GENETICS AND ENVIRONMENTAL MUTAGENESIS SOCIETY

FALL 2012 MEETING

# AIR QUALITY AND HEALTH IMPACTS

THURSDAY NOVEMBER 8, 2012

FRIDAY CENTER
UNIVERSITY OF NORTH CAROLINA-CHAPEL HILL

# Air Quality and Health Impacts

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| 8:00AM – 8:45AM | Registration and Continental Breakfast |
| 8:45AM – 9:00AM | Welcome: Nagu Keshava, Ph.D., GEMS President |
| 9:00AM – 9:45AM | **Emerging technologies for exposure assessment: making the exposome a reality.**<br>*David Balshaw, Ph.D.*<br>*National Institute of Environmental Health Sciences,*<br>*National Institute of Health* |
| 9:45AM – 11:30AM | Posters, sponsor exhibits and coffee |
| 11:30AM – 12:15PM | **Air Pollution Epidemiology in a Multi-pollutant World**<br>*Lucas Neas, Ph.D., National Health and*<br>*Environmental Effects Research Laboratory*<br>*U.S.Environmental Protection Agency* |
| 12:15PM – 1:00PM | LUNCH |
| 1:00PM – 1:10PM | Recognition of Sponsors |
| 1:10PM – 1:30PM | Student/Postdoc Talk |
| 1:30PM – 2:15PM | **Connecting Climate, Air Quality, and Human Health: Application Using Global Atmospheric Models**<br>*Jason West, Ph.D.,*<br>*Department of Environmental Sciences and Engineering*<br>*University of North Carolina* |
| 2:15PM – 2:30PM | Recognition of Past Presidents and Announcement of Awards |
| 2:30PM – 3:30PM | Business Meeting |
| 3:30 PM | Adjourn |

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# ABSTRACTS

## Comparative Mutagenicity of Diesel and Biodiesel Emissions

E Mutlu[a,b], SH Warren, C King, MI Gilmour, DM DeMarini
[a]U.S. EPA, RTP, NC, USA, [b]UNC School of Medicine, Chapel Hill, NC

Biofuels are increasingly being developed as alternatives to petroleum-derived fuels, but published research is contradictory regarding the mutagenicity of emissions from biodiesel relative to petroleum diesel. Thus, we performed bioassay-directed fractionation and analyzed the polycyclic aromatic hydrocarbon (PAH) levels of a composite sample of diesel-exhaust particles (C-DEP) generated on site from petroleum diesel with a 30-kW 4-cylinder Deutz BF4M1008 diesel engine connected to an air compressor. We also evaluated combustion particles generated using petroleum diesel (B0) and those from soy-based biodiesel where the soybean oil accounted for 20, 50, or 100% of the fuel (B20, B50, B100) in a Yanmar L70 diesel engine. We extracted particles with dichloromethane (DCM) and sequentially fractionated the extracts with solvents of increasing polarity. We determined the percentage of extractable organic material (EOM), solvent-exchanged extracts into dimethyl sulfoxide, and evaluated them for mutagenicity in *Salmonella* strains TA100 and TA98 +/-S9. For C-DEP, >50% of the mass eluted in fraction 1, but it was not mutagenic. The 2$^{nd}$ fraction had 60% of the TA100+S9 activity, indicative of PAHs. The 3rd fraction contained 60% of the TA98-S9 activity, indicative of nitroarenes. For whole extracts of emissions from B0 (petroleum diesel) and biodiesel, the mutagenic emission factors (revertants/MJ X $10^5$) in TA100+S9 were 2.95 (B0), 0.98 (B20), 0.51 (B50), and 0.38 (B100). These data for biodiesel indicate that under our combustion conditions, the emissions from biodiesel are less mutagenic than those from petroleum diesel. [Abstract does not necessarily reflect the views or policies of the U.S. EPA.]

**The role of DNA polymerase eta in oxidative stress response and mutagenesis**

Kimberly N. Herman, Scott D. McCulloch
North Carolina State University

Oxidative stress is induced by exposure to chemicals or radiation and can increase levels of reactive oxygen species (ROS). ROS can produce DNA lesions including 8-oxoguanine (8-oxoG), which has been linked to mutagenesis, cancer and aging. Cells can respond to this damage using translesion synthesis (TLS) carried out by Y family DNA polymerases. These polymerases have open active sites, allowing them to copy past DNA adducts that block replication. DNA polymerase eta (pol η) is known to bypass cyclobutane pyrimidine dimers (CPD), and more recently, 8-oxoG. Intriguingly, while *in vitro* bypass of 8-oxoG by pol η appears to occur with nearly 50% error rate, *in vivo* experiments suggest it acts to suppress mutagenesis, similar to CPD.

