

Division of Chemical Toxicology

Program, 236th ACS National Meeting,
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Program Chair, Kaushik Mitra

SUNDAY MORNING

General Papers, Oral Presentations

Kaushik Mitra, Organizer

1. Protective role of L-carnitine in albino rats treated with different concentrations of acrylamide
Hemdan I. Mahmoud, and Hosni Sh. Abdel Salam, Agric

L-carnitine (4-N-trimethylammonium-3-hydroxybutyric acid) has proved to be found in human nutrition and also synthesized from dietary amino acids. Carnitine as a one of the important natural products, it forms ester with fatty acids for transport across the mitochondrial membrane. Carnitine has many features, since it is widely distributed in the body. The research works on the ability of carnitine in protecting against acrylamide the neurotoxicant and carcinogen. L-carnitine (300 mg/kg bw) has a positive result against (50, 200 and 500 mg acrylamide/L). Up to 5 weeks, carnitine showed amelioration in vitamin C, serum glucose, cholinesterase activity, LDL : HDL ratio and liver function. On the other side, carnitine didn't enhance the antioxidant amount in the presence of acrylamide concentrations.

2. Factors affecting the amount of free-base nicotine in mainstream cigarette smoke and the determination of same by SPME
Mingliang Bao, Peter J Joza¹, William S. Rickert, and John H. Lauterbach

Some experts have advised monitoring both free-base nicotine (FBN) content and total nicotine content of mainstream cigarette smoke (MSS) for understanding addictive effects of new/modified cigarette products. Solid-phase microextraction (SPME) of FBN of the MSS particulate matter has emerged as the preferred technique for the determination of FBN in MSS. The SPME technique assumes that the particulate matter and the vapor phase of the aerosol are in equilibrium at collection time and that SPME processes do not alter composition and temperature of the aerosol. These are important assumptions since partitioning of FBN between vapor and particulate phases of MSS follows Pankow's theory of absorptive partitioning and the concentration of FBN in the headspace is controlled by Raoult's Law. We will discuss how changes in particulate-phase water content markedly affected amount of FBN found with the SPME technique and how regulations might be written to increase the relevance of FBN determinations.

3. Analysis of 21 polycyclic aromatic hydrocarbons in smokeless tobacco by gas chromatography-mass spectrometry
Peter W. Villalta, Aleksandar Knezevich, Joni Jensen¹, Dorothy K. Hatsukami, and Stephen S. Hecht

Smokeless tobacco contains 28 known carcinogens and causes precancerous oral lesions and oral and pancreatic cancer. We recently analyzed 8 different polycyclic aromatic hydrocarbons (PAH) in moist snuff, extending for the first time in over 2 decades the range of chemicals analyzed in U.S. smokeless tobacco beyond nicotine and nitrosamines. Fire-curing of tobacco is the most likely source of its contamination with PAH, some of which are strong toxicants and carcinogens. In this study, we used gas chromatography-mass spectrometry to analyze 21 different PAH in some of the most popular U.S. smokeless brands. The sum of 19 PAH detected in 15 products analyzed here averaged 6.85 (± 1.22) $\mu\text{g/g}$ wet weight. Among the detected PAH, eight are IARC carcinogens, their sum averaging 0.46 (± 0.31) $\mu\text{g/g}$ wet weight. This is the first study to render PAH one of the most prevalent groups of carcinogens in smokeless tobacco, along with tobacco-specific nitrosamines.

4. Biosimulation of drug induced liver injury. Harvey Clewell, Alison Harrill, Scott Siler, Richard Ho, and Ananth Kadambi

The objective of this research effort is to develop a dynamic, mechanistic computer model of liver homeostasis and drug-induced liver injury (DILI). Three drugs were selected for initial model development based on availability of rodent/human data, well-characterized metabolism, and different modalities of DILI: acetaminophen, isoniazid, and valproic acid. The model includes detailed biological representations of the key processes involved in DILI: drug metabolism, energy homeostasis, ROS formation, mitochondrial dysfunction, cholestasis, steatosis, necrosis, apoptosis, and tissue regeneration. The first stage of the platform focuses on intracellular interactions and signaling cascades that precipitate hepatocellular apoptosis and necrosis, while concurrently modeling the responses of nonparenchymal cells, such as Kupffer cells, endothelial cells, stellate cells, NK cells and NKT cells. Model parameters are being estimated using curated data from the literature along with data from patients who have experienced DILI, multiple-strain mouse studies, and multiple cell-type in vitro liver models.

5. A new green chemical and chemistry ANSI standard: What's it all about. Jennifer L. Young.

The ACS Green Chemistry Institute® has initiated a program to develop a multi-attribute green chemical and chemistry American National Standards Institute (ANSI) standard. A broad group of stakeholders met for the first time in Washington, DC in early March to begin the process. Two workgroups have been created to address chemical properties and the process used to manufacture the chemical. The standard is designed to include life cycle analysis. This talk will review details of the proposed standard and progress of the two workgroup. The goal is to craft a draft by late fourth quarter for public comment.

6. Microarray biochips for high-throughput metabolic toxicity assessment of drugs. Prashanth Asuri, Moo-Yeal Lee, Jessica Ryan, Michael Hogg, Bilge Eker, Sana Butt, David Rozzell, Douglas S Clark, and Jonathan S. Dordick

The clinical progression of new chemical entities (NCEs) to pharmaceuticals remains hindered by both the cost and slow pace of technology development in toxicology and clinical safety evaluation. This is particularly true for in vitro approaches as alternatives to animal testing that can be used in the early phases of drug development. Solidus Biosciences, Inc. has developed microarray biochip platforms that can adequately mimic human metabolism and assess cell-specific toxicity of NCEs and their metabolites. The Data Analysis Toxicology Assay Chip (Datachip) is a miniaturized 3D cellular array chip that is used in conjunction with the complementary Metabolizing Enzyme Toxicology Assay Chip (Metachip) that consists of various combinations of human metabolic enzymes arrayed on functionalized glass slides. The Metachip simulates the systemic drug metabolism in the human liver and as a result, the Metachip/Datachip platform can be used for in vitro toxicological assessment of NCEs and their enzyme-generated metabolites in high-throughput. As a 3D cell culture method, the Metachip/Datachip platform offers clear advantages over more conventional in vitro 2D cell culture assays, since it is well established that a 3D matrix provides a more tissue-like environment for toxicology assessment than 2D cell cultures. Moreover, since cytotoxicity and metabolism assays are typically performed in 96- or 384-well plates and well-plate experiments are costly, the miniaturization of both cell toxicity and metabolism assays will significantly reduce the cost of in vitro assays to predict toxicity. Finally, the Metachip can be tailored to mimic the reactivity of drug candidates in different segments of the population, thereby allowing the prediction of the variances in the metabolic toxicity of various drug candidates among different population groups.

7. Fish: A nonmammalian model for determining a "No Observable Effect Level" (NOEL) for crystal formation in kidneys after exposure to melamine and cyanuric acid. Renate Reimschuesse, Eric Evans, Cynthia B. Stine, Tamara Mayer, Nicholas Hasbrouck, and Charles Gieseker

The practice of adulterating feed ingredients with melamine, and/or related triazine analogs such as cyanuric acid, ammeline, or ammeline, was brought to light in 2007 when pets were sickened and died after eating contaminated pet food. Subsequent laboratory studies showed that cats, pigs and fish fed both melamine and cyanuric acid form renal melamine-cyanurate crystals. We report here the first studies to determine a NOEL level for crystals formation. Fish were exposed to 0.1, 1, 2.5, 5, 10 and 20 mg/kg melamine simultaneously administered with cyanuric acid at identical doses. The NOEL in catfish for crystal formation was 10 mg/kg bw (single dose) and 2.5 mg/kg for 7 daily doses. The effect of administering melamine first followed with cyanuric acid at a later time point was also investigated. Kidneys of some fish given cyanuric acid a week after being given melamine (both compounds at 20 mg/kg bw) contained crystals.

8. Intrastrand crosslinks between G and T bases: Role of nitrosoperoxycarbonate in the formation of

DNA lesions and their removal by nucleotide excision repair mechanisms. **Conor Crean**, Byeong Hwa Yun, Konstantin Kropachev, Marina Kolbanovskiy, Nicholas E. Geacintov, and Vladimir Shafirovich

Peroxynitrite produced by inflammatory cells rapidly combines with carbon dioxide to yield unstable nitrosoperoxy carbonate. Exposure of DNA containing 5'-GCT and 5'-GT sequences to the authentic peroxynitrite or peroxynitrite generator, 3-morpholinylsyringonimine (SIN-1) in buffer solutions (pH 7.4) containing bicarbonate anions/carbon dioxide, which favors the formation of nitrosoperoxy carbonate, generates intrastrand cross-links, 5'-G*CT* and 5'-G*T*. In these cross-links, guanine and thymine are linked by covalent bonds between the C8 atom of G and N3 atom of T bases. The G*CT* lesions positioned in double-stranded DNA were medium to excellent substrates of Nucleotide Excision Repair (NER) in cell-free extracts from human HeLa cells, while the G*T* cross-linked product was only weakly incised. The presence of structural distortions rather than the presence of a bulky lesion appears to be sufficient for eliciting the efficient recognition and excision of such lesions by human NER factors. Supported by NIEHS Grant 5R01 ES011589-08.

9. Transnitrosation of thioredoxin by S-nitrosoglutathione in vitro. **Charles G. Knutson**, Katherine T. Barglow, Michael A. Marletta, John S. Wishnok, and Steven R. Tannenbaum.

Controlled S-nitrosation of protein thiols is an important signaling mechanism in mammalian cells. Thioredoxin (TRX) is a key mediator of transnitrosation reactions between proteins, and is nitrosated on Cysteine 73 in cells. However, the mechanism by which TRX becomes nitrosated remains unresolved. We developed an LC-MS-tof method to monitor the nitrosation of TRX by the classic NO donor, S-nitrosoglutathione (GSNO), and simultaneously quantify TRX, GSNO, and glutathione (GSH and GSSG). Recombinant TRX (10 μ M, MW 14126.67) reacted rapidly with 1.0 μ M GSNO producing a singly nitrosated TRX (MW 14154.63) within 1 minute of reaction. When 10 μ M GSNO was reacted with TRX, doubly and triply nitrosated TRX was observed. Identical reactions performed in the presence of 1.0 mM GSH yielded virtually no nitrosated TRX. Thus, in cells where the [GSH] \gg [GSNO], TRX is unlikely to be nitrosated by free GSNO alone, which suggests protein assisted mechanism

10. Apoptosis signal-regulating kinase 1 (ASK1) is a sensor-trigger for stress signaling. **Simona G. Codreanu**, Jeremy S. Myers, Hansen L. Wong, and Daniel C. Liebler

Oxidants and electrophiles trigger apoptosis in part through the activation of the mitogen-activated kinase pathway and its terminal kinase c-jun N-terminal kinase (JNK). We found that a biotin-iodoacetamide probe, the lipid oxidation product 4-hydroxynonenal (HNE) and the acetaminophen metabolite, N-acetyl-p-benzoquinoneimine (NAPQI) all covalently modify and activate ASK1, a MAP3K implicated as an upstream JNK regulator. Mass spectrometry analysis of ASK1 modified with the electrophiles in vitro and in vivo mapped adduction sites in domains critical for ASK1 regulation, including the binding site for the negative ASK1 regulator thioredoxin 1 (Trx1). Adduction did not inhibit ASK1 kinase activity, but disrupted the ASK1-Trx1 complex in electrophile treated cells and impaired binding of Trx1 to purified ASK1. These findings identify ASK1, rather than Trx1, as the damage sensor for electrophilic drug metabolites and implicate ASK1 as both a sensor and a trigger of electrophile stress.

11. Advanced glycation end products of DNA in diabetes and cancer: A molecular link?. **John Termini**, Gerald E. Wuenschell, Daniel Tamae, Punrajit Lim, and Tim Synold

Elevated glucose uptake can result in the chemical modification of biopolymers via reactions with carbohydrate-derived methylglyoxal (MG) to form advanced glycation end products (AGEs). Nucleic acid-AGEs have been known for over 40 years, yet their biological distribution and potential health impact have not been explored. Recent development of suitable ESI-MS/MS methods has made it possible to measure levels of DNA-AGEs in biological samples. We have found that the predominant DNA-AGE N²-carboxyethyl-2'-deoxyguanosine (CEdG) can be detected and quantitated as a pair of diastereomers in urine, plasma and tissue. We will present data to support the idea that CEdG may be an important biomarker of glycolytic stress. For example, studies in rat models of Type 1 and Type 2 diabetes have revealed significantly elevated levels of urinary and tissue CEdG relative to normal controls. Comparisons of CEdG levels in tissue extracted DNA from a variety of human tumors and matched adjacent tissue has revealed significant differences consistent with the involvement of the Warburg effect. Data will also be presented on the stereospecific induction of mutations by (R, S) CEdG in normal and DNA repair deficient human fibroblasts. The possible significance of these findings to the recently recognized link between metabolic disease and elevated cancer risk will be discussed.

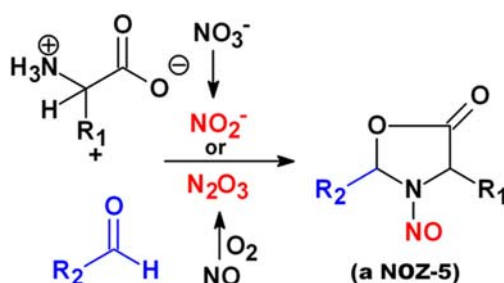
SUNDAY AFTERNOON

Founders Award Symposium

Stephen S. Hecht, Organizer

12. Carcinogenic nitrosamines from amino acids? Using the chemtox toolkit in cancer prevention. Richard N. Loeppky.

Nitrosamines are among the most potent carcinogens. Because nitrosamines form endogenously, and in the human macro- and micro-environments from ubiquitous nitrogen precursors more research and vigilance is required to reduce and to prevent cancers arising from them. New developments, often informed by past approaches, show that the ever evolving tools of chemical toxicology provide excellent resources for accomplishing this task. Here we illustrate the use of this toolkit in our recent explorations of the properties of N-nitroso-1,3-oxazolidin-5-ones (NOZ-5s) and their possible formation from amino acids, aldehydes, and nitrosating agents. NOZ-5s are produced from the facile nitrosation and subsequent cyclization of amino acid aldimines, a process that models suggest could occur in humans. NOZ-5 hydrolysis produces reactive diazonium ions through transient α -hydroxynitrosamines. Several NOZ-5s have been shown to be mutagenic, and alkylate guanine nucleosides. Yet NOZ-5s have sufficient stability to persist in foods, in tobacco, and in other products.



