatic beta cell, neural stem cells and hematopoietic stem cells in old mice. Therefore, the age-induced increase of $p16^{INK4a}$ appears to limit repair and regeneration in these tissues in old but not young rodents. These data suggest that expression of the *Ink4a/Arf* tumor suppressor locus is a biomarker and effector of mammalian aging.

ORAL PRESENTATIONS

T1

TUMOR PROMOTION AS A TARGET FOR CHEMOPREVENTION OF LUNG CANCER

S. Dance, J. Moore, N. Kock, A. Townsend, and M. Miller, WFUSM, Winston-Salem, NC 27157

Our laboratory has developed a bitransgenic mouse lung tumor model that constitutively expresses the human Ki-*ras*^{G12C} allele in a doxycycline (DOX)-inducible and lung-specific manner. This model appears to recapitulate the earliest stages of lung tumorigenesis as the lung lesions that developed were predominantly benign hyperplastic lesions and adenomas. We have initiated studies using this animal model to determine the effects of non-steroidal anti-inflammatory drugs (NSAIDs) on lung tumor progression. One week after the initiation of DOX administration (500 μg/ml in the drinking water) to up-regulate mutant RAS transgene expression, mice were treated with 6 weekly i.p. injections of the lung tumor promoter butylated hydroxytoluene (BHT, 200 mg/kg). Results obtained from short term studies in which mice were euthanized 24 hr after the last BHT injection suggest that when given in the diet, Sulindac can inhibit lung tumor progression. Co-treatment with DOX and BHT increased tumor multiplicity to 4.2±1.3 (n=6; 100% incidence) compared with treatment of DOX alone 2.5±0.5 (n=5; 100% incidence). Sulindac (100mg/kg) decreased tumor multiplicity in DOX/BHT mice to 2.0±2.0 (n=4; 75% incidence). Statistical analysis using ANOVA revealed that BHT caused a marginally significant increase in lung tumor multiplicity in DOX/BHT mice compared to DOX only mice (p=.073). Sulindac, however, was associated with a significant (p=0.033) chemopreventive effect by decreasing tumor multiplicity in BHT/DOX mice. Immunohistochemical analysis demonstrated increased COX-2 and iNOS expression in the focal hyperplastic lesions within the lung of the DOX/BHT mice. The DOX only mice had hyperplastic lung foci without associated COX-2 or iNOS expression while the BHT only mice (0% incidence) exhibited COX-2 and iNOS expression confined to alveolar macrophages. Neither COX-2 nor iNOS were expressed in Clara and alveolar type II cells in Sulindac/DOX/BHT mice. The data suggest that even at a very early time-point, the NSAID Sulindac has an inhibitory effect on the promotional phase of lung carcinogenesis, resulting in decreased tumor multiplicity.

T2

BIOMARKERS OF OXIDATIVE DAMAGE DUE TO PCB EXPOSURE IN *CORBICULA* CLAMS IN THE LAB AND THE FIELD (BRIER CREEK, NC)

D.W. Lehmann[1], D. Shea[1], J. Levine[2], J.M. Law[2]
[1] Department of Environmental and Molecular Toxicology, NC State University
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Brier Creek, a NPL listed site in central North Carolina, is heavily contaminated with polychlorinated biphenyls (PCBs) and related compounds. The impacted zone spreads from the source through a recreational use reservoir (Lake Crabtree). Initial EPA testing has determined that PCB, dioxin, and furan levels are at or above limits with even a single meal of fish (600g)

per month. Our research aims to define the oxidative stress on Asiatic Clams (*Corbicula*) in order to determine the cellular and biochemical changes that result from exposure to aged PCBs in the environment. Oxidative activity due to PCBs has been shown to cause damage to cellular macromolecules leading to cancer, reproductive changes, and other maladies. *Corbicula* were chosen due to their commonality, competition with native, endangered species, sediment association, and use in biomarker studies around the world. Our specific hypothesis that exposure to environmentally relevant PCB concentrations causes oxidative damage in bivalves is supported by our results from both field and controlled laboratory exposures. PCB exposure caused significant changes in total oxygen scavenging capacity, glutathione systems, and low molecular weight lipid soluble antioxidant levels which correlated to distance from source in *Corbicula* sp. Histopathology also showed severe changes consistent with cell membrane damage including edema, multifocal necrosis, as well as marked gonadal atrophy. By assaying different levels of cellular organization and comparing the variety of effects, interpretation can be made regarding population effects. Controlled laboratory exposures showed increases in antioxidant systems due to exposure to Arochlor 1260. In field studies, there was a decrease in low molecular weight antioxidants and related enzyme systems, as well as TOSCA. Interestingly, TOSCA showed a mild protective effect at polluted downstream sites. This is indicative of the need to overcome antioxidant defenses in order to cause overt cellular damage.