Since pol η can bypass 8-oxoG efficiently but with poor fidelity *in vitro*, we investigated pol η response in cells to oxidative-stress-induced DNA damage. We demonstrated that pol η acts as a suppressor of oxidative stress-induced mutagenesis, likely due to bypass of 8-oxoG. We examined pol η-expressing and -deficient cells, and evaluated responses to various ROS-inducing treatments. We evaluated the cytotoxicity of these treatments and found that while pol η did not greatly alter cell survival, the mutation rate of surviving cells was markedly decreased when measured using hypoxanthine phosphoribosyl transferase (HPRT) mutation assay; showing the presence of pol η suppresses mutations compared to deficient cells. These results demonstrate that pol η suppresses oxidative stress-induced mutations, suggesting that polymerase fidelity *in vitro* data is only part of the larger picture of lesion bypass.

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**REV1 plays critical roles for nonlinear dose-response relationships for abasic sites and/or $S_N2$-alkylating agent lesions that cause *PIGO* mutations in DT40 cells.**

Xu Tian, James A. Swenberg, Jun Nakamura

Many Superfund chemicals have public concerns and clean-up regulations based primarily on risk assessments related to carcinogenicity. Cancer is induced by both genotoxic and nongenotoxic chemicals. Genotoxic chemicals induce chemical specific DNA adducts that can lead to gene and chromosomal mutations. However, we find that mutations occur even in the absence of exogenous mutagens, possibly being caused by endogenous DNA damage. As a result of these background mutations, exogenous mutagen exposure often has a nonlinear dose response. This has important implications for cancer risk assessment and Superfund site clean-up standards. To better understand mechanisms of the nonlinear dose response curve in mutations induced by exogenous chemicals, we developed a novel mutation assay in DT40 chicken cells via screening PigO gene mutations using proaerolysin treatment. Isogenic DT40 cells deficient in DNA repair pathways are a great tool to investigate mechanisms of mutations caused by Superfund chemicals. We used methyl methanesulfonate (MMS) as a model mutagen and found that MMS caused mutations with a nonlinear (hockey-stick-shaped) dose-response curve in DT40 cells. We found that about 55% of the mutations are located at A:T sites with a high frequency of A/T to T/A mutations. We hypothesized that A/T to T/A conversions found in MMS exposure-derived mutant cells are due to base excision repair intermediates, such as abasic sites and single strand breaks induced by N3-methyladenine. It has been reported that *REV1* coordinates translesion DNA synthesis (TLS) polymerase switching. To address effects of REV1 on nonlinear dose-response relationships, we exposed REV1-/- DT40 cells to MMS. REV1-/- cells were sensitive to MMS but no increased mutations were detected. These results indicate that mutations induced by MMS are REV1-dependent. REV1 is required for TLS of abasic sites or single strand breaks caused by endogenous abasic sites and/or $S_N2$-alkylating agents and may result in an increase in *PIGO* mutations.

## Evaluating Single Pollutant and Multipollutant Traffic Indicators in Different Urban Environments Based on their Spatiotemporal Variability

Michelle M. Oakes[1], Tom Long[1], Lisa Baxter[2], Jorge Pachon[3], Ted Russell[4]
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Recent health studies have shown advantages of using multipollutant metrics instead of traditional single pollutant metrics to target traffic impacts. In this study, a variety of single pollutant (NOx, CO, and EC) and multipollutant metrics are evaluated as surrogates of traffic pollution in urban environments (e.g., Atlanta, Denver, and Houston) over a multi-year period. Emission-based integrated mobile source indicators (EB-IMSIs) are used as multipollutant metrics in this study. EB-IMSIs are calculated for diesel and gasoline mobile sources using ambient concentrations and emissions information of traffic pollutants (NOx, CO, and EC) in each location. For each metric, trends in spatial (cross-monitor correlations) and temporal (weekly and day to day) variability are characterized and compared. In general, the day to day and weekly variability of EB-IMSIs follow the variability of single pollutant mobile source tracers, indicating that these multipollutant metrics can capture expected temporal variability of urban scale traffic pollution. In several urban areas, higher spatial correlations are observed in select multipollutant indicators compared to their single pollutant counterparts. These results indicate that EB-IMSIs represent urban-scale mobile source impacts better than some single pollutant indicators, especially in areas where temporal and spatial variability of specific single pollutant indicators are greatly influenced by non-traffic sources. Overall, these results may help to identify specific air quality and health studies that largely benefit from the use of multipollutant metrics. The views expressed in this work are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

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## The Role of Environmental Exposures on Fragile Site Breakage in the Generation of Oncogenic *RET/PTC* Rearrangements.