13. Estrogen receptor as Trojan horse: Role of quinones in estrogen carcinogenesis. Judy L. Bolton

Endogenous estrogens as well as estrogens present in estrogen replacement formulations have been implicated in hormone dependent cancers; however, the carcinogenic mechanism(s) remains both controversial and elusive. One mechanism could involve metabolism of estrogens to catechols which are then oxidized to o-quinones which lead to oxidation and/or alkylation of DNA. The equine estrogen, equilenin, present in the popular estrogen replacement formulation Premarin is metabolized to 4-OHEN-o-quinone causing DNA damage. It is proposed that the estrogen receptor (ER) may contribute to estrogen carcinogenesis by transduction of the hormonal signal, and as a "Trojan horse" transporting genotoxic estrogen metabolites to the nucleus to complex with DNA, enhancing DNA damage. 4-OHEN was found to be an estrogen of nanomolar potency in cell culture using a luciferase reporter assay; and using a chromatin immunoprecipitation (ChIP) assay was found to activate ER α binding to estrogen-responsive genes in MCF-7 cells. Imaging of ROS induced by 4-OHEN showed accumulation selective for the nucleus of ER α (+) cells within 5 min. These data support ER α acting as a Trojan horse transporting 4-OHEN to the nucleus, to accelerate the rate of ROS generation and thereby amplify DNA damage. The Trojan horse mechanism may be of general importance beyond estrogen genotoxins. Supported by CA130037.

14. Mechanisms of polycyclic aromatic hydrocarbon activation. Trevor M. Penning

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous environmental pollutants that are multi-species and multi-organ carcinogens. Benzo[a]pyrene (B[a]P) a representative PAH has recently been upgraded by the International Agency for Research on Cancer as a human carcinogen based on its mutagenic and tumorigenic properties, epidemiological and mode-of-action data. However, B[a]P and other PAH are inert molecules and require metabolic activation to exert their deleterious properties. Three pathways of PAH activation have been proposed: formation of diol-epoxides (P450-mediated); formation of radical-cations (peroxidase mediated); and formation of ortho-quinones (AKR-mediated). The latter pathway provides a link between PAH-activation and the generation of reactive oxygen species increasing the spectrum of DNA-adducts possible. Each of these pathways will be discussed from a perspective of B[a]P activation in human lung cells.

15. Repair and replication of bulky DNA adducts derived from tobacco-specific nitrosamines. Thomas E. Spratt

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is one of the most potent carcinogens in tobacco smoke. NNK damages DNA by forming methyl and 4-(3-pyridyl)-4-oxobutyl (POB) adducts.

While the fate and importance of methyl DNA adducts has been well understood, little is known of the significance of the POB adducts. Recently it has been shown that NNK produces four major bulky adducts, 7-POB-dGuo, O⁶-POB-dGuo, O²-POB-Cyt and O²-POB-dThy. We have evidence in human cell culture that O²-POB-dThy is the least efficiently repaired adduct. *In vitro* and *ex vivo* experiments indicate that 7-POB-dGuo and O²-POB-dCyt are repaired by both the BER and NER pathways. O²-POB-dThy was synthesized and incorporated into oligodeoxynucleotides. Replication experiments with E coli DNA polymerase I and Sulfolobus solfataricus P2 DNA polymerase IV demonstrate that dTTP is preferentially incorporated opposite the O²-POB-dThy. These results are consistent with the importance of O²-POB-dThy in NNK-induced lung cancer.

16. Tobacco carcinogen biomarkers for investigating tobacco and cancer. Stephen S. Hecht

Tobacco carcinogen biomarkers are metabolites, DNA adducts, or protein adducts of specific tobacco toxicants or carcinogens. There are three phases of validation of these biomarkers: analytical, relationship to tobacco exposure, and relationship to disease. The first phase is accomplished using standard analytical chemistry approaches, the second by carrying out studies in tobacco users who stop, and the third through epidemiologic investigations. We have carried out studies with a panel of biomarkers representing major toxicants/carcinogens in tobacco smoke. The biomarkers and their sources (in parentheses) are: urinary nicotine metabolites (nicotine); urinary mercapturic acids (benzene, 1,3-butadiene, acrolein, crotonaldehyde, ethylene oxide); tobacco-specific nitrosamine metabolites [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosornicotine (NNN)], 1-hydroxypyrene and phenanthrene tetraol (polycyclic aromatic hydrocarbons); leukocyte DNA adducts (acetaldehyde and formaldehyde). All biomarkers have been validated in the first two phases and two (NNAL and cotinine) in all three phases. Recent studies of tobacco carcinogen biomarkers will be presented

MONDAY MORNING

General Papers: Young Investigator Session

Kaushik Mitra and Shana Sturla, Organizers

17. Toxicity of nanomaterials: Can "computational nanotoxicology" be helpful?. Bakhtiyor F. Rasulev, Danuta Leszczynska, and Jerzy Leszczynski

Nanoscale materials find use in a variety of different areas, such as electronic, magnetic and optoelectronic, biomedical, pharmaceutical, cosmetic, energy, environmental, catalytic, and materials applications. Use of nanomaterials in various industries is projected to increase dramatically in the future and environmental contamination by these materials is expected. Because of peculiar physical and chemical features, the study of nanoparticles as potential toxic agents requires an interdisciplinary approach, involving multiple aspects ranging from physics and chemistry to biology and medicine. Assuming that peculiar properties of nanoparticles depend from the physicochemical features and unusual size of particle, the complex study, including structural, physicochemical and size effects analyses are required to evaluate their toxic effects. This complex approach can be considered as computational nanotoxicology approach, which includes contributions of the following approaches:

- Quantum-Chemical methods;
- Protein-Ligand Docking Approaches;
- Quantitative Structure-Activity Relationship approaches (QSAR)

Some view to this problem and recent results are discussed.

18. The research of ROS generated by single wall carbon nanotube with capillary electrophoresis. Lei Ren, and Wenwan Zhong

With a home-made capillary electrophoresis (CE) system, we have found that single wall carbon nanotube (CNT), CNT-PEG and CNT-COOH could oxidize dichlorodihydrofluorescein (H2DCF) into fluorescent dichlorofluorescein (DCF), a fluorescent probe which are used to indicate the level of global reactive oxygen species (ROS). Their activities from high to low are CNT>CNT-PEG>CNT-COOH. Several specific suppressors for different ROS were tested for their ability to suppress the oxidation of H2DCF by CNT or CNT-PEG. From their effects on the reaction, we concluded that the reaction intermediate could be peroxy radical ROO_o generated from the adsorption of oxygen on the CNT

surface. This conclusion was further supported by the experiment of removing O_2 from the solution with N_2 . Adsorption of proteins such as BSA onto the CNT surface could inhibit the reaction as well. Our study helps to understand better about ROS generation of CNT in cells and identify possible ways to prevent it.

19. Free radical chemistry of anesthetics in water: Investigating the toxicity of degradation products.

Timothy J Feliciano, Stephen P. Mezyk and James J. Kiddle

It has long been recognized that human exposure to degradation products of volatile anesthetics can lead to toxicity. Because most of the commonly used general anesthetics are aliphatic compounds with carbon-halogen bonds the intermediates produced upon metabolism of these compounds are significant. Current evidence indicates that the majority of metabolic pathways for activation of these compounds lead to the formation of reactive electrophilic intermediates. Since there is a well-established relationship between drug and xenobiotic-derived reactive intermediates and chemical-induced carcinogenesis, a thorough understanding of the formation, reactivity, and fate of anesthetic derived reactive intermediates is necessary to fully elucidate their toxicity. Oxidative reactions dominate under physiological conditions. We therefore hypothesized that there is a relationship between the reactivity of these anesthetics with oxidative radical reactions, notably those of the hydroxyl radical ($^{\bullet}OH$). To test this hypothesis, absolute reaction rate constants for $^{\bullet}OH$ reaction with four commercially available anesthetics (Halothane, Isoflurane, Enflurane, and Sevoflurane) have been determined. The oxidation of these anesthetics was conducted using electron pulse radiolysis techniques, utilizing the hydroxyl radical generated in N_2O -saturated aqueous solution, at both room and physiological temperatures. As no significant transient absorption was found upon the oxidation of these anesthetics, the rate constant values were determined using thiocyanate competition kinetics. For these four anesthetics, the rate constants were found to be effectively the same. We therefore conclude that the hydroxyl radical oxidation of these anesthetics would not fully account for the differences in toxicity observed.

20. Understanding redox regulation in membrane associated cytochrome P450s and the FMN domain of nitric oxide synthase.

Aditi Das, Huiying Li, Yelena Grinkova, Hiruy Sibhatu, Joumana Jama, Thomas L. Poulos, and Stephen G. Sligar

Human cytochrome P450s play a critical role in drug metabolism. There are multiple P450s in the liver, and the exact control of redox potential by drug binding to the cytochrome P450s is not well understood. For soluble cytochrome P450 CYP101, it has been shown that there is a linear free energy relationship between heme redox potential and the spin state of the ferric protein. However, the universality of this relationship has been challenged for the mammalian enzymes. Most cytochrome P450s are integral membrane proteins, and detailed redox potential measurements have proved difficult due to protein aggregation or the necessary presence of detergent. Hence, we utilize a soluble nanometer scale membrane bilayer disc (Nanodisc) to stabilize monomeric human cytochrome P450 3A4. We show that substrate binding can effect the redox potential of the heme protein. A linear free energy relationship is observed, analogous to that noted for the soluble P450s, indicating a common mechanism for this linkage and providing a means for control of electron input in response to the presence of a metabolizable substrate. In a separate study, effect of the deletion of a key amino acid residue on the redox potential of FMN domain of nitric oxide synthase (NOS) was evaluated. In NOS and cytochrome P450 reductase (CPR), the FMN sq/hq redox potential is lower than that of the ox/sq couple and hence it is the hq form of FMN that delivers electrons to the heme (ox = oxidized, sq = semiquinone, hq = hydroquinone). By deleting the residue Gly810 from the FMN binding loop in neuronal NOS (nNOS), the FMN ox/sq redox potential became lower than the sq/hq couple similar to P450 BM3. Therefore, the G810 residue plays an important role in controlling the redox potential of FMN domain in NOS. (Supported by NIH Grant GM31756 and NSF EEC-0647560)

21. Comparative metabolism of B[a]P and B[a]P-7,8-Diol in the HBEC-KT and Beas-2B human bronchial epithelial cell lines: Implications for routes of PAH metabolism in noncancerous lung tissue.

Mary E. Kushman, Amy M. Quinn, and Trevor M. Penning

To further understand PAH metabolism in normal bronchial epithelial cells, we studied $[3H]$ -B[a]P and $[3H]$ - (\pm) -B[a]P-7,8-dihydrodiol metabolism along with P450 and AKR expression profiling and functional enzyme assay in HBEC-KT or Beas-2B cells. Constitutive and induced AKR1C activity, but not P450 activity, was observed at the functional enzyme level in HBEC-KT cells. Beas-2B cells expressed detectable P4501A1 and 1B1 mRNA and functional P450 activity, but not functional AKR1C activity. Metabolism of 1 micromolar $[3H]$ -B[a]P and $[3H]$ - (\pm) -B[a]P-7,8-dihydrodiol was observed in naive and TCDD-induced Beas-2B cells, but not in HBEC-KT cells. $[3H]$ -B[a]P metabolism in Beas-2B cells resulted in B[a]P-1,6-dione as the predominant daughter metabolite. $[3H]$ -B[a]P-7,8-dihydrodiol was metabolized to the four BP-tetrols, with BP-tetrol 1 predominant, consistent with P4501B1 expression. The AKR product B[a]P-7,8-dione was not detected. Our results suggest Beas-2B cells may serve as a

suitable model for profiling PAH metabolism in lung epithelial cells (supported by 1R01-ES015857 to TMP).

22. Detection of ethylene dibromide mediated DNA-adducts of O6-alkylguanine-DNA alkyltransferase. Goutam Chowdhury, Anthony E. Pegg, and F. Peter Guengerich

The DNA repair protein O6-alkylguanine-DNA alkyltransferase (AGT, MGMT) is a repair protein involved in the repair of the promutagenic lesions O6-methyl guanine and to a lesser extent O4-methyl thymine by irreversibly transferring the methyl group to an active site cysteine in a stoichiometric, direct damage reversal pathway. Interestingly, AGT is also known to cause mutations by forming protein-DNA crosslinks in the presence of bis-electrophiles like ethylene dibromide and butadiene diepoxide. The active site cysteine of AGT reacts with ethylene dibromide to form an episulfonium ion that generates crosslink in the presence of DNA. N-7 of guanine has been shown to be one of the sites of crosslink formation, although indirect evidence suggests the presence of other sites. A tandem mass spectrometric based approach for the detection of proteolytic digest of DNA-CH₂CH₂-AGT has been developed to detect the presence of DNA-protein crosslinks. Using this approach non-labile adducts of DNA-CH₂CH₂-peptide in GC and AT rich oligonucleotides were detected. Sites of crosslink formation in DNA were also investigated. (Supported in part by USPHS R01 ES010546, P30 ES000267 and Merck Fellowship)

23. Identification of intracellular modified proteins by the lipid peroxidation aldehyde DODE. Peter G. Slade, Michelle V Williams, Viral Brahmabhatt, John S. Wishnok, and Steven R. Tannenbaum

The hydroperoxide of linoleic acid (13-HPODE) degrades to 9,12-dioxo-10(E)-dodecenoic (DODE) which readily modifies proteins forming protein-carbonyls. The goals of this study were to identify cellular proteins modified by DODE and verify the usefulness of DODE as a biomarker of oxidative stress. MCF7 breast cancer cells were treated with 13-HPODE-biotin. Modified proteins were enriched by avidin affinity and identified by 2D-LC-MS/MS. Cells were treated with 13-HPODE. Proteins-carbonyls were biotinylated with an aldehyde reactive probe (ARP) and modified proteins enriched by avidin affinity and identified by 2D-LC-MS/MS. DODE modified proteins were located by 2D-SDS-PAGE and Western blot and identified by in-gel digestion and LC-MS/MS. Analysis of the proteins identified demonstrated a significant overlap between methods and it was concluded these were the major proteins modified by DODE in MCF7 cells. Further studies are continuing to identify the site of DODE modification.

24. DNA-protein crosslinks induced by diepoxybutane. Elisabeth M. Loecken and F. Peter Guengerich

The bis-functional electrophile diepoxybutane is a carcinogenic metabolite of the industrial chemical butadiene. The cross-linking of proteins to DNA by bis-electrophiles may contribute to mutagenesis. We sought to characterize DNA lesions formed by reactions involving diepoxybutane and GSH. Stable GSH-epoxide adducts are produced upon GST incubation, and treatment of DNA with diepoxybutane in the presence of GSH yields DNA cross-links. In addition, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and histone H2b were identified in a mass spectral screen for bis-electrophile-reactive proteins. We confirmed formation of protein adducts by mass spectrometry and gel mobility assays demonstrated cross-link formation in vitro. However, both GAPDH and histone H2b failed to enhance mutagenesis by bis-electrophiles when overexpressed in *Escherichia coli*. The lack of enhanced mutagenicity by GAPDH may result from reduced DNA binding. Differential repair and bypass of DNA-protein cross-links could also explain the lack of mutagenic enhancement observed with both proteins.