T3

MEDAKA FISH AS A MODEL FOR DEVELOPMENTAL ETHANOL TOXICITY: INVESTIGATION OF WINDOWS OF HEIGHTENED SENSITIVITY

S. Oxendine[1,2], D.E. Hinton[3], J. Cowden[1] and S. Padilla[1]
[1] U.S. EPA, RTP; [2] Curr. in Toxicol., UNC-CH, Chapel Hill; [3] Nicholas School of the Environ., Duke Univ., Durham, NC.

Ethanol (EtOH) is a well-known developmental toxicant that produces a range of abnormal phenotypes. While the toxic potential of developmental EtOH exposure is well characterized, the effect of the timing of exposure on the extent of toxicity remains unknown. Fish models such as the Japanese medaka, *Oryzias latipes,* provide a convenient system for investigating the effects of developmental EtOH exposure. In this study, medaka embryo toxicity tests were used to assess temporal variations in EtOH toxicity. Fertilized eggs were collected at the 64-cell stage and incubated during early, middle or late gestation (*e.g.*, 0-3, 3-6 or 6-9 days post fertilization) with various sublethal concentrations of EtOH (0.1, 0.5 or 1%) (n=20 per dose). Viable embryos were then photographed on the day of hatching and time to hatch, outer eye distance and total body length were used to assess toxicity. Dose-related hatching delays and growth inhibition were consistently observed in treated embryos (*e.g.*, approximately 45% of controls hatched by day 9, whereas only 17% of embryos treated with 1% EtOH hatched by that time). Hatching delays were most pronounced when exposures occurred early in development. EtOH-induced growth inhibition, however, appeared to be most pronounced when exposures occurred late in development (*e.g.*, 1% EtOH only decreased total body length by 8.8% if exposure occurred on days 0-3, but that decrement doubled to 17.6% when exposure occurred on days 6-9). The observed temporal variations in EtOH-induced growth inhibition may be related to stage-specific pharmacokinetic effects, as the EtOH dose was slightly higher in embryos treated late in development when compared to those treated earlier in development. In general, these data suggest that critical periods for heightened sensitivity to developmental EtOH exposure may vary

according to the endpoint used to assess toxicity. *This is an abstract of a proposed presentation and does not reflect EPA policy.*

T4

A NEW MODEL FOR DNA METHYLATION-DEPENDENT EPIGENETIC REGULATION OF GENE EXPRESSION

A.G. Rivenbark[1], W. D. Jones[2], and W. B. Coleman[1]
[1] Curriculum in Toxicology, Department of Pathology and Laboratory Medicine, UNC Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC 27599. [2] Expression Analysis, Durham, NC 27713.

To identify epigenetically-regulated genes in breast cancer, MCF-7 cells were exposed to 250nM 5aza or 5aza + 50nM TSA for 3 weeks followed by a 5 week recovery period after treatment withdrawal and gene expression patterns were examined by microarray analysis. We identified 20 genes that are associated with a ≥2-fold increase in expression in response to the demethylating treatment but returned to control levels after treatment withdrawal. RT-PCR verified that the genes identified were expressed at low or undetectable levels in control MCF-7 cells but increased expression in treated cells. Most of these putative epigentically-regulated genes in MCF-7 cells do not contain CpG islands. However, prototype CpG-deficient genes were shown to be methylation-sensitive (subject to CpG methylation and responsive to demethylating agents), suggesting that not all targets of DNA methylation in breast cancer conform to the conventionally-accepted definition of an epigenetically-regulated gene. We propose a new model for methylation-dependent regulation of gene expression and a new classification for methylation-sensitive genes based upon promoter CpG sequence characteristics, including genes with: (i) typical CpG features (CpG islands), (ii) intermediate CpG features (weak CpG islands), and (iii) atypical CpG features (no CpG islands). This new model recognizes that all promoter CpG dinucleotides represent legitimate targets for DNA methylation and suggests that the methylation of specific CpG dinucleotides in critical domains of regulatory regions can result in gene silencing. This model challenges the paradigm that methylation-sensitive genes will contain a conventional CpG island as the target of methylation in gene silencing.

T5

GENERAL ENHANCEMENT OF MUTAGENIC POTENCY OF VARIOUS MUTAGENS DUE TO DELETED GENES IN THE *ΔUVRB* STRAINS TA98 AND TA100 OF *SALMONELLA* COMPARED WITH STRAINS CONTAINING ONLY A POINT MUTATION IN *UVRB*

C. Swartz[1], N. Parks[2], R. Schaaper[3], and D. DeMarini[2]
[1]UNC, Chapel Hill, NC 27599, [2]ECD, USEPA, RTP, NC, 27711 and [3]LMG, NIEHS, RTP, NC 27709