Christine E. Lehman, Laura W. Dillion, Yuh-Hwa Wang
Wake Forest School of Medicine, Winston-Salem, NC

The rates of thyroid cancer, and more specifically papillary thyroid carcinoma (PTC), have dramatically increased in the United States and other countries throughout the past several decades, and incidences are now nearly three times those of the early 1970s. This upsurge in thyroid cancer is not well understood, and while diagnostic methods have improved, these cannot solely account for this increase. One known risk factor is exposure to ionizing radiation; however, the majority of thyroid cancer patients have no history of radiation exposure. We recently observed that in human thyroid cells, chemical induction of DNA breakage at chromosomal fragile sites generates the oncogenic *RET/PTC1* rearrangement found in sporadic PTC. Fragile sites are regions of the genome prone to DNA breakage and often coincide with mutations found in cancer. Exposure to environmental agents including pesticides and cigarette smoke also induce DNA breakage at fragile sites. Therefore, we investigated whether exposure to environmental chemicals, benzene and diethylnitrosamine (DEN), lead to breakage within fragile sites and ultimately *RET/PTC* rearrangements. To date, we have shown that treatment of thyroid cells with benzene and DEN significantly increases DNA breakage at both fragile sites *RET* and FRA3B, compared to the non-fragile region. We have also demonstrated that normal thyroid cells of PTC patients display increased DNA breakage within *RET* compared to normal thyroid cells from non-PTC patients, suggesting the possibility of differential chemical exposures. This data suggests that environmental exposure to various fragile site-inducing chemicals may lead to cancer-specific rearrangements found in cancers such as PTC.

## Environmental Chemicals Increase Fragile Site Breakage in Regions Associated with Leukemia-Causing Gene Rearrangements

Ryan G. Thys, Christine E. Lehman and Yuh-Hwa Wang
Wake Forest School of Medicine, Winston-Salem, NC

Gene rearrangements are associated with many cancers, including leukemia. The initial event for rearrangement to occur is DNA breakage, but in patients with no history of radiation exposure the cause of breakage is unknown. Recently, we showed that over half of gene pairs involved in cancer-specific rearrangements contain at least one gene within fragile sites; therefore, we investigate whether fragile site-inducing conditions can lead to formation of leukemia-causing rearrangements. Common fragile sites are found in all individuals and breakage at these sites can occur following exposure to various chemicals and environmental factors such as benzene and diethylnitrosmine (DEN). Benzene, a known carcinogen, and DEN are found in gasoline and cigarette smoke, among other sources. While the mechanism of fragile site breakage is still not fully understood, all fragile sites studied to date are predicted to form stable secondary structure, which has the potential to stall replication fork progression, leading to breakage. Our work focuses on the *MLL* gene within fragile site FRA11G, which is rearranged with over 60 gene partners in AML and ALL. Nearly half of the gene partners of *MLL* are also located within fragile sites. Using ligation-mediated PCR, we find that benzene and DEN cause a significant increase in breakage within the *MLL* breakpoint cluster region, and these breakpoints occur near those seen in patients with *MLL*-rearranged leukemias. Also, by employing the Mfold secondary structure prediction program, we find that chemically-induced breakpoints and patient breakpoints are located near areas of potential secondary structure formation.

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## Evaluation of the Ames II and 96-well *In Vitro* Flow Micronucleus (MN) Assay using 25 Toxcast™ Chemicals.

Andrew D.Kligerman[1], Marilyn J. Aardema[2] ,Keith A. Houck[1], Robert R Young[3], Leon F. Stankowski, Jr.[3], Kamala Pant[3], Timothy E Lawlor[3],
[1]US Environmental Protection Agency, Office of Research and Development, Research Triangle Park, NC; [2]Marilyn Aardema Consulting, Fairfield, OH ; Chief Scientific Advisor Toxicology, BioReliance by SAFC, Rockville, MD; [3]BioReliance by SAFC, Rockville, MD