25. Exocyclic deoxyadenosine adducts of diepoxybutane: Synthesis, structural elucidation, mechanistic studies and effects on DNA structure. Uthpala Seneviratne, Sergey Antsyovich, Rebecca Guza, Melissa Goggin¹, Thakshila Darshani Dissanayake, Adam Moser, Darrin M. York, and Natalia Tretyakova¹

1,2,3,4-Diepoxybutane (DEB) is the ultimate carcinogenic metabolite of 1,3-butadiene. DEB preferentially modifies guanine nucleobases within DNA, but induces a large number of A to T transversions, suggesting that strongly mispairing adenine lesions are also formed. In the present study, we identified three novel, potentially promutagenic exocyclic DEB-dA lesions: N⁶,N⁶-(2,3-dihydroxy-1,4-butadiyl)-2'-deoxyadenosine (N⁶,N⁶-DHB-dA, 2), 1,N⁶-(2-hydroxy-3-hydroxymethyl-1,3-propanodiy)-2'-deoxyadenosine (1,N⁶- α -HMHP-dA, 3) and 1,N⁶-(1-hydroxymethyl-2-hydroxy-1,3-propanodiy)-2'-deoxyadenosine (1,N⁶- α -HMHP-dA, 4). The structures and stereochemistry of the novel DEB-dA adducts were determined by a combination of UV and two-dimensional NMR spectroscopy, tandem mass spectrometry, and stereospecific synthesis. To examine the effects of the exocyclic DEB-dA adducts on DNA structure and stability, DNA duplexes containing site-specific 2 and 3 were prepared by a post-oligomerization approach and analyzed by UV melting and CD spectroscopy. HPLC-ESI+-MS/MS analyses of DNA hydrolysates demonstrated that 3 and 4 (major) were present in DEB-treated double stranded DNA and in liver DNA of laboratory mice exposed to 1,3-butadiene by

inhalation. Under physiological conditions, 3 and 4 can be inter-converted by a reversible unimolecular pericyclic reaction favoring more thermodynamically stable 4.

26. Reversible quinone methides alkylation of DNA. Huan Wang and Steve E. Rokita

Quinone methide intermediates (QMs) have been implicated in a range of biological processes and can effectively alkylate numerous cellular components, including DNA. The reversibility of some QM reactions has recently been illustrated with a bis-functionalized QM-acridine conjugate (bisQMP). The presence of a strong nucleophile such as dA preserves the ability of bisQMP to cross-link DNA under aqueous conditions, whereas other deoxynucleotides show no similar capacity. Oligonucleotides too can preserve QM intermediates by forming intrastrand adducts for later transfer to complementary sequences resulting in selective formation of interstrand cross-links. An oligonucleotide composed of only G and T has a strong ability to trap bisQM and facilitate its cross-linking to a complementary sequence. However, this activity is greatly suppressed when G is replaced by either A or C.

27. Crystal structures of ternary complexes of the Y-class *Sulfolobus solfataricus* DNA polymerase Dpo4 with a template containing a reduced analog of the acrolein-derived deoxyguanosine adduct N²-(3-Hydroxyl-propyl)-deoxyguanosine. Ganesh Shanmugam, Ivan D. Kozekov, Carmelo J. Rizzo, Martin Egli, and Michael P. Stone

When placed complementary to deoxycytosine in DNA, the γ -hydroxy-1,N²-propano-2'-deoxyguanosine (γ -OHPdG) adduct derived from acrolein undergoes ring-opening to the N²-(3-oxopropyl)-dG aldehyde or its hydrate. This is anticipated to facilitate Watson-Crick hydrogen bonding with incoming dCTP during DNA replication, explaining why γ -OHPdG is weakly mutagenic. We present structures of the reduced N²-(3-hydroxyl-propyl)-dG adduct, a chemically stable model for the N²-(3-oxopropyl)-dG aldehyde, in complex with the *Sulfolobus solfataricus* DNA polymerase Dpo4. Two template:primers were examined: 5-d(TCACXGAATCCTTCCCCC)-3':5'-d(GGGGAAGGATTC)-3'(template-I) and 5'-d(TCATXGAATCCTTCCCCC)-3':5'-d(GGGGAGGATTC)-3' template-II), where X is N²-(3-hydroxyl-propyl)-dG. For both, the incoming nucleotides dGTP or dATP did not pair with the reduced adduct, but instead with the 5'-neighbor template dC (template-I) or dT (template-II), utilizing Watson-Crick geometry. Thus both complexes were of the type II structure described for ternary complexes of the Dpo4 polymerase with native DNA. Supported by NIH grant ES-05355 (C.J.R., M.E., and M.P.S.).

28. Translesion DNA synthesis across the M1dG lesion catalyzed by Y-family human DNA polymerase kappa? Leena Maddukur, Robert L. Eoff, Carmelo J. Rizzo, F. Peter Guengerich, and Lawrence J Marnett

M1dG (pyrimidopurinone deoxyguanosine adduct, 3-(2'-deoxy-beta-D-erythro-pentofuranosyl)pyrimido-[1,2-a]purin-10(3H)-one), a major and stable DNA-adduct of MDA (malondialdehyde) and basepropenal is known to block replication in vitro. The ability of polymerase ? to bypass M1dG lesions has been tested. Steady-state kinetic parameters and LC/MS/MS analysis showed that the insertion of dCTP opposite the M1dG lesion is most favorable event (only 50-fold less efficient than dCTP opposite dG). Pol κ also inserts dGTP and dTTP opposite the M1dG lesion, 7- to 8-fold less efficiently than dCTP incorporation. These data suggests that pol κ may bypass M1dG lesions more accurately and efficiently than pol κ . One possible explanation for this accurate bypass is might be the involvement of N-clasp domain of pol κ , which helps the polymerase to encircle the DNA at the primer-template active site and could conceivably be involved in the ring opening of M1dG through stabilization of the nascent dCTP:M1dG base pair.

29. Thermodynamic and real time analysis of aminofluorene-DNA adduct/polymerase interaction at the replication fork. VG. Vaidyanathan, and Bongsup Cho

We hypothesize that sequence-dependent adduct heterogeneity at the replication fork influences the thermodynamics and kinetics of polymerase binding affinity. In this study, we carried out a systematic thermodynamic and real time investigation of DNA/polymerase interactions. The dynamic FNMR/DSC results on two prototype aminofluorene-modified 16-mer sequences (TG*A and CG*A) exhibited a distinct incremental sequence effect on the stacked (S)/B-type conformational heterogeneity at the replication fork during a simulated trans-lesion synthesis (n-1 ~ n+6). Induced circular dichroism (ICD) data revealed the presence of the S/B heterogeneity in accordance with the NMR data. The same sequences were either incorporated into a 67-mer template-primer substrate in a solution for isothermal titration calorimetric (ITC) analysis, or immobilized directly on a biosensor chip for surface plasma resonance (SPR) detection. These substrates were then treated with various polymerases (Klenow, Dpo4, etc). The results from these experiments provided valuable insight into the molecular interaction between the damaged DNA and polymerases.

30 Structure and activity of the Y-class *Sulfolobus solfataricus* DNA polymerase Dpo4 with

primer: Templates containing aflatoxin adducts. [Surajit Banerjee](#), Kyle L. Brown, and Michael P. Stone

DNA alkylation by aflatoxin B1 (AFB1) yields the N7-deoxyguanosine adduct trans-8,9-dihydro-8-(N7-guanyl)-9-hydroxyaflatoxin B1. Its hydrolysis gives the persistent and highly mutagenic formamidopyrimidine (FAPY) derivative. The FAPY derivative equilibrates between α and β deoxyribose anomers; the β anomer is associated with the high mutagenicity of this lesion in *E. coli*, whereas the α anomer blocks DNA replication. We report the structure of the *Sulfolobus solfataricus* DNA polymerase Dpo4 with the template:primer 5'-TCATTGAATCCTTCCCCC-3':5'-GGGGGAAGGATTC-3', where G is the site of adduction with either the AFB1-N7-Gua or the AFB1-FAPY adduct. For both adducts, the 5'-intercalation of the AFB1 moiety relative to the alkylated dG is maintained during DNA replication. The structures with the Dpo4 polymerase show that the mutagenic β anomer of the lesion is maintained within the active site. The structural studies are correlated with replication bypass experiments in vitro. Supported by NIH grant CA-55678 (M.P.S.).

MONDAY AFTERNOON

Advances in Aquatic Toxicology: Alternative Non-traditional Endpoints

Bryan Brooks and Richard Brain, Organizers

31 Introduction and background for alternative nontraditional endpoints in aquatic toxicology [Bryan W. Brooks](#) and Richard A. Brain

32 Quest for new endpoints is a Sisyphean task [Mark L. Hanson](#)

In our hunt for endpoints that might appear to be more responsive than ones we currently use, we have to be aware of where this search will lead. By defining utility as 'sensitivity', we open the door to an almost endless exercise. Just as one endpoint is shown to respond at a lower exposure than previous examples and arguments are made for its adoption, the ball rolls back down the hill as a different rate of gene expression, or enzyme inhibition, or metabolite production is found to respond at yet lower concentrations. Without an understanding of the biological and ecological relevance of the new responses being measured, it is difficult to see how they can replace the standard responses that are linked to growth, reproduction and development, let alone find a place in the standard risk assessment framework.

33 Impacts of environmental contamination on the reproductive ecology of marine invertebrates [Ceri Lewis](#)

The majority of marine macro-invertebrate species reproduce by releasing their eggs and/or sperm into the water column so that fertilization takes place externally. These broadcast spawning strategists are particularly susceptible to exposure to water-borne contaminants. Successful fertilization is a critical step in the life history of a species, but particularly so for broadcast spawning marine invertebrates where sperm is often limiting and fertilization success is rarely 100% even under optimal environmental conditions. We examine the vulnerability of sperm from the infaunal polychaete worm *Arenicola marina* and the benthic bivalve mollusc *Mytilus edulis* to toxicity from environmental pollutants and relate these impacts to population fertilization ecology. Exposure to the water soluble fraction of crude oil (WAF) at concentrations equivalent to 3.8 $\mu\text{g L}^{-1}$ total PAH was found to reduce sperm motility with the equivalent effect of increasing sperm dilution by a factor of 104. This resulted in significantly reduced sperm: egg collision rates, negatively impacting upon both fertilization kinetics and fertilization success and effectively enhancing population sperm limitation. In contrast, the genotoxic PAH benzo(a)pyrene caused significant damage to sperm DNA but did not impact upon sperm swimming behaviour or reduce fertilization success. In studies to deduce the mechanism of these effects, sperm were found to have a significantly reduced capacity for DNA repair and anti-oxidant defence compared to oocytes and somatic cells. We discuss these findings in relation to the selective pressure acting on spawning behaviour and larval competition in broadcast spawners living in contaminated environments.

34 Effects of organophosphate and organochloride exposures on zebrafish biogenic amine transporter proteins, GABAA receptors, and behavior in novel environments [Georgianna G. Gould](#)

Neurophysiological models of pesticide exposure are needed for occupational health, environmental exposure and ecosystem impact studies. Due to their mapped and malleable genomes, and

amenability to low cost-high volume screening, zebrafish are excellent candidates for this function. Pesticide exposures, particularly to organophosphates or organochlorides, can affect serotonin, dopamine and/or norepinephrine neurotransmission in the mammalian brain. Zebrafish biogenic amine system anatomy and development has been well characterized, and while brain morphology differs, parallel regions share many similarities with mammalian systems. Further, active binding sites for biogenic amines appear to be largely conserved among zebrafish and mammals. Yet it is unclear to what extent specific mammalian and zebrafish binding properties and receptor mediated pathways are shared, and if they are similarly targeted by pesticides. Consistent with findings in mammals, herein we demonstrate that sub-chronic (3-week) exposure of the organophosphate chlorpyrifos (10 ug/day) reduces serotonin uptake sites, while exposure to the organochloride dieldrin (10 ug/day) increases dopamine uptake sites, as measured by radioligand binding to brain homogenates. However, neither chlorpyrifos nor dieldrin were bound with high affinity to either serotonin or dopamine transporters. It is possible that the effects of these two pesticides on biogenic amine transporters are mediated through GABAergic systems. Consistent with mammalian studies, zebrafish acetylcholinesterase activity was inhibited by exposure to either pesticide at 10 ug/day. Further, both pesticides altered the behavior of zebrafish in novel environments, consistent with what has been observed in rodent models. Taken together, these findings suggest that the response of mammalian and zebrafish biogenic amine systems to these pesticides is so similar, that zebrafish should be further employed in pesticide exposure studies.

35 From proteomics to pipefish: Pioneering practical procedures and providing potential prototypes which partake in paternal provisioning Jennifer L. Ripley

Extensive fish kills, widespread hypoxic events and identification of intersex fish exemplify the last decade in the Chesapeake Bay watershed. In our search to identify potential culprits for the observed, altered physiology and to foresee physiological disruption from emerging contaminants, we employ both novel techniques and unique model organisms. First, we will discuss our evaluation of a proteomics approach to gage smallmouth bass health in the Shenandoah River, VA, USA. Second, we will share our research on Syngnathid pipefishes in which the male broods the developing embryos in a placenta-like structure until free-swimming fry are released. Current projects including capillary electrophoresis to identify changes in steroid hormones in fish from the South Branch Potomac River, WV and latex casts of Syngnathid brood pouch vasculature will also be discussed.

36 Behavioral testing: An underutilized tool in the field of environmental toxicology Dalma Martinovic

Behavioral techniques derived from behavioral ecology and pharmacology can be applied to detect and characterize changes in animals living in the environment exposed to pollutants, but remain under-utilized in the field of ecotoxicology. Behavioral endpoints may be exceptionally useful for detection of effects of the emerging contaminants such as endocrine disrupters and various pharmaceuticals, many of which often have only subtle effects on usual ecotoxicological endpoints (e.g., survival, reproduction), thus making them difficult to detect with the standard bioassays. In addition to being a sensitive endpoint, behavior also represents an integrated response of an organism to its environment, and it is directly linked to individual survival and reproductive fitness. As such, unlike many of the commonly evaluated biochemical endpoints, it can be linked to population and ecosystem level effects. The purpose of this presentation is: 1) to demonstrate the substantial potential of behavioral endpoints for assessment of effects of emerging pollutants, 2) to examine potential role of the modern genomic and metabolomic tools in elucidating behavioral impairments and 3) to explore the linkages between individual-level behavioral effects and effects at population and/or ecosystem levels.