The two most common strains used in Ames mutagenicity assays, TA98 and TA100, contain a *ΔuvrB* mutation designed to enhance the mutagenicity of compounds, presumably due to the loss of the nucleotide excision repair system. We showed previously that the *ΔuvrB* mutations in these strains resulted in the deletion of 47 genes in TA100 and 119 genes in TA98. Studies involving one of us (R.M.S.) in a *ΔuvrB* strain of *Escherichia coli* showed that deletion of

molybdenum cofactor biosynthesis genes, which are also deleted in TA98 and TA100 of *Salmonella* as part of their respective *ΔuvrB* mutations, enhanced the mutagenicity of the base analog N^6-hydroxylaminopurine (HAP). To explore the consequences of these gene deletions on mutagenesis in TA98 and TA100, we constructed homologues of TA98 and TA100 containing point mutations in *uvrB* or the molybdenum cofactor biosynthesis genes *moeA* or *moaA*. Using the plate-incorporation assay, we then tested in these strains the following mutagens, which were representative of various chemical classes: MX (a water disinfection byproduct), 1-nitropyrene, HAP, 2-amino-N^6-hydroxylaminopurine (AHAP), benzo(a)pyrene, 4-aminobenzene, 2-acetylaminofluorene, and Glu-P-1 (a heterocylic amine food mutagen). Consistent with previous studies in *E.coli*, HAP and AHAP were pure base substitution mutagens and were active only in the absence of a molybdenum cofactor biosynthesis gene. All other compounds assayed in this study required the absence of uvrB for enhanced mutagenicity. Potencies in point mutant strains compared to parent TA98 or TA100 varied depending on the compound and the identity of the mutated gene(s). Thus, the absence of 47 or 119 genes in TA100 and TA98, respectively, may enhance the mutagenic potency of a variety of mutagens compared to the potency such mutagens might exhibit in strains possessing these genes and having only a point mutation in *uvrB*. [Abstract does not necessarily reflect the policy of the US EPA.]

T6

MUTAGENESIS BY EXOCYCLIC ALKYLAMINO PURINE ADDUCTS IN *ESCHERICHIA COLI* AND HEK293 CELLS

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Exocyclic alkylamino purine adducts, including N^2-ethyldeoxyguanosine, N^2-isopropyldeoxyguanosine, and N^6-isopropyldeoxyadenosine, occur as a consequence of reactions of DNA with toxins such as the ethanol metabolite acetaldehyde, diisopropylnitrosamine, and diisopropyltriazene. However, there are few data addressing the biological consequences of these adducts when present in DNA. We hypothesized that N^2-ethyldeoxyguanosine and N^2-isopropyldeoxyguanosine are mutagenic lesions. Therefore, we assessed the mutagenicities of these single, chemically-synthesized exocyclic alkylamino adducts when placed site-specifically in the supF gene in the reporter plasmid pLSX and replicated in *Escherichia coli* and HEK293 cells. No significant difference in the mutant fraction of N^2-ethyldeoxyguanosine or N^2-isopropyldeoxyguanosine-containing vs. control constructs without deoxyuridine in the complementary strand was observed after replication in either HEK293 cells or *E. coli*. In *E. coli*, when deoxyuridine was present in the complementary strand, the mutant fractions for N^2-ethyldeoxyguanosine and N^2-isopropyldeoxyguanosine were 2.3- and 10-fold higher than control, respectively (0.6% for control, 1.4% for N^2-ethyldeoxyguanosine, 5.7% for N^2-isopropyldeoxyguanosine; $p = 0.04$ for N^2-ethyldeoxyguanosine, $p = 0.003$ for N^2-isopropyldeoxyguanosine). No significant difference in the mutant fraction of N^6-isopropyldeoxyadenosine-containing vs. control constructs was observed (0.6% for control, 1.2% for N^6-isopropyldeoxyadenosine; $p = 0.13$). In HEK293 cells, when deoxyuridine was present in the complementary strand, the mutant fractions for N^2-ethyldeoxyguanosine, N^2-isopropyldeoxyguanosine, and N^6-isopropyldeoxyadenosine were 6-, 4-, and 4-fold higher than control, respectively (1.00% for control, 6.47% for N^2-ethyldeoxyguanosine, 4.06% for N^2-

isopropyldeoxyguanosine, and 3.84% for N^6-isopropyldeoxyadenosine). The mutation spectra generated by the N^2-ethyl and -isopropyldeoxyguanosine adducts (adduct site-targeted GC:TA transversions, adduct site single base deletions, and single base deletions three bases 3' to the adduct) contrasts sharply with the mutation spectrum generated by O^6-ethyldeoxyguanosine (95% adduct site-targeted transitions). We conclude that N^2-ethyl and -isopropyldeoxyguanosine are mutagenic adducts in *Escherichia coli* and in HEK293 cells and that their mutation spectra differ markedly from that of O^6-ethyldeoxyguanosine.