ToxCast™ is a multi-year effort to develop a cost-effective approach for the US Environmental Protection Agency to prioritize thousands of chemicals that need toxicity testing. Initial evaluation of more than 650 high-throughput, robotic, microwell-based endpoints with little or no metabolic activation showed that most of these assays lacked specificity and sensitivity for detecting direct-acting genotoxicants. In an attempt to understand how to improve this approach for genotoxicity testing, we evaluated the specificity and sensitivity of 25 selected Phase I and Phase II chemicals from the ToxCast™ program using standard genotoxicity endpoints. These chemicals were chosen because of their known genotoxicity, their responses in specific ToxCast™ assays, or available data from the literature and databases. The tests used were the medium throughput Ames II and *in vitro* flow MN assays conducted ±S9. Results to date indicate that, as expected, the Ames II assay showed an excellent correlation with published data and industry submissions. Overall concordance was 89 and 82% (with and without S9, respectively). The MN assay also had good concordance of 78 and 73% (with and without S9, respectively); however, the number of conclusive calls was significantly smaller due to concentrations limits and cytotoxicity. Chemicals were also tested in a 96-well *in vitro* alkaline single cell gel electrophoresis (Comet) assay, but there were insufficient data from the literature to evaluate performance of this assay. Taken together, these studies offer a promising approach to higher throughput genotoxicity testing. [This is an abstract of a proposed presentation and does not necessarily reflect US EPA policy.]

**Mode of Action Ontology: An Application Ontology for Risk Assessment Data Mining**

Kyle Painter*, Lyle Burgoon
*Oak Ridge Institute for Science and Education/US EPA, RTP, North Carolina

Automated natural language processing (NLP) will make the practice of risk assessment more efficient. In the future, computer algorithms will use NLP to screen the literature for appropriate studies, identify relevant data, and help synthesize mode of action (MOA) evidence across several studies. However, before we can utilize the data from NLP searches, ontologies must be built to facilitate efficient data storage and sharing using existing standards. We developed the Mode of Action Ontology (MOAO) to support automated NLP discovery, integration, and management of mode of action knowledge. MOAO is a semantic model that 1) describes MOA knowledge, 2) easily combines data from multiple sources, 3) makes MOA knowledge more transparent and easily available to the public via semantic web interfaces, and 4) will allow computers to discover latent information and relationships via logical inference. MOAO is the first ontology we are aware of that describes MOA information for toxicological purposes. MOAO is an application ontology, borrowing terms from existing ontologies produced for biological sub-specialties. This allows maximum integration with outside data. MOAO can be a model for how other sub-domains in toxicology can reap the benefits of ontologies without the overhead of constructing one from scratch. We leverage the work by the Open Biological and Biomedical Ontologies (OBO) Foundry and the Basic Formal Ontology (BFO) to facilitate integration of our ontology with others. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

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**Oxidative stress causes PIGO mutations in DT40 cells with nonlinear dose-response relationships in oxidized bases and single strand breaks**

Vyom Sharma, Xu Tian, Leonard Collins, James A. Swenberg, Jun Nakamura

One of major public concerns about Superfund chemicals is their carcinogenicity. While genotoxic agents cause chemical specific DNA adducts, non-genotoxic carcinogens often induce oxidative DNA damage in addition to cytotoxicity. Our project has demonstrated that polychlorinated biphenyls, a non-genotoxic carcinogen, cause an increase in oxidative DNA damage over background level of DNA damage (endogenous DNA damage). We hypothesize that this endogenous oxidative DNA damage introduces additional mutations, resulting in nonlinear dose response curves in mutations caused by environmental chemicals. As one of our approaches to address mechanisms of nonlinear dose response curves in mutations, we chose $H_2O_2$ as a model compound. $H_2O_2$ caused PIGO mutations in DT40 cells with a nonlinear dose-response curve similar to that of the induction of 8-oxo-dG adducts and single strand breaks. Our preliminary data for the spectra on $H_2O_2$-induced PIGO mutations revealed deletions and T to C transitions which might indicate the presence of oxidized thymidine adducts and abasic sites. The data also suggests that 8-oxo-dG is unlikely to play an important role in the oxidative stress induced mutations at low doses. In addition, results from DNA damage response analysis in isogenic DT40 cells exposed to $H_2O_2$ showed hyper-sensitivity of RAD18-, REV1-, POLk- and POLh-deficient cells in cell survival. These data suggest that error-prone translesion DNA synthesis pathway may be involved in oxidative stress-induced PIGO mutations.