37 Alternative models for fish ecotoxicity testing Michelle R. Embry

Though still largely reliant on traditional whole animal tests, current and new legislation (such as the EU REACH legislation and the Canadian Categorization of the Domestic Substances List) is leading to the testing of a large number of chemicals. Chemical management programs coupled with societal forces to reduce, refine, or replace (the 3-R's) animal testing has initiated the development of novel assays and refinement of existing assays that can serve as alternative models for fish ecotoxicity testing. Several well-established methodologies already exist and are undergoing validation. These include the fish embryo test (FET) as a replacement for the OECD 203 fish acute toxicity test, and cellular and subcellular methods are under development as alternatives to the OECD 305 fish bioconcentration (BCF) test. New alternative techniques to evaluate chronic toxicity and endocrine disruption are being discussed, along with the integration of additional (and potentially more sensitive) endpoints into existing tests. Finally, relevant alternative test protocols are being pursued using fish cell lines to measure the uptake across membranes to represent bioavailability. This presentation will provide an overview of the landscape of interactions ongoing in fish alternatives, with the goal of highlighting areas for future globally coordinated scientific efforts related to ecotoxicity testing.

38 Considerations of advancements in aquatic toxicology and nontraditional endpoints for regulatory use: Perspective from Office of Water Joseph R. Beaman

Under the United States Clean Water Act (CWA), EPA is required to take a number of actions to protect and restore the ecological integrity of the Nation's water bodies. Specifically under section 304(a) of the CWA, EPA must develop and publish ambient water quality criteria (AWQC), which are recommended guidance by States and authorized tribes to establish water quality standards for their water bodies. AWQC for aquatic life (aquatic life criteria, ALC) developed under Section 304(a) reflect the "latest scientific knowledge" concerning "all identifiable effects" of the pollutant in question. In 1985, EPA published Guidelines that have provided uniformity and transparency in the derivation methodology of ALC for a large number of compounds among several classes of chemicals. Recently, considerable attention has been generated by the ecological effects of a widely ranging group of chemicals collectively termed, in this document, contaminants of emerging concern (CECs). Some of these CECs present challenges for the application of the Guidelines to ALC development. This is clearly evident for some classes of emerging contaminants such as pharmaceuticals and personal care products exhibiting specific modes of action (such as endocrine disrupting activity or other toxic mechanisms) that require additional consideration when applying the Guidelines. An intra-agency workgroup addressed issues relating to criteria development for CECs and concluded that the basic framework and conceptual underpinnings of the Guidelines are no less applicable than to other chemicals. Further, the "Good Science" clause of the Guidelines provides the flexibility to adopt procedures different than the standard procedures when such alternatives are scientifically justified. In that regard, the workgroup identified a number of possible modifications or alternate interpretations that might aid those developing criteria for CECs to do so in a resource efficient manner that takes best advantage of existing knowledge.

39 Panel Discussion on integration of alternative non-traditional endpoints in ecological risk assessment Richard A. Brain and Bryan W. Brooks

This time will include a panel discussion of presentors in the symposium. The presentors will specifically be asked to identify major advantages and obstacles to integrating alternative non-traditional endpoints in prospective and retrospective ecological risk assessments.

TUESDAY MORNING

Human Drug Metabolites in Safety Testing: Guidelines & Strategies

F. P. Guengerich, Organizer

40 FDA guidance on the safety testing of drug metabolites: Scientific recommendations with flexible interpretation Aisar Atrakchi

Knowledge of how a drug is metabolized provides significant information necessary to adequately understand the behavior of a drug in the body and impacts its clinical safety. Differences in metabolism between humans and animals are not new nor unusual. Dealing with such differences during drug development is discussed in the recently finalized FDA guidance to industry on the safety testing of drug metabolites (February 2008). Identification of drug metabolites has become more prevalent due to significant advancements in analytical technologies. Metabolites may contribute to the therapeutic efficacy and/or the toxicity of a drug. The FDA guidance makes recommendations concerning when and under what circumstances to test a metabolite that is present at quantitatively higher levels in humans but not formed or, is found at much lower levels in animals. The FDA guidance supports and encourages a rational and scientific approach to the design of nonclinical studies that may be needed to investigate the clinical safety of a human metabolite.

41 Strategies to obtain exposures of metabolites in preclinical species through plasma pooling and quantitative NMR in the absence of radiolabeled compounds and chemically synthesized metabolite standards Abdul Mutlib, Karthick Vishwanathan, Kathlene Babalola, Jack Wang, Robert Espina, Linning Yu, Adedayo Adedoyin, Rasmy Talaat, and JoAnn Scatina.

The "Safety Testing of Drug Metabolites" guidance issued by the US FDA has highlighted the importance of identifying and quantitating significant circulating metabolites in human plasma during early drug development. To demonstrate the coverage of these metabolites in preclinical safety species, exposure levels (AUC) of these metabolites are needed. However, quite frequently synthetic standards of

metabolites are not available and, hence, obtaining their AUC values can be a challenge. In this presentation, we demonstrate how NMR, LC/UV/MS and plasma pooling methods can be used to obtain AUCs of metabolites in the absence of synthetic standards. NMR was demonstrated to be useful in quantitating biologically generated metabolites, which were subsequently used as "reference standards" for further quantitative studies. A practical solution is presented that enables us to obtain a quantitative assessment of metabolite exposure in humans and coverage in toxicology species, hence circumventing the use of synthetic metabolite standards and radiolabeled compounds.

42 Assessing the metabolism of drugs in the context of assuring safety R. Scott Obach,

In any given example of adverse effects of a drug, the possibility exists that a metabolite or metabolites could be mechanistically involved. Thus, there has been considerable interest in knowledge of human metabolite profiles of new chemical entities and the comparison of these profiles to those observed in animal species used in toxicology assessments. It is well known that chemically reactive metabolites that can react with tissue macromolecular nucleophiles (e.g. the quinoneimine metabolite of acetaminophen) can be responsible for toxicities that are unrelated to the target effect of the parent compound. However, examples of circulating chemically stable metabolites causing a toxicity that is unrelated to effects already possessed by the parent compound are unknown. Furthermore, gaining a quantitative profile of circulating metabolites almost always requires the administration of radiolabelled parent compound to humans and careful and logical interpretation of such data must be done so as to ensure that abundant metabolites are addressed while unnecessary scrutiny is not placed on low concentration metabolites. Other less definitive approaches can be used to address whether human circulating metabolites are present in animals and these will be illustrated. To address the possibility that a new compound could generate a chemically reactive metabolite, many investigators apply in vitro assays such as nucleophile trapping studies or measurements of metabolism-dependent covalent binding to protein. However, while such assays can raise the possibility that a compound is capable of being bioactivated, they are a poor indicator of the potential for toxicity. This shows that there is considerable research needed to bridge the gap between metabolic bioactivation of drugs to reactive intermediates and toxicity outcomes.

43 Strategic considerations for addressing the safety assessment of human drug metabolites Clay B. Frederick

The 2008 FDA regulatory guidance on the Safety Testing of Drug Metabolites presents significant strategic and scientific challenges for drug development. The most significant strategic issue relates to the feasibility of quantitatively evaluating the profile of circulating human drug metabolites early in development relative to the metabolite profiles observed in the nonclinical safety assessment species. Ideally, this interspecies comparison will provide assurance that the drug metabolites were adequately evaluated in the nonclinical safety assessment studies. The use of UPLC-HRMS technology for these interspecies metabolite profile comparisons will be discussed. The derivative scientific concern relates to the capacity of nonclinical species to generate sufficient plasma concentrations of human metabolites to meet the regulatory requirements. To potentially increase the formation of human drug metabolites in nonclinical species, genetically-engineered nonclinical species containing human metabolic enzymes are being evaluated. Progress in the evaluation of 'humanized' safety assessment models for drug development will be discussed.

TUESDAY AFTERNOON

Drug-Induced Mitochondrial Dysfunction and Human Disease: New Insights and Applications

Kevin Leach and David Thompson, Organizers

44 Strategies to reduce NCE attrition due to mitochondrial toxicity: Novel screening methods Yvonne Will

Mitochondria produce almost all the energy in cells, but also chronically expose the cell to cytotoxic free radicals. Many widely prescribed therapeutics undermine mitochondrial function by interfering with DNA replication or expression, and more acutely, by uncoupling or inhibiting oxidative phosphorylation, leading to a variety of organ toxicities such as hepatic, cardiac, muscle, kidney and CNS. Early identification of new chemical entities (NCE) that perturb mitochondrial function is therefore of

significant importance in drug discovery if attrition due to toxicity is to be avoided. Within the past three years we have developed high throughput capable assays (HTS) to assess mitochondrial function at the organelle and cellular level. These include oxygen sensors to measure mitochondrial respiration in isolated mitochondrial and cells, immunocapture of individual electron transport chain proteins that can identify inhibitors of mitochondrial electron transport and metabolic profiling using solid oxygen and pH sensors. We discuss the strength and limitations of new HTS applicable screens and provide recommendations of where to position these assays within drug development

45 Is underlying mitochondrial dysfunction a susceptibility factor in idiosyncratic drug-induced liver injury? Urs A. Boelsterli

Idiosyncratic drug-induced liver injury (DILI) is not only a significant clinical problem but also poses a major challenge for the pharmaceutical industry due to its unpredictable nature. The underlying determinants of susceptibility in patients are not understood. However, cellular studies and animal models have revealed that many of the DILI-causing drugs target mitochondria, causing mitochondrial permeabilization and cell death. In addition, mitochondria are sine-qua-non amplifiers of drug-induced cell death in hepatocytes. Therefore, preexisting conditions that sensitize cells to mitochondria-mediated injury may be an important risk factor for developing liver disease. We used a mouse model to test the hypothesis that a discreet and clinically silent deficiency in the mitochondrial antioxidant defense system in otherwise phenotypically normal mice can greatly potentiate mitochondrial stress and lead to overt cell injury in the liver. Studies with heterozygous Sod2^{+/-} mice, but not wild-type mice, demonstrate that a number of DILI drugs, but not their safer bioisosteric analogs, can be converted into hepatotoxic compounds in this mouse model when given at low dose for an extended period of time. The underlying mechanisms are complex and include cumulative oxidative injury and decreases in mtDNA-encoded protein subunits, reaching the mitochondrial threshold and triggering mitochondria-mediated cell death. However, only the translation of these findings to the clinical situation can ultimately provide the proof of concept.

46 Insights into drug activity and toxicity based on a chemical dissection of mitochondrial function Bridget K. Wagner

Understanding OXPHOS function and regulation, particularly within the context of the entire cell, has important implications for managing many human diseases. Traditional approaches to studying energy metabolism in the mitochondrion have focused either on the kinetics of ATP synthesis in isolated mitochondria or on transcriptional control of mitochondrial components. Our goal was to combine physiologic and genomic profiling of intact cells in order to probe OXPHOS function and regulation in response to thousands of small-molecule perturbations. We systematically combined four cell-based assays of OXPHOS physiology with multiplexed measurements of nuclear and mtDNA gene expression across ~2,500 small-molecule perturbations in cultured muscle. We observed that a subset of HMG-CoA reductase inhibitors, combined with propranolol, can cause mitochondrial toxicity, yielding potential clues about the etiology of statin myopathy. We also discovered that structurally diverse microtubule inhibitors stimulate OXPHOS transcription, while suppressing reactive oxygen species, via a transcriptional mechanism involving PGC-1 β and ERR α . This integrated approach provides a rich description of cellular mitochondrial state, reporting on more stable changes in the organelle, which can be useful for studying its longer-term adaptations. Our compendium is freely available, and can be used as a discovery tool both for understanding mitochondrial biology and toxicity and for identifying novel therapeutics.

47 Development-limiting pancreatic toxicity due to a novel mitochondrial mechanism David C. Thompson

Development-limiting exocrine pancreatic toxicity (mitochondrial swelling and atrophy) was observed in a pre-clinical safety study with a drug candidate, which prompted a series of investigative studies in an effort to uncover the potential mechanism. The lesions were both species and target organ specific. Studies on respiration, swelling and cytochrome c release in isolated mitochondria from various tissues suggested an indirect mechanism that was unique to the pancreas. Follow-up in vivo studies confirmed the proposed mechanism and identified new chemical substrates for further clinical development.

TUESDAY EVENING

General Posters

Kaushik Mitra, Organizer

6:00 - 10:00

48 Steric and electrostatic effects at the C2 atom substituent influence replication and miscoding of the DNA deamination product deoxyxanthosine and analogs by DNA polymerases Huidong Zhang, Urban Bren, Ivan D. Kozekov, Carmelo J. Rizzo, and F. Peter Guengerich

Deoxyinosine (I) and deoxyxanthosine (Xa) are both formed in DNA in vivo by deamination of A and G, respectively. The replicative bacteriophage T7 DNA polymerase/exonuclease⁻ (pol T7⁻) and translesion DNA polymerase *Sulfolobus solfataricus* pol IV (Dpo4) were used as two models to discern the miscoding mechanisms. I showed similar catalytic efficiency to guanine for either polymerase. Xa was highly miscoding of four dNTPs by both polymerases. Our studies with electronegative analogs showed that the strong attenuation of binding and of catalytic activity can be explained by a combination of the steric and electrostatic clash of the charged oxygen atom (of Xa, or a halogen in 2-FI or 2-BrI) with the O2 atom of dCTP, as opposed to either bulk or perturbation of purine ring electron density alone. (Supported by Grants R01 ES010375, P01 ES005355, P30 ES000267, S10 RR019022)

49 Aristolochic acid, a human carcinogen Francis Johnson, Masaaki Moriya, Radha R. Bonala, Sivaprasad Attaluri, Charles R. Iden, and Arthur P. Grollman Abstract

Aristolochic acid nephropathy (AAN) is a global disease that occurs frequently in Balkan countries and in Asia and occasionally in the USA. AAN is characterized by a progressive interstitial renal fibrosis associated in ~50% of the cases with upper urothelial cancer. AAN is caused by the chronic ingestion of Aristolochia herbal remedies and by ingestion of bread prepared from flour contaminated by aristolochic acids I and II. We have synthesized de novo the dA and dG adducts of AA II, incorporated them into oligomeric DNA and established their mutagenic spectra, using site-specific techniques. The synthetic methods and biological results will be presented.