P1

THE ROLE OF PHOSPHATIDYLSERINE AND CALCIUM IN THE REGULATION OF FACTOR IX_A

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Blood coagulation consists of two converging pathways, the extrinsic and intrinsic pathways. Factor IX_a activated by the intrinsic pathway forms the "intrinsic Xase complex" together with factor $VIII_a$ and Ca^{2+} on the platelet membrane surface. Factor IX_a plays a crucial role in blood coagulation, since absence of its activity results in the bleeding disorder, hemophilia B. The purpose of this project is to reveal new information about the regulation of factor IX_a by phospohatidylserine (PS). We will use a soluble form of PS, C6PS (1,2-dicaproyl-*sn*-glycero-3-phospho-L-serine) which is a unique and powerful tool that will allow me to examine specific binding interactions that would be difficult to study on a membrane surface.
It was reported that factor IX_a binds very tightly (K_d~12 nM) with PS containing membranes in the presence of Ca^{2+}. Recent data from the Lentz laboratory has shown that a soluble form of phosphatidylserine, C6PS, binds to factor IX_a (K_d~ 2 μM) in the presence of 5 mM calcium. My work to date has accomplished the following 1) Both proteolytic and amidolytic activity of factor IX_a is regulated by soluble PS in the presence of calcium 2) 2-3 mM calcium is optimal for both amidolytic and proteolytic activity of factor IX_a, 3) Circular dichroism (CD) spectrometry shows that both the CD-spectra and α -helicity of factor IX_a changes substantially with the addition of C6PS at 5 mM calcium. Fitting the plot of differential molar coefficient of factor IX_a at 208 nm with C6PS concentration gives a K_d of ~ 10 μM that matches well with the K_d obtained from fluorescence titration. My result proves that the tight PS binding (K_d~2-10μM) in factor IX_a regulates the structural changes while the weak PS binding site (K_d~130-160 μM) regulates the activity. All these results prove factor IX_a is regulated by soluble PS. (National Science Foundation HRD 9978874).

P2

STRAIN-SPECIFIC SENSITIVITY TO INDUCTION OF MURINE LUNG TUMORS FOLLOWING *IN UTERO* EXPOSURE TO 3-METHYLCHOLANTHRENE

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We previously demonstrated that different strains of fetal mice were more sensitive to lung tumor induction by 3-methylcholanthrene (MC) than were adults. Offspring from either a D2 x

B6D2F1 backcross or from parental Balb/c mice exhibited a similar high incidence of lung tumors 6 to 12 months after transplacental exposure to MC. We thus compared the effects of *in utero* treatment with MC on lung tumor induction in the offspring of intermediately susceptible Balb/c (Bc), resistant C57BL/6 (B6), and reciprocal crosses between these two strains. Pregnant mice were treated with 45 mg/kg of MC on day 17 of gestation. Tumor incidence, multiplicity, and Ki-*ras* mutational spectrum were determined in the offspring 12-18 months after birth. Tumor incidences in Bc mice and reciprocal crosses between the two strains were 83% and 100%, respectively, while B6 mice demonstrated a remarkable resistance to tumorigenesis, with a tumor incidence of 11%. Tumor multiplicities in Bc, B6Bc, BcB6, and B6 mice were 3.1 ± 3.2, 5.5 ± 3.4, 4.8 ± 2.9, and <0.1, respectively, counting only lesions that were discrete, individual nodules. Ki-*ras* mutations in the lung tumors, which occurred chiefly in the K^s allele (96%), were found in 79 to 81% of B6Bc and BcB6 mice, 64% of Bc mice, and 50% of B6 mice, with Val^{12}, Asp^{12}, and Arg^{13} mutations associated with more aggressive tumors. Expression of *Cyp1a1* and *Cyp1b1*, which are involved in the metabolic activation of MC, were examined by real time RT-PCR and similar kinetics of induction and decline of both RNAs were observed in all four strains. Maximal induction of both RNAs occurred 2 to 8 hrs after injection with MC in both organs in all 4 strains. *Cyp1a1* induction was 2 to 5 times greater in the fetal liver (7000- to 16,000-fold) than fetal lung (2000- to 6000-fold), and significantly higher than *Cyp1b1* induction, which ranged from 8- to 20-fold for both organs. The only significant strain-specific effect seen was a particularly poor induction of *Cyp1b1* in B6 mice, especially in the fetal lung. We also determined whether strain-specific differences in Phase II metabolism could account for the differences in fetal susceptibility. Using CDNB as a substrate, we found that treatment with MC had no effect on the levels of GST enzyme activity in either organ in any of the 4 strains of mice. Western blot analysis demonstrated very low levels of expression of *GST* isoforms , , , and in both lung and liver supernatants from all 4 strains. With the exception of *GST*μ, which showed a 3.5-fold induction in the lung of Bc mice 48 hrs after exposure, there were no significant effects seen among the other isozymes. DNA adduct formation and repair were also assessed at several time points between 1 to18 days (2 weeks post-natal) post-injection. Few differences were noted between the four strains. Collectively, while high *Cyp1a1* induction and low, uninducible levels of phase II conjugating enzymes probably accounts for the increased overall susceptibility of the fetus to transplacentally-induced tumor formation, metabolic differences among the 4 strains and differences in adduct formation could not account for differences in lung tumor susceptibility observed in B6 mice relative to the other strains. These results suggest that another dominantly acting genetic locus may influence lung tumorigenesis specifically following *in utero* exposure to carcinogens during fetal development. (Supported by EPA STAR grant R829428-01-0)
The research described in this article does not necessarily reflect the views of the Environmental Protection Agency.