## *Chrm2* Is A Candidate Susceptibility Gene For Hyperoxic Lung Injury In A Murine Model Of Bronchopulmonary Dysplasia

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**Background:** Reactive oxygen species contribute to the pathogenesis of many pulmonary disorders, including bronchopulmonary dysplasia (BPD), a respiratory condition affecting preterm infants. In BPD, treatment involves respiratory support with high oxygen levels, and oxidative stress is an adverse side effect that has been associated with vascular damage and impaired lung development and function in a subset of infants. Genetic polymorphisms in a few candidate genes have been associated with BPD susceptibility, however the genetic basis of differential susceptibility remains poorly understood. In this study, we used haplotype association mapping (HAM) to identify the role of genetic background in differential susceptibility to oxidant injury in developing lungs. **Methods:** Time-mated pregnant inbred mice were allowed to deliver spontaneously. Time-mated pregnant outbred mice were used as foster dams during exposure. At post-natal day 1 (P1), neonates were pooled and randomly reassigned to litters exposed to 100% $O_2$ or air for 72hr. Dams were provided with food *ad libitum*. Following exposure, neonates were necropsied at P4, and bronchoalveolar lavage fluid analysis (protein concentration, total cells, neutrophils, and lymphocytes) and histopathology were measured as disease phenotypes. All exposures were conducted at NIEHS and approved by IACUC. HAM of response phenotypes was done using SNPster. **Results and Conclusions:** Significant inter-strain variation was found for all phenotypes and heritability estimates ranged from 33.6% to 55.7%. Further genetic analysis by SNPster identified significant quantitative trait loci on chromosomes 1, 2, 4, 6, 7, and 9, and candidate susceptibility genes were identified (*Cntnap5a*, *Cntnap5b*, *Cyp2j5*, *Cyp2j6*, *Cyp2j9*, *Cyp2j11*, *Mgmt*, *Fat3*, and *Chrm2*). *Chrm2* (cholinergic receptor, muscarinic 2, cardiac) is known to have a role in lung physiology and disease. A SNP (rs30378838) in *Chrm2* causes an amino acid substitution (P265L) in exon1 and, relative to neonate strains homozygous for the wild-type allele, significantly reduced hyperoxia-induced lung inflammation was found in strains with the mutant allele ($p<0.0001$). Further, targeted deletion of *Chrm2* caused significantly ($p<0.001$) reduced lung permeability and edema following neonatal hyperoxia exposure compared to wild-type controls. Thus, we developed a genetic model of lung injury with similarities to BPD. We also found a novel susceptibility gene that may significantly improve the current understanding of neonatal lung injury.

**SWI/SNF-related chromatin remodeling complexes are required for full activation of the DNA-damage response (DDR) and proper segregation of the genome**

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DNA is bound by histones into nucleosomes. Histones provide an interface for instructing proteins when and how to interact with DNA, and nucleosomes can modulate the accessibility of DNA depending on the degree of chromatinization. SWI/SNF-related complexes are multi-subunit chromatin remodelers known for their ability to slide or evict histones. They use BRAHMA (BRM) or BRAHMA-related gene 1 (BRG1) as the catalytic ATPases for their chromatin remodeling functions. An emerging question is whether chromatin remodelers, well known for their contributions to transcription, are also required for major aspects of DNA metabolism. We have evaluated whether SWI/SNF-related complexes influence the ability of cells to respond to DNA damage. We exposed a HeLa cell line that constitutively expresses shRNAs targeting BRG1 or BRM to a variety of DNA-damaging agents and assessed activation of the DNA damage response (DDR) and cell viability. Compared to the control cell line, deficiency for SWI/SNF-related complexes blunted the DDR response – including activation of H2AX – to DNA-damaging agents that activate ATM and/or ATR. Ongoing experiments have been designed to distinguish whether the amount of DNA damage incurred in cells deficient for SWI/SNF-related complexes is different from the parent cell line or whether γ-H2AX spreading at sites of damage is attenuated in cells lacking BRG1 and BRM. Our results suggest that chromatin remodeling by SWI/SNF-related complexes may contribute to the efficacy of the DDR. In a separate set of experiments, we discovered that co-depletion of BRG1 and BRM via siRNA from normal human fibroblasts expressing the catalytic subunit of telomerase (NHF-hTERT) produced incomplete partitioning of the genome, resulting in an unusual abnormal nuclear morphology. Subunits of SWI/SNF-related complexes are mutated or lost in a variety of cancers, including lung cancers, rhabdoid tumors, ovarian clear cell carcinomas, endometrial carcinomas, renal carcinomas, and breast cancers. This work may help to inform the selection of chemotherapeutics for tumors deficient for SWI/SNF functions and to further characterize important contributions of chromatin remodelers to proper segregation of the genome.