50 Evaluation of hyaluronic acid-based ocular viscosurgical devices (OVDs) using an in vitro human corneal epithelial cell line model Jessica Cobb, Alexandra Lee, Roger Tran-Son-Tay, Malisa Sarntinoranont, and Hoan-My D. Luu

Abstract text not available

51 Application of the direct silylation GC-MS scan technique for identifying potential toxicants in reference smokeless tobacco products John H. Lauterbach and Deborah A. Grimm,

Some health experts are recommending that smokers who refuse to quit or refuse to use nicotine replacement therapy switch to low nitrosamine, Western-style smokeless tobacco products (STP). However, relatively little public information on the detailed chemistry of such products; and there is evidence for toxicants in STP other than the so-called GothiaTek analytes. Consequently, we used the direct silylation GC-MS scan technique, which is known to provide identifications and semi-quantitative data, on acids, humectants, sugars, and certain other compounds, for the partial characterization of the three types of reference STP [loose-leaf chewing tobacco (1S1 and 2S1), dry snuff (1S2), moist snuff (1S3 and 2S3)]. In general, the total ion chromatograms and mass spectra obtained were reflective of published data on these products and the underlying tobacco chemistry. However, there were unexpected compounds found and tentative identifications have been made. These compounds may have originated during many years of storage.

52 Banned in Nashville: The presentation that would have been too hot for the 62nd Tobacco Science Research Conference John H. Lauterbach

Health experts have called for regulatory limits on several classes of cigarette mainstream smoke toxicants collectively known as the Hoffmann analytes. However, the 40+ analytes selected were those deemed important and measurable in the early 1990s. At the 61st Tobacco Science Research Conference (TSRC), we presented a paper (#38) suggesting certain dicarbonyls, cyanohydrins, and free radicals be added to the Hoffmann list before it was used for regulatory purposes. In April 2008, Burns et al. (Tob. Control 17:132-41) called for ceilings for certain Hoffmann analytes based on literature data. We had planned to present a critique of Burns et al. at the 62nd TSRC (Nashville, TN), but our abstract was reportedly rejected for our use of literature data and in silico estimates instead of new (and costly) experimental data. Our rationale for additions to the Hoffmann list and their relevance to potential regulation of cigarette designs will be given.

53 Identification of plasma protein targets of 4-hydroxynonenal and their sites of modification utilizing click cycloaddition, photocleavable linkers, and shotgun proteomics Hye-Young Kim, Keri A. Tallman, Simona G. Codreanu, Daniel C. Liebler, and Ned A. Porter

The prototypical lipid electrophile 4-hydroxynonenal (HNE) is thought to contribute to protein damage in toxicities and diseases associated with oxidative stress. A challenge in characterizing protein damage by HNE and similar lipid electrophiles is the difficulty of detecting adducts in the presence of excess unmodified protein. We have previously applied the HNE analog omega-alkynyl-HNE (aHNE) and Click chemistry to biotinylate and capture aHNE protein adducts. However, bulky tags resulting from Click cycloadditions often preclude mapping sites of aHNE modification. Here we introduce a photocleavable azido-biotin, which enabled enrichment of aHNE adducts either at protein or peptide level to identify target proteins and their adduction sites in the complex mixture of proteins. We describe here a protein catch and photorelease strategy, for enrichment of aHNE adducted proteins, and a peptide catch and photorelease strategy, for enrichment of aHNE adducted peptides following proteolytic digestion. The isolated adducted proteins and peptides were analyzed by LC-MS/MS. We applied this approach here to identify plasma protein targets of aHNE. We further mapped one or more specific adduction sites in 14 plasma proteins adducted with aHNE. The application of a photocleavable biotin-azide reagent enables combination of Click chemistry-based proteomics analyses with site-specific mapping of modifications.

54 Identifying the common binding modes of flavonoids to cytochrome P450 3A4 Sarangan Ravichandran

Enzyme cytochrome P450 3A4 is mostly expressed in liver and intestine and plays a major role in the metabolism and detoxification of a wide-range of compounds including several drug molecules and chemical carcinogens. Over the period of several years, a significant number of drugs are withdrawn due to toxicity especially in the liver. Recently natural substances from several food sources and fruit juices such as black raspberry, wild grape, black mulberry and red wine have been shown to modulate P450 function thereby increasing the risk of drug-food interaction and toxicity. The major common chemical constituents in these natural substances are flavonoids such as anthocyanins, proanthocyanidins and phenolic metabolites and they have been speculated to play the role of inhibitors in P450 systems. Existing modeling studies of P450 inhibition never considered flavonoids and this new information has been used in this study to build a pharmacophore model for P450 3A4 inhibition. The model from this study differs from the existing models in several aspects, and the current study will discuss the similarities and differences between the models.

55 Comparative mutational profiles of the environmental mammary carcinogen 6-nitrochrysene and its [R,R]- and [S,S]-trans-1,2-dihydroxy-1,2-dihydro-6-nitrochrysene metabolites in a lacI mammary epithelial cell line Yuan-Wan Sun, Joseph B. Guttenplan, Wieslawa Kosinska, Jacek Krzeminski, Shantu Amin, and Karam El-Bayoumy

The metabolite trans-1, 2-dihydroxy-1, 2-dihydro-6-nitrochrysene (1, 2-DHD-6-NC) is the proximate carcinogen/mutagen of 6-nitrochrysene (6-NC), a potent mammary carcinogen in the rat. The goal of this study was to evaluate the effect of stereochemistry of 1, 2-DHD-6-NC on the mutagenicity of 6-NC. We first resolved the 1, 2-DHD-6-NC isomers using chiral stationary phase HPLC and determined the absolute configuration by circular dichroism and optical rotation methods. Using the cII gene of lacI mammary epithelial cells in vitro, we then examined the mutation fractions and mutation spectra of [R,R] and [S,S]-enantiomers. Our results showed that [R,R]-is much more potent mutagen than the [S,S]-isomer and mutation spectra induced by [R,R]- are similar to those obtained from 6-NC in vivo in the mammary gland of rats treated with 6-NC. The results of mutation profiles of [R,R]-, [S,S]- and 6-NC appear to support a major contribution of the [R,R]-enantiomer in the mutagenicity of 6-NC.

56 Perfluoro toxicity (LC50 inhalation) in rat and mouse using QSAR Barun Bhatarai and Paola Gramatica

PFOS and PFOA are a class of perfluoro-compounds (PFCs) which are categorized by US-EPA and EU-REACH as toxic chemicals. PFCs are widely distributed in the environment, of which some are classified as 'emerging pollutants'. These pollutants are known to be associated with environmental and health-related toxicities. This raises alarms for other commercially used PFCs, on which very few experimental data on environmental and bio-toxicity are available. Moreover, toxicity profiles are different for different animals and species. QSAR is applied to understand the inter-species toxicity of PFCs by modeling inhalation (LC50) data in the rat and mouse. Training and test set compounds were prepared on the available data by splitting using: Self-organizing-map and random-selection-through-activity-sampling⁴. These sets are used to derive statistically robust and predictive models with well defined 'applicability domain' for all PFCs retrieved from database and journals. The descriptors involved, and inter-species similarities and differences observed will be discussed.

57 CYP11A1-mediated bioactivation of a met kinase inhibitor associated with rat adrenal cortical toxicity William G. Humphreys, Donglu Zhang, Ashok Gupta, Richard Westhouse, Guoxiang Shen, Yueping Zhang, Alban Allentoff, Robert Borzilleri, Aouatef Bellamine, Punit Marathe, and Oliver Flint

A Met kinase inhibitor was associated with dose- and time-dependent vacuolar degeneration and

necrosis in adrenal cortex following oral administration in rats. Pretreatment with aminobenzotriazole (ABT), a P450 inhibitor, ameliorated the toxicity. Following oral dose of C-14 labeled compound, two hydroxylated metabolites were identified as prominent species only found in the adrenal tissue. In addition, a high level of radioactivity was covalently bound to adrenal tissue proteins, 40% of which was localized in the adrenal mitochondrial fraction and the covalent binding could be reduced by ABT-pretreatment. The hydroxy-metabolite found in vivo and protein covalent binding were detected in incubations with the adrenal mitochondrial fraction in presence of NADPH, which was not affected by addition of glutathione or a CYP11B1 inhibitor, metyrapone, but was inhibited by ketoconazole and a CYP11A1 inhibitor, aminoglutethimide. These results are consistent with a CYP11A1-mediated bioactivation mechanism to generate a reactive species leading to protein covalent binding.

58 Safety evaluation of Asparagus racemosus: A commonly used herb of Ayurvedic medicine in Charles Foster rats Debabrata Chanda, K Patider, A Pal, Suaib Luqman, D U Bawankule, D Mani, and N P Yadav

Asparagus racemosus Willd., one of the most important medicinal plant is regarded as a 'rasayana' in the Ayurvedic system of medicine and has been recommended as galactagogue, aphrodisiac, anodyne, diuretic and nerve tonic since time immemorial necessitating its incorporation in a number of important Ayurvedic formulations like Shatavarikalpa, Phalaghrita and Vishnutaila. However, data regarding safety or toxicity of *Asparagus racemosus* in animal system is lacking. Hence the present experiment envisaged acute and sub-acute toxicity of *Asparagus racemosus* root aqueous extract in adult Charles Foster rats following the guidelines of OECD including parameters like observational, hematological, biochemical and pathological studies. Except serum creatinine level in acute study and SGPT activity and serum creatinine level in sub-acute study, all the observational, hematological and biochemical parameters studied showed non-significant changes. Histopathological examination of hepatic sections of sub-acute samples showed mild inflammatory and fatty changes at higher doses.

59 Presenting procedure for safe operation of exothermic reactions in chemical industries Fatemeh Abniki, Dolat Abad, Shahrake Vahdat, and Ehsan Bakhshi

In this research with regarding importance of safe performance of chemical reactions, a practical procedure is presented which allows deciding for each type of reaction system considering different solvents and concentrations, if the reaction can be carried out safely in the existing apparatus. The procedure is essentially based on the characteristics of the reaction by the achievable adiabatic temperature rise and assumption for a simplified formal reaction kinetic from which the maximum pressure in the apparatus and the maximum vapor production rate can be calculated. Also, there is a minimum amount of information necessary if a new reaction is introduced into a multipurpose reactor. At least, the thermal stability of all reactants, including side and consecutive products has to be investigated. An essential part of the proposed practical procedure for safety handling exothermic chemical reactions in multipurpose plants is the design of the pressure relief device on the basis of incomplete information about the reaction. It is described with conservative assumption about the reaction kinetic behavior. Herewith, a formal kinetic model is developed, which is based only on the adiabatic temperature rise of the reaction. On basis of this model, the required relief area can be calculated. In case of reactions with adiabatic temperature rises higher than 100 K, it is recommended to carry out a single case investigation. Then, it might even result that pressure relief is unsuitable. Generally, the proposed safety concept for multipurpose plants is applicable for most of the reaction systems used in chemical industry on condition that they are carried out in solvents. The procedure allows evaluating if a new reaction fits to the safety concepts of an existing plant.

60 Ultrasensitive quantitation of carboplatin-DNA adducts provides mechanistic insights into breast cancer drug resistance Teesta Jain, Tao Li, Miaoling He, Chong-Xian Pan, and Paul T. Henderson

Carboplatin is widely used in treating metastatic breast cancer as part of second-line chemotherapy. It would be useful to identify resistance to carboplatin before exposing patients to chemotherapy in order to allow personalized therapy. Toward this goal, experimental data are presented that indicate carboplatin microdoses (1/100th the therapeutic dose) are useful in predicting drug resistance at the cellular level. Accelerator mass spectrometry (AMS) allowed correlation of [¹⁴C] carboplatin microdose data with response to chemotherapy in a variety of breast cancer cell lines. AMS could distinguish sensitive from resistant cells based on the level of carboplatin-induced DNA damage, drug-DNA repair kinetics, drug uptake, efflux and intracellular inactivation. DNA damage caused by the microdoses and the therapeutic dose of carboplatin were linearly proportional, indicating that microdoses of carboplatin can be used to predict therapeutic chemoresistance in a variety of breast cancer cells, which may have relevance for tumor studies.

61 Chemical aspects of acylfulvene bioactivation to a cytotoxic reactive intermediate Kathryn E. Pietsch, Xiang Yu, James F. Neels, Jiachang Gong, and Shana J. Sturla

Understanding molecular mechanisms of cytotoxicity is vital to the development of more effective chemotherapies. Acylfulvenes (AFs) are a class of semisynthetic analogues of the natural product illudin S. Minor structural changes between the parent compound and AFs have resulted in a more favorable selectivity profile in preclinical chemotherapy assays. AF cytotoxicity involves alkylation of biological targets, including DNA and cellular proteins. While AFs are capable of direct alkylation, reductive bioactivation to an electrophilic intermediate is correlated with enhanced cytotoxicity. Alkenal/one oxidoreductase (AOR) is a cytosolic enzyme implicated in activating AF in cells that are sensitive to the drug. This study aims to elucidate chemical aspects of acylfulvene activation mechanisms. We compared enzymatic versus chemical activation pathways for AF involving NADPH-dependent AOR or sodium borohydride, respectively. These two processes result in isomeric reactive intermediates. Despite structural differences, these isomers appear to have similar biological activity and give rise to similar patterns of DNA modification. Cell-based studies, utilizing human embryonic kidney cells transiently transfected with an AOR-overexpressing vector, were conducted to test the hypothesis that a chemically activated AF does not require further bioactivation to be cytotoxic. On the basis of this study, we anticipate that the chemically activated form of AF will serve as a useful tool for evaluating protein and nucleic interactions, and understand their contributions to cytotoxicity, independent of bioactivation.

62 Development of a sensitive method for the observation and characterization of unknown adducts in human peripheral blood leukocyte DNA Silvia Balbo, Peter W. Villalta, Siyi Zhang, and Stephen S. Hecht

We developed a mass spectrometric method utilizing neutral loss of 2'-deoxyribose ($[M + H - 116]^+$) for the observation of unknown DNA adducts. A low flow (300 nL/min, 75 μ m ID column) separation utilizing a trap column for relatively large injection volumes (8 μ L) along with nanospray ionization was used to attain the sensitivity necessary for the detection of low levels of DNA adducts. A mixture of 6 deoxyguanosine adducts was used to optimize the separation and to assess the sensitivity of the approach. The analytical method was applied to compare DNA from different blood cells, from smokers and non-smokers. Lymphocytes and neutrophils are the two most abundant cell subpopulations in peripheral blood with large differences in lifespan (several months and 2-3 days, respectively). The identification of adducts in these cells could potentially reflect distinct exposure effects and provide information on formation and repair of the adducts.

63 Unfolding thermodynamics of a non-self complementary DNA dodecamer duplex containing single or multiple dT to dU substitutions Barry Gold, Irine Khutsishvili, and Luis A. Marky

Deoxyuracil (dU)odA base pairs can arise by misincorporation of dUTP into DNA or from deamination of dC and DNA replication of the resulting dUodG pair. dU can base pair similar to dTodA, but is rapidly removed from DNA by uracil-DNA glycosylase (UNG). To understand the process by which UNG finds dU, we investigated the thermodynamic contributions of the ionic and hydration environments of dAoT and dAoU in DNA. We report that the incorporation of dU yielded duplexes with lower stabilities, lower folding heats, higher uptake of counterions and lower immobilization of water. The results show that the reduced stabilities of the dU substituted duplexes are predominantly due to unfavorable enthalpy terms. The inclusion of dU produces more hydrophilic DNA duplexes with reduced stacking contributions. Therefore, the exclusion of the methyl group from dT causes an exchange of structural for electrostricted water molecules. Supported by grants MCB-0315746 (NSF) and RO1CA29088 (NIH).