P3

COMPARISON OF COMMERCIALLY AVAILABLE RNA AMPLIFICATION KITS

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Many molecular assays, such as microarrays, require relatively large quantities of RNA for hybridization. This is a major limitation when dealing with small quantities of mRNA obtained from small biopsies, primary cell cultures or laser capture microdissection (LCM). RNA amplification therefore becomes a necessary step when working with these limited samples. There are several commercially available amplification kits on the market, most of which use T7 RNA polymerase driven transcription of cDNA obtained from total RNA samples, to amplify large amounts of cRNA. These methods maintain a representation of the starting mRNA population and can yield 1000-fold or more amplification. In this study we evaluated four RNA amplification kits for overall amplification, consistency between technical replicates, consistency between 2-round amplification and 1-round amplification, and reliability of expression patterns when verified by real-time qPCR.

P4

CYTOTOXICITY OF ESTROGEN AND ITS CATECHOL METABOLITES IN CELLS EXPRESSING DIFFERENT CYTOCHROME P450 PHASE I ENZYMES

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Estrogens have been associated with the development of breast cancer in humans. There has recently been an increase in interest as to how metabolism of estrogen relates to breast carcinogenesis. The phase I cytochrome p450 enzymes (CYP) are thought to play a key role in the metabolism of estrogen (E_2) to its catechol metabolites 4-hydroxyestradiol ($4OHE_2$), 2-hydroxyestradiol ($2OHE_2$) and quinones (E_2-2,3-quinone and E_2-3,4-quinone). The highly reactive quinones are thought to induce mutagenic DNA lesions. The mutagenic potential of these metabolites may be reduced by conjugation to glutathione (GSH) either spontaneously, or by the phase II glutathione-S-transferase (GST) enzymes.
This study aims to determine the cytotoxic and mutagenic effects of estrogen and its catechol metabolites, on cells expressing a single cytochrome p450 enzyme. We used V79 hamster lung fibroblast cells that were stably transfected with one of the following p450s; CYP1A1, CYP1B1, or CYP3A4, as well as the untransfected V79MZ parent cells. The cytotoxic effects of estrogen, 2OHE, and 4OHE, were determined by a dose-response experiment. Estrogen was slightly more cytotoxic in cells expressing 3A4 (IC_{50}=8.7±0.6 uM), but not significantly more than non-p450 expressing V79MZ parent cells (IC_{50}=14.3±2.2 uM). The hydroxy metabolite 2OHE was the most cytotoxic overall and significantly more cytotoxic in cells expressing CYP1B1 (IC_{50}=2.9±0.8 uM) when compared to V79MZ cells (IC_{50}=6.7±0.7 uM), and not significantly cytotoxic in either 1A1 cells (IC_{50}=6.3±0.6 uM), or 3A4 cells (IC_{50}=4.1±0.7 uM). The 4OHE was again more cytotoxic in 1B1 cells (IC_{50}=5.3±1.3 uM) when compared to V79MZ cells (IC_{50}=8.2±1.0 uM), and not significantly more cytotoxic in either 1A1 cells (IC_{50}=7.6±0.6 uM)

or 3A4 (IC_{50}=6.3±0.6 uM). Other studies have implicated CYP1B1 as the key p450 enzyme in estrogen metabolism. It is possible that the lack of sensitivity to the probable catechol metabolites may be sue to slow rates of activation and/or spontaneous reaction with GSH or protein. Mutagenicity studies are currently underway.

P5

EXPLORING THE MODE OF ACTION FOR PHTHALATES AND THE ROLE OF CPLA2 IN STEROID SYNTHESIS

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Phthalic acid diesters are ubiquitous environmental contaminants resulting from their use in plastics. Human exposure occurs primarily via drinking water and food consumption, though it can also result from iv treatments and blood transfusions. In male rats, di-n-butyl phthalate (DBP) and di-(2,4) ethylhexylphthalate (DEHP) disrupt sexual development through testosterone inhibition. Vital to the estimation of human risk is an understanding of the mechanism by which these chemicals act on the biological system. From our review of available literature and a comparison of observed phthalate dose-response in in vivo and in vitro studies, we postulated that the monophthlate metabolites of DBP and DEHP (MBP, MEHP) may interfere with arachidonic acid (AA) signaling in the Leydig cell leading to reduced steroidogenesis. In an effort to refine this hypothesis, we have performed a number of assays designed to answer specific questions about the potential interaction between phospholipase A2 (an AA-releasing enzyme) and the monophthalates. Steroidogenesis assays were performed in cultured Leydig cells using phthalates and known PLA2 inhibitors. In addition to MEHP, MBP and CQ (a general PLA2 inhibitor), inhibitors of iPLA2 and sPLA2 isozymes were tested for their ability to disrupt steroidogenesis. Despite the lower affinity of CQ for its substrates, it was a stronger inhibitor of progesterone than either the iPLA2 or sPLA2 inhibitors. This suggests that cytosolic PLA2 (cPLA2) is important in steroidogenesis. Furthermore, when AA (the preferred product of cPLA2 hydrolysis) was added to the media of CQ-dosed cells, the progesterone production returned to control levels. Immunoblot analysis of luteinizing hormone-stimulated Leydig cells, showed increased expression of the cPLA2 protein in the presence of MEHP, while expression of the phosphorylated (active) form decreased. This suggests that the monophthalates may interfere with activation of cPLA2, thereby reducing cellular AA and subsequent testosterone synthesis.

P6

DOSE-RESPONSE COMPARISON OF MICRONUCLEATED RETICULOCYTE FREQUENCIES IN RODENT PERIPHERAL BLOOD WITH FOUR GENOTOXIC AGENTS BY FLOW CYTOMETRY AND SLIDE-BASED ENUMERATION

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We are using four known genotoxic agents to compare the frequencies of micronucleated reticulocytes (MN-RET) in B6C3F_1 mouse and Fischer 344 rat peripheral blood using flow cytometry and slide-based enumeration. Acrylamide (ACM), ethyl methanesulfonate (EMS), cyclophosphamide (CP), and vincristine sulfate (VS) are being used to evaluate the degree of correspondence between measurements of MN-RET generated by flow cytometry (FCM) and those obtained using standard light microscopy. ACM, EMS, and CP were each administered by oral gavage once daily on 4 consecutive days and blood samples were obtained approximately 4 hr after the final treatment. VS was administered by intraperitoneal injection on 3 consecutive days and blood samples were obtained approximately 26-28 hr after the final treatment. MN-RET frequencies were determined for each sample based on the analysis of 2,000 (microscopy) and 20,000 (FCM) reticulocytes. FCM and slide-based measurements of mouse MN-RET were not significantly different for ACM, EMS, and CP (using paired t-tests), with overall Pearson correlation coefficients of 0.90 (n=75) indicating a high degree of correspondence between the two methods. Both FCM and slide-based methods of scoring produced very similar group means, though the means determined from slide-based methods had much larger standard errors. An additional analysis was performed on each sample to provide a quantitative description of MN size by FCM only. ACM, EMS, and CP all maintained a steady MN size measurement with increased dosage, while VS exhibited an increase in MN size. This reflects VS's known ability to interfere with the mitotic spindle apparatus creating MN consisting of whole chromosomes, while a genotoxic mechanism of chromosome breakage would be associated with smaller MN. The FCM system of data collection offers several advantages over microscopy-based scoring, including analysis of a greater number of cells, much faster data acquisition, MN size determination, and increased objectivity.

P7

CYTOTOXICITY AND MUTAGENICITY OF 5-METHYLCHRYSENE AND ITS DIHYDRODIOL METABOLITE IN V79MZ CELLS STABLY TRANSFECTED WITH HUMAN CYP1B1 AND/OR HUMAN GSTPI/MU.

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5-Methylchrysene (5-MC), a methylated polycyclic aromatic hydrocarbon (PAH), is believed to contribute significantly to induction of lung cancer in smokers. Cytochrome P-450s (CYP) are phase I enzymes known to activate PAHs to more reactive electrophilic metabolites. Glutathione-S-transferases (GSTs) are phase II enzymes that conjugate and detoxify reactive intermediate metabolites of PAHs. The aim of the present study is to investigate the cytotoxicity and mutagenicity of 5-MC and its dihydrodiol intermediate (5-MC-1,2-diol) in V79MZ cells stably transfected with either human CYP1B1 (h1B1) alone or double transfected with h1B1 and human GSTpi/mu (hGSTpi-26, hGSTpi-40 or hGSTmu-12 clones). We measured the cytochrome P-450 enzyme activity in all h1B1 and hGSTpi/mu transfected cells to ascertain that all cell lines had equal enzymatic activity.
5-MC was relatively more cytotoxic in all h1B1 and double transfected hGSTpi/mu clones as compared to V79MZ control cell (IC_{50} of 5-MC in h1B1 = 3.2 ± 0.5, hGSTpi-26 = 3.0 ± 0.4, hGSTpi-40 = 2.4 ± 0.4, hGSTmu-12 = 1.8 ± 0.2 and V79MZ = 5.8 ± 0.4 μM). However, 5-MC-1,2-diol (a dihydrodiol metabolite of 5-MC parent compound) was significantly more cytotoxic in