64 Simultaneous determination of inositol and inositol phosphates in complex biological matrices using quantitative ion-exchange chromatography/tandem mass spectrometry Xiaodan Liu, Peter W. Villalta, and Shana J. Sturla

The simultaneous measurement of myo-Inositol (Ins) and myo-inositol phosphates (InsPs) in cells and plant sources can impact the understanding of their role in nutrition, cellular processes and diseases, and how they are modulated by diet. An anion-exchange chromatography/tandem mass spectrometry method for the simultaneous quantitation of Ins and different naturally occurring phosphorylated inositol compounds has been developed. Separation was achieved in 30 min and the analytes were identified by selective reaction monitoring using a triple quadrupole mass spectrometer in negative ion electrospray ionization mode. Adenosine 5'-monophosphate was used as a general internal standard for quantitation. The limit of detection is 0.25 pmol with a signal-to-noise ratio of 10:1 for all analytes. Using this approach, Ins and InsPs were measured in three different plant samples and in cultured cells, illustrating significant differences in the distribution of inositol compounds in food samples compared to cells and between cell types.

65 Tolerance of Cr (VI) and Hg (II) on differential concentration by Eichhornia crassipes in hydroponic culture Rajkishore Patel

The phytoremediation of Cr (VI) and Hg (II) has been studied from a synthetic solution by an aquatic

plant *Eichhornia crassipes*. Plants were cultured in a modified Hoagland's nutrient solution at pH 6.8 supplemented with 0, 0.75, 1.50, 2.50, and 4 mg Cr/l as K₂Cr₂O₇ and 0, 5, 10, 15, and 20 mg Hg/l as HgCl₂. They were separately harvested after 3, 6 and 9 days. Plants exposed to Cr (VI) and Hg (II) showed significant decrease in the biomass productivity and total chlorophyll content with increase in metal concentration and exposure time. The accumulation of metals in the plants increases but not linearly with the exposure time and metal concentration. The maximum values of bio-concentration factor (BCF) for Cr (VI) and Hg (II) were found to be 734.3 and 856.2 mg/l, respectively. The toxicity symptoms increased when the exposure time and metal concentration were increased.

66 High-throughput metabolic toxicity assessment of compounds using the metachip-datachip platform **Prashanth Asuri**, Jessica Ryan, Moo-Yeal Lee, Guangyu Zhu, Michael Hogg, Douglas S Clark, Jonathan S. Dordick, and David Rozzell

Although conventional 2D cell culture assays have been valuable for identification of toxic drug candidates, it is well established that a 3D matrix will serve as a more realistic environment for toxicology assessment than 2D cell culture surfaces, since the majority of cell types grow in 3D in vivo. And, since cytotoxicity and metabolism assays are typically performed in 96- or 384-well plates and well-plate experiments are costly, the miniaturization of both cell toxicity and metabolism assays will significantly reduce the cost of future strategies based on in vitro assays to predict toxicity. Moreover, in a recent workshop conducted to assess the merit of using in vitro cytotoxicity tests for predicting the acute oral lethality of chemicals in humans and animals, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) concluded that the available 2D in vitro cell assays would require further development. Thus, Solidus Biosciences, Inc. has developed a miniaturized 3D cell-culture array (the Data Analysis Toxicology Assay Chip, or DataChip) that was used in conjunction with a complementary human metabolizing enzyme-containing microarray (the Metabolizing Enzyme Toxicology Assay Chip or MetaChip) to screen compounds and their metabolites for toxicity. As a first proof of concept, the DataChip/MetaChip platform has been used to screen 30 model compounds profiled in the ICCVAM workshop, for toxicity against Hep3B human hepatoma cells. The DataChip/MetaChip rapidly assessed the toxicity of each of the 30 compounds, and the cytotoxicity profiles generated under varying metabolic conditions were representative of the in vivo rodent LD50 data published in the workshop. Therefore, the DataChip/MetaChip serves as a high throughput in vitro assay tool for predictive toxicology that can contribute to satisfying the demands of emerging regulatory requirements.

67 DNA base sequence effects in nucleotide excision repair of benzo[a]pyrene diol epoxide-derived N²-guanine lesions in DNA by the prokaryotic UvrABC system **Jian Ren**, Konstantin Kropachev, and Nicholas E. Geacintov

The removal of bulky DNA adducts by nucleotide excision repair (NER) mechanisms depends on a number of factors that include the chemical structure of the lesion, its conformation in double-stranded DNA, and base sequence context. The effects of flanking bases on NER are poorly understood. We have compared the removal of 10S ((+)-trans-B[a]P-N₂-dG adducts (G*) by the UvrABC NER system in different XG*Y sequence contexts in 43-mer duplexes where X, Y = T, A, or C (all other base pairs were identical). Compared with the eukaryotic NER system, UvrABC proteins are less complex and thermostable, and might provide insights into general principles of structure-function relationships. The flanking bases exert variable activities on DNA repair with the NER activity being highest in the case of TG*T, and lowest in the case of AG*A. We thank Dr. B. Van Houten's laboratory for supplying the UvrABC proteins. Research supported by CA099194.

68 Mutagenesis studies with synthetic nucleoside triphosphates **Hailey L. Gahlon** and Shana J. Sturla

DNA damage arising from exposure to both endogenous and exogenous alkylating agents, if not effectively repaired, may lead to mutation. Upon enzyme-mediated metabolic activation, carcinogenic nitrosamines, which may be derived from dietary and environmental exposures, can initiate carcinogenesis by this mechanism. A common structural motif associated with nitrosamine-mediated DNA alkylation involves the formation of bulky O₆-alkylguanine adducts. These DNA adducts are found physiologically at very low levels, and in part because of this, it has been difficult to evaluate their specific contributions to carcinogenesis. We have pursued a strategy involving synthetic nucleoside analogs to probe the occurrence and properties of O₆-benzylguanine (O₆BnG) in DNA as a representative bulky adduct. Previous studies have shown that the Y-family polymerase dpo4 can undergo translesion DNA synthesis past these bulky O₆BnG adducts. We will present data for studies we have carried out to address aspects of polymerase-mediated synthesis of DNA involving synthetic O₆BnG-modified templates and potentially complementary synthetic nucleoside triphosphates. Data will be presented concerning the preparation and characterization of required nucleic acid components, and results of testing the selectivity and efficiency of polymerase-mediated DNA primer extension.

69 Toxicity study of gold nanoparticles in Salmonella typhimurium TA102 Shuguang Wang, Rasheeda Lawson, Paresh Chandra Ray, and Hongtao Yu

Gold (Au) nanoparticles are a versatile material whose unique size- and shape-dependent properties make it ideal for a wide-range of electronic and biological applications. The impact of nanomaterials on human health and the environment has gained much attention in the last decade. In this research, a 16 nm gold nanoparticle was synthesized using hydrogen tetrachloroaurate (HAuCl₄) and sodium citrate. The stability of Au nanoparticles under light irradiation or in the presence of phosphate buffer was studied by absorption spectroscopy and Transition Electron Microscopy (TEM). The absorption of Au nanoparticles does not change after 1 h irradiation by a 300 W Xenon lamp and after 24 h treatment with 20 mM phosphate buffer, indicating that Au nanoparticles are stable both under light irradiation and phosphate buffer treatment. It is found that Au nanoparticles are photomutagenic in Salmonella typhimurium TA102, the presence of Au nanoparticles and light irradiation causes the bacteria to revert to its natural state. However, the photomutagenicity is only slightly dependent on the concentration of the Au nanoparticles. Due to this unusual behavior, we also tested the photomutagenicity of sodium citrate and Au (III) solution. Under our experimental conditions, Au(III) solution is not mutagenic, but is photomutagenic at the concentrations between 0.15-0.75 μM . It becomes strongly toxic at 3.7 μM and the toxicity is enhanced by light irradiation. Sodium citrate appears to be photomutagenic as well, and the photomutagenicity changes slightly with the increasing concentration. In addition, neither Au(III) nor Au nanoparticles can produce singlet oxygen due to light irradiation. Therefore, the apparent photomutagenicity of Au nanoparticle solution is due to the remnant Au(III) or sodium citrate, not Au nanoparticles themselves. Acknowledgement: National Science Foundation grants NSF-PREM #DMR-0611539 and REU DMR #0755433. Keywords: Gold nanoparticle, hydrogen tetrachloroaurate, trisodium citrate, photomutagenicity

70 Formation of acrylamide at 37C: Role of oxidative stress Eden Tareke, Goncalo Gamboa, Thomas Heinz, and Syed Ali

Significant increase ($p < 0.05$) in acrylamide Hb adduct levels was observed in mice treated with chemicals that induce oxidative stress. Since there is no plausible metabolic pathway that may lead to direct formation of acrylamide from the chemicals, the finding suggests that acrylamide may probably be formed endogenously as a result of free radical formation. This observation is supported by in vitro studies that showed that acrylamide is formed at 37C in a solution of glucose and asparagine upon addition of H₂O₂. In the solution acrylamide levels increased with incubation time. These results prove the formation of acrylamide at physiological temperature. The study raises the question of possible endogenous formation of acrylamide. Therefore, the study warrants more studies to investigate the possible endogenous formation of acrylamide and to understand the effect of long term chronic oxidative stress on the internal dose of acrylamide.

71 A thermodynamic study of DNA with 8-oxoguanine lesions Barry Gold, Sreelekha Singh, and Luis A. Marky,

The oxidation of DNA is a major pathway for DNA damage, which if not repaired can lead to mutations and an increase in the incidence of cancer. The predominant lesion formed in DNA exposed to oxidizing agents is 8-oxoguanine (oxoG). The oxoG lesion is removed from the nucleotide pool by the hydrolysis of oxoGTP by MutT and from ds-DNA by 8-oxoguanine-DNA glycosylase (OGG). Structural analyses of DNA with oxoG by NMR and X-ray crystallography show that the base pairing is very similar to a Watson-Crick G-C pair. However, the NMR provided evidence for a 3-5 °C decrease in thermal stability. The origin of this destabilization was not experimentally addressed. To understand how oxoG affects DNA stability, we initiated a thorough thermodynamic study to understand how the oxoG-C base pair affects DNA stability, hydration and cation binding. Our UV melting and differential scanning calorimetry studies demonstrate that G to oxoG substitution affords duplexes with lower stabilities, lower folding heats, lower uptake of counterions and lower immobilization of water molecules. Assuming a local effect of the oxoG lesion, the reduced stability of the duplex vs. the random coil states for oligomers with oxoG-C indicates that the extrahelical state of oxoG is relatively more stable vs. the fully stacked duplex than it is in the case of an extrahelical G in a G-C base pair. This is explained in terms of its higher flexibility, which is due to a lower binding of both ions and water molecules. (support: NIH RO1CA29088 and NSF MCB-0315746).

72 Inflammation-induced changes in the serum metabolome Erin G. Prestwich, I. Ramesh Babu, Koli Taghizadeh and Peter C. Dedon

One mechanism linking chronic inflammation to human disease involves the generation of chemical and biological mediators, including cytokines and reactive oxygen and nitrogen species, by immune cells at sites of inflammation. In the search for biomarkers of inflammation, we have studied inflammation-induced changes in the serum metabolome in the SJL mouse model of nitric oxide over-production,

which mimics the inflammatory process in humans. Deproteinized serum was analyzed using LC-MS and LC-MS/MS methods, and comparative analysis of the resulting spectra was performed on the basis of m/z values and retention time, targeting metabolites that changed two-fold or more. The results reveal significant differences in levels of several eicosanoids, amino fatty acids, sterols, and phospholipids of the inflamed mice. The potential role of these lipid species and other metabolites as biomarkers of inflammation will be discussed.

73 Biochemical effects of 1,4-dioxo-2-phosphorylbutane (DOB), an abasic lesion resulting from the oxidation of DNA [Lirui Guan](#), and Marc M. Greenberg

1,4-Dioxo-2-phosphorylbutane (DOB) is produced in DNA as a result of cleavage of the C4'-C5' carbon-carbon bond following hydrogen atom abstraction from the C5'-position. Using ternary complexes in which DOB is produced from a synthetic precursor, we show that the lesion produces interstrand and intrastrand cross-links. The yields of these products are dependent upon the surrounding nucleotide sequence. Kinetic studies indicate that in the ternary complex, DOB produces cross-links and undergoes β -elimination to give 1,4-dioxobutene. 1,4-Dioxo-but-2-ene alkylates the DNA.

74 Correlations between the characteristics of fjord region dibenzo[a,l]pyrene diol epoxide-guanine and -adenine adducts in double-stranded DNA and nucleotide excision repair [Adam Schwaid](#), Zhi Liu, Yijin Tang, Fabian A. Rodriguez, Alexander Kolbanovskiy, Yuqin Cai, Shuang Ding, Shantu Amin, Suse Broyde, and Nicholas E Geacintov

The structural characteristics and physical properties of DNA adducts derived from the binding of the highly tumorigenic fjord region dibenzo[a,l]pyrene diol epoxides to N2-guanine and N6-adenine in double-stranded DNA are of significant interest. These bulky DNA adducts exhibit striking differences as substrates for the human nucleotide excision repair (NER) apparatus; the stereochemically different N2-guanine adducts are generally excised with good efficiencies, while the N6-dA adducts are very poor NER substrates. Significant differences are also observed in the thermal stabilities and UV absorbance and fluorescence properties of these adducts site-specifically inserted into 11-mer oligonucleotide duplexes of defined sequence contexts. These observations, coupled with those derived from a more limited set of NMR solution structures, indicate that some of the intercalative adduct conformations impart unusual stabilities to the duplexes that are correlated with the unusual resistance of some of these adducts to human NER. Supported by NIH grants CA099194 (NEG) and CA28038 (SB).