cells transfected only with h1B1 (IC_{50} = 0.058±0.001 μM compared to ≥3 μM in control V79MZ cells). Cells transfected with both (h1B1 and hGSTpi/mu) were approximately 2-3 fold less sensitive to 5-MC-1,2-diol cytotoxicity (IC_{50} hGSTpi-26 = 0.164±0.018, hGSTpi-40 = 0.174±0.022 and hGSTmu-12 = 0.149±0.015 μM). Mutagenicity of 5-MC and 5-MC-1,2-diol were also studied in V79MZ and h1B1-transfected cells. Both 5-MC and 5-MC-1,2-diol were mutagenic in h1B1-transfected cells. 5-MC-1,2-diol was relatively more mutagenic as compare to 5-MC in h1B1-transfected cell (5-MC-1,2-diol = 675, 5-MC = 367 mutants). Mutagenicity studies are currently underway in V79MZ cells transfected with both h1B1 and hGSTpi/mu. These results clearly demonstrate that activation of 5-MC and its 1,2-dihydrodiol metabolite (5-MC-1,2-diol) to cytotoxic and mutagenic species is catalyzed by hCYP1B1. Further these results indicate that 5-MC-1,2-diol is relatively more cytotoxic and mutagenic than the parent compound (5-MC). Finally, our findings also suggest that hGSTpi/mu might play a significant role in detoxification of dihydrodiol metabolite of 5-MC.

P8

MUTAGENICITY IN *SALMONELLA* OF SULFUR-CONTAINING POLYCYCLIC AROMATIC HETEROCYCLES AND THEIR DIHYDRODIOL DERIVATIVES

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Polycyclic aromatic sulfur heterocycles (PASH) are common constituents of cigarette smoke, fossil fuel-derived materials, and their combustion byproducts. Many PAHs are known mutagens and carcinogens. However, unlike their nonsulfur-containing counterparts, relatively little is known of their mechanism of action. We synthesized dihydrodiol and sulfone derivatives of benzo[*c*]phenanthrene (B[*c*]Ph) and two 5-ringed, thiophene-fused phenanthrenes and tested their mutagenicity in the *Salmonella* (Ames) plate-incorporation assay in strains TA98 and TA100. The goal of the study was to examine the influence of the presence and position of the sulfur heteroatom on the mutagenic activity of B[*c*]Ph and to deduce the nature of the possible active intermediates. With the exception of phenanthro[*3,4-b*]thiophene sulfone, all of the compounds tested were weak mutagens in TA98 (<20 rev/μg). The sulfone was also the most potent compound in strain TA100 (~1000 rev/μg). Phenanthro[*3,4-b*]thiophene, a compound with the sulfur distal to the angular benzo-ring, was 10-fold more mutagenic than its carbocyclic analog B[*c*]Ph (580 vs. 54 rev/μg). The mutagenic activity of phenanthro[*4,3-b*]thiophene, a compound with the sulfur proximal to the angular benzo-ring, was markedly reduced compared to that of B[*c*]Ph. Not surprisingly, the benzo-ring 3,4-dihydrodiol of B[*c*]Ph, which induced 250 rev/μg, was 5-fold more potent than B[*c*]Ph. Likewise, the analogous dihydrodiol of phenanthro[*4,3-b*]thiophene induced 240 rev/μg, which was 34-fold more potent than its parent compound. In contrast, the dihydrodiol of phenanthro[*3,4-b*]thiophene was ~7-fold less mutagenic than its parent compound. The K-region dihydrodiols showed little or no mutagenicity. These data suggest that PASH are potent base substitution mutagens, and may contribute significantly to the mutagenicity of tobacco smoke and industrial and vehicular emissions. The possibility that different phenanthrothiophenes may be activated through different mechanisms is currently under investigation. [HCS and SK supported, in part, by Philip Morris USA, Inc. Abstract does not necessarily reflect the policy of the US EPA.]