75 Dibenzo[a,l]pyrene diol epoxide-adenine but not -guanine adducts are resistant to nucleotide excision repair in human cell extracts [Konstantin Kropachev](#), Marina Kolbanovskiy, Fabian A. Rodriguez, Yuqin Cai, Shuang Ding, Lu Zhang, Shantu Amin, Suse Broyde, and Nicholas E. Geacintov

Dibenzo[a,l]pyrene (dB[a,l]P) is a fjord polycyclic aromatic hydrocarbons (PAH) and is one of the most potent chemical carcinogens known in rodent model systems. Like benzo[a]pyrene that is metabolized in vivo to the bay region diol epoxides BPDE, dB[a,l]P is metabolized to the analogous fjord region dB[a,l]P diol epoxides (dB[a,l]PDE). Both types of diol epoxides form covalent N2-dG and N6-dA adducts in DNA with BPDE reacting predominantly with guanine in DNA, while dB[a,l]PDE forms similar quantities of guanine and adenine adducts. We have investigated the repair of the adducts by the human nucleotide excision repair (NER) apparatus in HeLa cell extracts. In contrast to the stereoisomeric BPDE-N2-dG, BPDE-N6-dA, and dB[a,l]PDE-N2-dG adducts that are removed by the NER system, the dB[a,l]PDE-N6-dA adducts are resistant to human NER. These differences are discussed in terms of the variable DNA conformations of these lesions. Supported by NIH grants CA099194 (NEG) and CA28038 (SB).

76 Effects of base sequence context on the nucleotide excision repair of AF- and AAF-C8-dG adducts in NarI sequence duplexes [Lu Zhang](#), Konstantin Kropachev, Marina Kolbanovskiy, Alexander Kolbanovskiy, Lihua Wang, Suse Broyde, and Nicholas E. Geacintov

Site-specifically modified oligonucleotides derived from the covalent binding of 2-aminofluorene (AF) to the C8-positions of guanines G4, G5, and G7 in the NarI mutation hot spot sequence 5'-d(CTCG4G5CG7CCATC) were synthesized. In this double-stranded sequence, the 2AF-C8-dG adducts exist as mixtures of two conformers, a syn-guanine base-displaced intercalated (S in the B. Cho notation) and an external anti-guanine external major groove (B) conformation in sequence-dependent proportions (B. Mao et al., *Biochemistry* 37, 95, 1998). The excision of these three sequence-isomeric lesions by the human nucleotide excision repair (NER) apparatus in HeLa cell extracts was investigated and compared to the NER efficiencies of the more bulky 2-(acetylamino)fluorene (AAF) adducts at G4, G5, and G7. The efficiencies of repair of the base-displaced intercalated AAF-C8-dG adducts was greater than that of the AF-C8-dG adducts and depended on base sequence context. Research supported by NIH grants CA099194 (NEG) and CA075449 (SB).

77 Formation of cysteine crosslinks via a sulfenic acid intermediate [Andrea H. Cummings](#), Kripa Keerthi,

and Kent S. Gates

Cysteine residues in proteins are readily oxidized to sulfenic acids. Sulfenic acids, in turn, can act as potent electrophiles that have been observed to form intrastrand protein crosslinks with neighboring amide or cysteine residues. Cysteine-tyrosine crosslinks have also been observed in proteins, but the mechanism(s) of their formation is not clear. Recently, we have provided chemical evidence that cysteine sulfenic acids have the potential to directly forge intrastrand protein crosslinks with tyrosine residues in proteins without the assistance of metal cofactors. We report here on the mechanism of this reaction. In addition, due to the facile nature of this reaction, we propose that phenylalanine residues may also be able to form intrastrand crosslinks with cysteine sulfenic acids. In the work presented here we investigated the intramolecular reaction between a sulfenic acid and a phenylalanine mimic and continued further mechanistic studies of the sulfenic acid with our tyrosine mimic.

78 Furan metabolites react with polyamines and their precursors Mathilde M. Sullivan, Martin B. Phillips, Ding Lu, and Lisa A. Peterson³

Furan, an environmental toxicant, is carcinogenic and hepatotoxic in rodents. The complete mechanism through which furan exerts its toxicity and carcinogenicity is unknown. It is activated by P450 enzymes to the reactive metabolite cis-2-butene-1,4-dial (BDA). BDA has been shown to react readily with glutathione (GSH) to form an intermediate that can then react further with cellular amines. The supernatant from furan-treated hepatocytes was analyzed by LC-MS/MS for glutathione-containing metabolites. Four of these metabolites were identified as GSH-BDA crosslinks with the polyamine spermidine and its precursor ornithine. Recent work suggests that the relative levels of polyamines may have a profound impact on cellular function and health, and that the disruption of homeostasis may have toxicological implications. The disruption of this balance may indirectly relate to the overall toxicity and/or carcinogenicity of furan [Supported by ES-10577].

79 Identification of furan metabolites derived from cysteine-cis-2-butene-1,4-dial-lysine crosslinks Ding L¹, Mathilde M. Sullivan, and Lisa A. Peterson

Furan toxicity and tumorigenicity in rodents are well documented and human risk to furan exposure is being evaluated. However, the mechanism of furan toxicity and carcinogenicity is unknown, partially due to insufficient knowledge about furan metabolic pathways. It is established that furan metabolism is initiated by cytochrome P450 catalyzed oxidation to cis-butene-1,4-dial (BDA), but the downstream metabolic pathways are unclear. LC-MS/MS analysis of urine from furan-treated rats indicated the presence of more than twenty metabolites. We have now characterized seven of them, all of which are derived from a cysteine-BDA-lysine crosslink intermediate. Both cysteinyl residue and lysinyl residue of this intermediate are subjected to further biotransformations, including N-acetylation, sulfur oxidation and oxidative decarboxylation. These data support a hypothesis that BDA is a reactive metabolite of furan that readily crosslinks cysteine and lysine residues in cells. A likely source of these metabolites are degraded protein adducts [Funded by ES-10577].

80 Impact of conformations of bulky DNA adducts on efficiencies of human nucleotide excision repair Dara Reeves, Marina Kolbanovskiy, Shuang Ding, Jacek Krzeminskiy, Yuqin Cai, Alexander Kolbanovskiy, Judy L. Bolton, Shantu Amin, Karam El-Bayoumy, Suse Broyde, and Nicholas E Geacintov

The effects of DNA conformation of different bulky lesions derived from the binding to DNA of metabolites of the equine estrogen metabolite 4-hydroxyequilenin (4-OHEN-dC), the heterocyclic amine food mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP-C8-dG), and the mammary carcinogen 6-nitrochrysene (6-NC-N2-dG) on the efficiencies of nucleotide excision repair (NER) in human HeLa cell extracts were investigated. In the case of the 4-OHEN-dC adducts, the Watson-Crick H-bonding face of dC is blocked, while in the case of the 6-NC-N2-dG adducts, the bulky 6-NC residue is assumed to be positioned in the minor groove, with two possible Watson-Crick H-bonds remaining. The PhIP-C8-dG adducts assume a base-displaced adduct conformation (M. Cosman et al.). In contrast to the 4-OHEN-dC and PhIP-C8-dG adducts that are efficiently excised, the 6-NC-N2-dG lesions are weak NER substrates. The persistence of these latter adducts in vivo could contribute to the known tumorigenic characteristics of 6-NC. Supported by CA035519 (K.El-B), CA112412 (NEG), and CA75449 (SB).

81 Incorporation of gemcitabine and cytarabine into DNA by DNA polymerase beta and ligase III/XRCC1 A. S. Prakasha Gowda, Joanna M. Polizzi, Subarna Hamid, Kristin A. Eckert, and Thomas E. Spratt

2', 2'-Difluoro-2'-deoxycytidine (gemcitabine, dFdC) and 1-β-D-arabinofuranosylcytosine (araC) are effective chemotherapy agents because they inhibit DNA synthesis through termination mechanisms involving replicative polymerases. We evaluated the action of these agents during DNA synthesis involving base excision repair. The kinetics of incorporation of dCTP, rCTP, dFdCTP, araCTP and 2'-fluoro-2'-deoxycytidine triphosphate (FdCTP) by DNA polymerase β into two gapped DNA substrates

were evaluated. The k_{cat}/K_m values ($\mu\text{M}^{-1}\text{min}^{-1}$) for the two DNA substrates are dCTP (6.0, 36) > araCTP (3.0, 0.13), dFdCTP (1.8, 0.05), FdCTP (0.6, 0.16) > rCTP(0.2, 0.0064). The ability of ligase III/XRCC1 to seal the was evaluated. The relative rates of ligation were dC (1), rC (1) > FdC (0.3) > araC (0.014) > dFdC (0.003). It is concluded that the DNA sequence influences the ability of the agents to be incorporated into the DNA but if incorporated ligase very inefficiently seals the nick. The resulting single strand break may provide a means by which these drugs can cause cytotoxicity via the BER pathway.

82 Nucleotide excision repair of cluster bulky DNA lesions Bongsup Cho, Yue Zou, Marina Roginskaya, and Benjamin A. Hilton

Cluster DNA damage refers to two or more lesions located in a single turn of the DNA helix. Unlike oxidative damage, very little is known about the repair of cluster bulky adducts. We determined how and whether tandem bulky adducts can be recognized and removed by the E. coli UvrABC system. The models used were bi-aminofluorene adducts separated by none, one, and two nucleotides, respectively, in the NarI hot spot sequences G1*G2*CG3*C, G1G2*CG3*C and G1*G2CG3*C. 51-bp DNA substrates were efficiently incised at an unusual site about 15 nucleotides 3' to the bi-adducts. However, no 5'-incision was observed in sharp contrast to the efficient 5'-incision of the mono-adduct substrate. Efficiency of the 3'-incision appeared to decrease with the number of nucleotides separating the bi-adducts. Our results suggests that NER alone is unable to remove the bi-adducts and a combination of different DNA repair pathways could be responsible for their repair in cells.

83 One-electron reduction of 13S-hydroperoxy-9Z,11E-octadecadienoic acid generates pentyl radicals that alkylate guanine neutral radicals in DNA Jie Shao, Conor Crean, Nicholas E Geacintov, and Vladimir Shafirovich

Oxidative modification of guanine bases by free radicals derived from the one-electron reduction of 13S-hydroperoxy-9Z,11E-octadecadienoic acid (13S-HPODE) was initiated by the selective two-photon ionization of 2-aminopurine (2AP) embedded in the oligonucleotide 5'-d(CC[2AP]TCGCTACC). The 2AP residue can be selectively excited by 308 nm XeCl excimer laser pulses, thus yielding 2AP^{•+} radical cations, together with hydrated electrons. In deoxygenated buffer solutions (pH 7.5) the hydrated electrons reduce 13S-HPODE to form unstable alkoxy radicals, which undergo fast β -scission that gives rise to pentyl radicals. In turn, the 2AP^{•+} radicals are strong one-electron oxidants that selectively oxidize the nearby guanine, thus forming G(-H)[•] radicals. The combination reactions of G(-H)[•] and pentyl radicals generate 8-pentyl- and N2-pentylguanine lesions. The latter were excised from the oxidized oligonucleotides by enzymatic digestion, and identified by LC-MS/MS using the authentic standards of the uniformly 15N-labeled 8-pentyl- and N2-pentyl-2'-deoxyguanosines. This research was supported by NIEHS Grant 5R01 ES011589-08.

84 Quantification of N²-carboxyethyl-2'-deoxyguanosine (CEdG): A putative biomarker of glycolytic flux Daniel Tamae, Gerald E. Wuenschell, Tim Synold, and John Termini

Metabolic disease encompasses hyperglycemia, hyperlipidemia, and chronic inflammation and complications from this syndrome present an ever-increasing burden on healthcare systems of the developed world. At the cellular level, metabolic disease increases oxidative and carbonyl stress, resulting in elevated oxidative damage and advanced glycation end-products (AGEs). Measurement of nucleotide AGEs presents an avenue for exploring the potential effects of glycolytic flux on genotoxicity. Using the stable-isotope dilution method and ESI-MS/MS, we have focused on the primary nucleotide AGE, N2-carboxyethyl-2'-deoxyguanosine (CEdG). Increase in glycolytic activity results in the production of an electrophilic α -oxoaldehyde, methylglyoxal (MG), which can react with duplex DNA to form CEdG. Quantification of both diastereomeric forms of CEdG (CEdG-R/-S) in biological fluids and tissue samples from a Type II diabetes rat model have yielded significantly elevated CEdG levels in kidney tissue. We will also present preliminary data from a breast cancer tissue culture model to measure CEdG in the context of the Warburg effect.

85 Scope and mechanism of formation of interstrand crosslinks that give rise to double strand breaks upon nucleotide excision repair Jonathan Sczepanski, Aaron Jacobs, Bennett Van Houten, and Marc M. Greenberg

C4-hydrogen atom abstraction in DNA leads to the formation of the C4 ϕ -oxidized abasic site (C4AP). This lesion was recently shown to produce two unique interstrand cross-links (ICLs) to a deoxyadenosine on the complementary strand. The structure of the ICL involving 2 ϕ -deoxyadenosine was determined by NMR and MS/MS. The mechanism was probed using kinetics, trapping studies, and other techniques. In addition, the scope of this reaction was investigated by placing C4AP in a variety of sequence contexts to determine its reactivity with other nucleobases. Nucleotide excision repair (NER) of the ICL was examined using the UvrABC proteins. Incisions by this enzyme system led to

misrepair of the DNA by generating double strand breaks. Breaks of this type can be more toxic than the ICL from which they originated.

86 Synthesis and biological characterization of minor groove binding methylating agents [Prema Iyer](#), Sreelekha Singh, Ajay Srinivasan, Barry Gold, and Gerard Mascara

We have previously reported the synthesis and characterization of {1-methyl-4-[1-methyl-4-(3-methoxysulfonylpropanamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido}propane (Me-lex), an O-methyl sulfonate ester, that efficiently and selectively generates N3-methyladenine (3-mA) due to its DNA minor groove equilibrium binding based on N-methylpyrrolecarboxamide subunits. In order to enhance alkylation activity and the associated toxicity, and to improve its physical properties, a series of Me-lex analogues have been synthesized and their DNA methylation and DNA binding properties determined, in addition to their toxicities in wild type E. coli and DNA repair mutants. (Supported by NIH grant RO1CA29088)

87 Synthesis and oxidation of 2-hydroxynevirapine, a metabolite of the HIV-1 reverse transcriptase inhibitor nevirapine [Alexandra MM. Antunes](#), Muna C. Sidarus, Frederick A. Beland, and M. Matilde Marques

Nevirapine (11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b: 2',3'-e][1,4]diazepin-6-one, NVP) is a non-nucleoside HIV-1 reverse transcriptase inhibitor used to prevent mother-to-child transmission of the virus. However, severe hepatotoxicity and serious adverse cutaneous effects have raised concerns about the safety of NVP administration. NVP metabolism yields several phenol-type derivatives, conceivably capable of undergoing further metabolic oxidation to electrophilic quinoid species that could react with bionucleophiles. The covalent adducts thus formed might be at the genesis of toxic responses. To test this hypothesis, we synthesized the phenolic metabolite, 2-hydroxy-NVP, and investigated its oxidation in vitro. Using potassium nitrosodisulfonate as a model oxidant, we obtained evidence for a fast, pH-dependent, generation of an electrophilic quinone-imine that readily underwent hydrolytic conversion to a fully characterized spiro derivative, with subsequent degradation of the NVP ring system. The same spiro intermediate was obtained from peroxidase-mediated oxidation of 2-hydroxy-NVP, which suggests that a similar transformation could occur in vivo.