P9

MULTIPLE-ENDPOINT CYTOTOXICITY AND GENOTOXICITY ASSAY IN MOUSE L5178Y CELLS WITH LIMITED COMPOUND REQUIREMENTS

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Chromosomal aberration assays use gram amounts of compound and significant amounts of personnel and time resources. The *in vitro* Comet and micronucleus assays are ideally suited as potential rapid screens for DNA and chromosomal DNA damage in mammalian cells and can aide in eliminating unacceptable results early in compound development, saving valuable time and limited resources. The ILS genetic toxicology program has established a low-compound requiring ($\leq$50 mg) medium-throughput *in vitro* assay that would enable the determination of 2 genotoxicity endpoints in the same exposed cell culture, DNA damage by the Comet assay and micronucleus induction. Initial studies have employed the L5178Y mouse lymphoma cell line and a series of positive controls for conditions with S9 (benzo(a)pyrene) and without S9 (ethylmethane sulfonate and mitomycin C). Briefly, cell cultures containing approximately 500,000 cells/mL are exposed to 8 or 12 concentrations of test article in 24-well dishes. After addition of the test article to an initial well, serial dilutions of the exposed culture are used to produce 1.5 to 2.0-fold differences in exposure concentrations between wells while maintaining the exposure population constant. After termination of exposure the test article aliquots are removed for the assessment of ATP levels as a measure of cytotoxicity and the Comet assay as a measure genotoxicity The remaining cell culture is allowed to grow for 20-24 hrs to collect cells for micronucleus determination and to determine population doubling as a measure of cytotoxicity for the micronucleus assay. This design enables the assessment of 2 complimentary genotoxicity endpoints and 2 measures of cytotoxicity under the same test article exposure conditions. The positive controls compounds induce positive responses within the same concentration range as conventional assays. The methods used for the development of this assay are amenable to many other cell types and for automation.

P10

PHASE I AND PHASE II ENZYME POLYMORPHISMS AND *P53* MUTATIONS IN BREAST CANCER

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Breast cancer is the second leading cause of cancer related deaths among American women. It is believed that known risk factors may account for less than half of the cases diagnosed. Some studies suggest that exogenous carcinogens as well as endogenous estrogens may be involved in the initiation of breast cancer. Several of the enzymes involved in the metabolism of these

carcinogens have polymorphisms with known functional implications either increasing activation of the compounds or decreasing detoxification. Multiple studies have focused on the association of these genes with risk, often producing conflicting and inconclusive results. Only some of these studies have focused on their association with damage at critical genetic loci, such as the tumor suppressor gene *p53*. We hypothesize that women with these polymorphisms will be at increased risk for having a mutation in *p53*. In this ongoing study, 166 Caucasian breast cancer patients have been analyzed for polymorphisms in *GSTM1*, *GSTT1*, *GSTP1*, and *CYP1B1* in genomic DNA and *p53* mutations in tumor tissue. Approximately 14% exhibit a mutation at *p53*. Although not statistically significant, our current results suggest an interesting trend for the *CYP1B1*119 polymorphism as those that have at least one Ser allele are more likely to have a mutation in *p53* [Crude odds ratio (OR) = 1.96; 95% Confidence Interval (95% CI) = 0.71-5.49]. A similar trend was also noted with the *GSTP1*105 polymorphism [OR= 1.52; 95% CI= 0.56-4.21]. The *GSTM1* and *GSTP1*114 polymorphisms appear to have a protective effect [OR=0.61; 95% CI= 0.23-1.62 & OR =0.58; 95% CI = 0.09-2.85 respectively]. These associations were not noted for the *CYP1B1*432 and *GSTT1* polymorphisms. Further analyses are required to determine the impact of these polymorphisms on mutations in *p53*. Overall, this study may help in the identification of women that are at increased risk and therefore would benefit most from early intervention either through chemoprevention or lifestyle modifications.

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Schaaper, R.	T5	
Sedelnikova, OA	S1	Invited Speaker
Sharpless, NE	S3	Invited Speaker
Shaw, CS	P10	
Shea, D	T2	
Sherill, GB	P10	
Sikka, H	P8	
Swartz, C	P8	Poster Post Doc
Swartz, C	T5	Talk
Torous, D	P6	
Townsend, AJ	T1, P2, P4, P7	
Upton, D	T6	Talk
Van Emburgh, BO	P10	Student Poster
Wang, X	T6	
Watkins, J	P10	

Willingham, MC	P10	
Winters, J	P9	
Witt, K	P6	
Wolfinger, R	P3	
Xu, M	P2	
Zimonjic, DB	S1	

Genetics and Environmental Mutagenesis Society

P.O. Box 13475, Research Triangle Park, North Carolina 27709
Web: www.ncneighbors.com/GEMS *email: gems@scientist.com*

MEMBERSHIP APPLICATION OR RENEWAL FORM

Name: ____

Affiliation: ____

Address: ____

City: ______________________ **State:** ______ **Zip code:** ______

Telephone No: ____

E-mail: ____

Please include email, which is used for GEMS meeting notifications and updates.

Membership Dues: Students - $5/yr; - regular$10/ yr
(Circle one)

Amt. Paid $__________
Total Paid $__________
X years paid ________

o Please check if you are a NEW MEMBER

Would you be willing to run for election as a Councilor or Officer of GEMS? Yes___ No___

Send to: Susan M. Ross, *Treasurer*
GEMS
P.O. Box 13475
RTP, NC 27709

o Please check if you need a receipt.

Please make checks payable to GEMS.