88 Thermal evaluation of a synthetic nucleoside probe for DNA damage products [Rahul R. Lad](#) and Shana J. Sturla

DNA damage in the form of DNA adducts significantly contributes to chemically induced mutagenesis and carcinogenesis, although adducts are formed at low levels and are substrates for repair. In this study, factors influencing DNA duplex stability for damaged DNA paired with a synthetic nucleoside probe were evaluated as a step toward devising new molecular strategies for adduct detection and evaluation of biochemical mechanisms. A synthetic diamionaphthalene-derived nucleoside (dNap) and various DNA adducts were incorporated in short oligonucleotide sequences. On the basis of duplex melting temperature (T_m), as an indicator of base pair stability, the synthetic nucleoside probe was able to discern O6-benzylguanine adduct-containing DNA from unmodified DNA. To evaluate the effect of adduct structure on base-pair stability, thermal stability studies were carried out with adduct analog O6-tetrahydronaphthylguanine, as well as other structurally varied damage products. Thermodynamics of DNA base pairing are affected by sequence context; therefore sequences having varied positions of probe and adducts were prepared and characterized. Data regarding the effects of adduct structure and sequence context on nucleoside probe: adduct base pair stability will be presented.

89 Thermodynamics of DNA with 7-deaza-, 7-aminomethyl-7-deaza- and 7-hydroxymethyl-7-deaza-dG: Effect of cationic charge in the major groove [Ruo Wen Wang](#), [Manjori Ganguly](#), [Luis A. Marky](#), and [Barry Gold](#)

The replacement of the 7-N atom on guanine with a C-H alters the electronic properties of the heterocyclic base and at least partially eliminates a potential major groove cation binding site, which could affect the organization of salts and water in the major groove. We report the characterization of synthetic DNA oligomers containing 7-deazaguanine (c7G) using a variety of biophysical approaches. To complement these studies on c7G, we also synthesized and introduced into DNA c7G residues that have an aminomethyl or a hydroxymethyl group at C-7. While the NMR and x-ray crystal structures of c7G are virtually indistinguishable from unmodified DNA, the thermodynamic results show that the incorporation of c7G has a significant effect on the dynamic structure of the DNA and reduces the stability due to a reduced folding heats that are associated with changes in hydration and reduced cation binding. In contrast, the tethering of a cation in the major groove using 7-aminomethyl-c7G affords DNA that is as, or more, stable than the corresponding unmodified DNA. The hydroxymethyl-c7G reduces DNA stability. These effects are associated with the folding enthalpies and hydration. The interpretation of how these modifications of G affect DNA structure and stability will be discussed. (Supported by NIH RO1CA29088 and NSF MCB-0315746)

90 Unusual DNA sequence effect on the aminobiphenyl-induced conformational heterogeneity Vipin Jain, Fengting Liang, Satyakkam Patnaik, Varsha Biyyala, and Bongsup Cho

Arylamine-modified DNA exists in two distinct B-type (B) and stacked (S) conformers. Our aminofluorene-modified NarI sequence (5'---GGCG*CT---3') exerts greater lesion flexibility over the G*CC context. This next flanking T effect appears to be a general phenomenon. We found that aminobiphenyl-modified duplexes (CCATCG*CXACC) with the same G*CT context (X=T) exhibited a 2:3 mixture of B- and S-conformers, whereas the isomeric duplex (X=A) exclusively produced the B-conformer. To better understand this dramatic sequence effect, we have studied two sequences with different lengths and sequence contexts: CCAXXG*XXACC (X=A or T) and CTTCTCGCXCTC (X = A or T). The structural and conformational origin of the flanking or the next flanking T effect was probed by dynamic FNMR, circular dichroism (CD), differential scanning calorimetry (DSC), and molecular modeling. The mutation and repair consequences of the long range T-effect will be discussed in terms of the sequence-dependent S/B heterogeneity.

91 Elucidating structural properties of dibenzo[a,l]pyrene-derived adenine adducts Yuqin Cai, Shuang Ding, Konstantin Kropachev, Adam Schwaid, Marina Kolbanovskiy, Fabian A. Rodriguez, Shantu Amin, N. E. Geacintov, and Suse Broyde

The high tumorigenic potency of the fjord-region PAH dibenzo[a,l]pyrene (DB[a,l]P) may be related to inefficient repair of certain of its adducts. Specifically, it has been shown that the 14R (+)- and 14S (-)-trans-anti-DB[a,l]P-N⁶-dA adducts are refractory to repair in a human HeLa cell assay (Buterin T. et al., 2000). In order to elucidate structural origins of this phenomenon, we have carried out molecular modeling and MD simulations of these two adducts. Our results show that these adducts adopt classical intercalation conformations with the 14S stereoisomer on the 3'-side and the 14R on the 5'-side of the damaged base. The structures are stabilized by favorable stacking interactions between the 5-ring DB[a,l]P moiety and flanking base pairs, which may prevent beta-hairpin intrusion by the NER recognition factor XPC (Min J.H. et al., 2007). Results are compared with structural and repair data for the 14R (+)- and 14S (-)-trans-anti-DB[a,l]P-N2-dG adducts (Supported by NIH)

92 NMR and computational studies of stereoisomeric 4-hydroxyequilenin-cytidine adducts in oligonucleotide duplexes: Opposite orientations of diastereomeric forms Shuang Ding, Na Zhang, Alexander Kolbanovskii, Anant Shastry, Vladimir Kuzmin, Yuqin Cai, Judy L. Bolton, Suse Broyde, Nicholas E. Geacintov, and Dinshaw J Patel

Components of the hormone replacement drug Premarin, implicated in initiation of breast cancer, metabolize to 4-hydroxyequilenin (4-OHEN); these react predominantly with dC to form unusual stereoisomeric, stable DNA adducts. We have utilized combined NMR and computational methods to elucidate structures of one stereoisomeric adduct pair, 4-OHEN-C3 and 4-OHEN-C4, in 11-mer DNA duplexes. In both cases the damaged cytosine adopts the anti glycosidic conformation and the equilenin rings span the width of the helix, with the non-planar distal rings containing the bulky methyl group protruding into the minor groove, and the two hydroxyl groups of the bridge ring being exposed to solvent on the major groove side. However, in the 4-OHEN-C3 adduct, the equilenin rings are oriented toward the 5'-end of the damaged strand, while they are 3'-directed in the 4-OHEN-C4 adduct. The duplexes are significantly distorted, with distortions propagated in the direction of the equilenin ring orientation in each case. The stereoisomer-dependent mutational properties are thus rationalized.

93 Polymerase-tailored variations in the water-mediated and substrate-assisted mechanism for nucleotidyl transfer: Insights from a study of T7 DNA polymerase Lihua Wang, Suse Broyde, and Yingkai Zhang.

The nucleotidyl transfer reaction catalyzed by DNA polymerases is critical for accurate transfer of genetic information and its malfunctioning can cause mutations leading to human diseases including cancer. Utilizing ab initio quantum mechanical/molecular mechanical calculations with free energy perturbation, we carried out an extensive investigation of the nucleotidyl transfer reaction mechanism in the high-fidelity replicative T7 DNA polymerase. We find an energetically feasible variant of the water-mediated, substrate-assisted (WMSA) mechanism previously delineated for Dpo4 (JACS, 129:4731) with features specific to the T7 DNA polymerase structure. However, a unifying theme in the WMSA mechanism is the cycling through crystal and solvent waters of the proton originating from the primer 3'-terminus to the αβ bridging oxygen of dNTP; this neutralizes the evolving negative charge as pyrophosphate leaves and restores the polymerase to its pre-chemistry state. These unifying features are likely requisite elements for nucleotidyl transfer reactions. Supported by NIH, NCI.

94 Proteomic characterization for determination of drug toxicity in liver bioreactor cultures Viral Brahmabhatt, Ajit Dash, Walker Inman, Linda G. Griffith, and Steven R. Tannenbaum

Hepatocytes are the principal cell type involved in xenobiotic metabolism. A variety of cell culture systems have been developed that maintain the in vivo architecture and function of hepatocytes for the duration of experimentation. Herein, we utilize two bioreactor systems: 1) consisting of hepatocytes alone; 2) a co-culture of hepatocytes with non-parenchymal cells. Sivaraman, et al., 2005, have previously described these systems. Our characterization of the bioreactor medium proteins reveals the presence of 39 common proteins and 37 and 28 distinct proteins in the hepatocyte mono-culture and co-culture bioreactor system, respectively. Hepatocyte inflammation may promote drug-related idiosyncratic reactions, hence, we are working on developing these systems for study of idiosyncratic drug-related toxicity. Ranitidine and Nefazodone, drugs that are known to cause idiosyncratic reactions, have been used for this initial study. Quantitative proteomic data comparing the changes in the bioreactor media for both systems will be presented.

95 Simultaneous quantification of free and protein-bound chlorotyrosine and nitrotyrosine in biological fluid and tissue by NICI GC/MS **Yu Zeng**, Ju Liu, Kari E. Schlicht, Amirah Khan, John S. Wishnok, and Steven R Tannenbaum

3-chlorotyrosine (ClTyr) and 3-nitrotyrosine (NTyr) are representative protein damage products arising from inflammation, therefore a sensitive and selective measurement of ClTyr and NTyr in biological fluids and tissue could provide insight into early diagnosis and evaluation of inflammatory disease. The method presented here is applicable to the measurement of both free and protein-bound ClTyr and NTyr in biological fluid and tissue samples. Sample preparation involves protein enzyme digestion, HPLC purification and derivatization. Mild digestion minimizes artifactual halogenation and nitration. ¹³C¹⁵N- and ²H-labeled ClTyr and NTyr, are used as internal standards for quantification, and ¹³C-labeled Tyr is spiked to monitor artifactual halogenation and nitration. Preliminary analysis of human serum from patients with two variations of inflammatory-bowel disease (IBD) and colon tissue from the C. rodentium mouse model of IBD, has revealed pg ~ ng/ml levels of free ClTyr and NTyr and pg ~ ng/mg levels of protein-bound ClTyr and NTyr.

96 Analysis of effects of dichloro-diphenyl-trichloroethane (DDT) on natural killer cells **Felicia Udoji** and Margaret M. Whalen

Environmental exposure to carcinogenic compounds, such as dichloro-diphenyl-trichloroethane (DDT), may lead to the accumulation and proliferation of mutated cells. Normally, cytotoxic cells, such as Natural Killer (NK) cells eliminate cancerous cells by initiating apoptosis in and/or lysing the cells (lytic function). However, in the presence of DDT, this process may be interrupted. This study investigates the effects of DDT on the function of NK cells. The ability of highly purified NK cells to lyse tumor cells was examined using a ⁵¹Cr-chromium release assay. Exposures to 2.5 and 1 micromolar DDT for 24 h decreased lytic function by approximately 80, and 20%, respectively. Subsequently, the ability of NK cells to bind to target cells was examined at these same exposures, to determine if a loss of binding capacity was contributing to the loss of lytic function. Results of the binding studies indicate that DDT exposures diminish the ability of NK cells to bind to tumor cells. Overall results suggest that DDT has a deleterious effect on the ability of human NK cells to eliminate cancer cells; potentially increasing an individual's susceptibility to cancer. Supported by NIH grant S06 GM008092-33.

97 Analysis of stable 4-hydroxyequilenin DNA adducts by liquid chromatography electrospray ionization tandem mass spectrometry in human breast cancer cells **Zhican Wang**, Praneeth Edirisinghe, Johann Sohn, Zhihui Qin, Nicholas E. Geacintov, Gregory R. J. Thatcher, and Judy L. Bolton

DNA adducts are potential biomarkers for assessing the risk of developing cancers. 4-Hydroxyequilenin (4-OHEN) is the major catechol metabolite of equine estrogens, which autoxidizes to a reactive o-quinone. Formation of stable stereoisomeric cyclic 4-OHEN DNA adducts has been reported; however, their removal by DNA repair processes in cells has not been characterized. These studies have been hampered by low product yields and reproducibility which could be partially due to the instability of 4-OHEN in aqueous media. This was overcome by treating cells with 4-OHEN diacetate rather than 4-OHEN, and a sensitive LC-MS/MS method was developed for detecting these adducts. A concentration-dependent increase in adduct levels was observed in MCF-7 cells after exposure to 4-OHEN diacetate. The stabilities of adducts was also investigated. This newly developed LC-MS/MS method allows detection and relative quantification of 4-OHEN DNA adducts in human cells which could be adapted for adduct detection in human samples.

98 Effects of exposures to dibutyltin on signaling pathways and cytosolic calcium ion levels in human natural killer cells **Sabah Odman-Ghazi**, and Margaret M. Whalen

Dibutyltin (DBT) is a widespread environmental contaminant, which has been found in human blood. Natural killer (NK) cells are immune cells that protect us from tumor development and viral infection. Previous studies have shown that DBT decreases the tumor-cell lysing function of NK cells. Additional

studies examining enzymes needed in the lytic process, indicated that DBT causes significant increases in mitogen-activated protein kinases (MAPKs) phosphorylation (activation) state. In this study we assessed the effects of exposures to DBT for 10 min, 60 min and 1 h on the activation state of the MAPK activators, mitogen activated protein kinase kinases (MKKs). NK cells exposed to 10, 5, 2.5, 1, and 0.5 μ M DBT for 10 min caused increased phosphorylation of the MAP2Ks; MEK1/2, MKK3/MKK6, MKK7. These data indicate that in vitro exposure to DBT activates enzymes of the MAPK pathway, which are known to be involved in the lytic function of NK cells. The effects of DBT exposures on cytosolic Ca²⁺ were also examined and it was found that both 10 and 5 μ M DBT caused significant but quite small increases in cytosolic Ca²⁺ after 30 and 60 min. The increases were about 20% above control levels for 10 μ M DBT and about 13% for 5 μ M DBT Supported by NIH grant S06GM00809233.

99 Tetrabromobisphenol A interferes with the immune function of human natural killer cells Esther Caroline Kibakaya, Krishna Stephen, and Margaret M. Whalen

Tetrabromobisphenol A (TBBPA) is used as a flame retardant and has been found in human blood samples. Human natural killer (NK) lymphocytes are able to lyse virally- infected and tumor cells. Agents that interfere with the ability of NK cells to lyse targets may increase tumor development or viral infection. We examined the effects of varying concentrations of TBBPA on the lytic function of human NK cells. Exposures to 5 and 1 μ M TBBPA for 24 hrs decreased lytic function about 90% and 39%, respectively. Examination of the ability of NK cells to bind to the tumor cells showed that after a 24 hr exposure to 5 μ M TBBPA, binding was inhibited by about 40%, while 1 μ M TBBPA caused no significant loss of binding function. These results indicated that a loss of lytic function is not necessarily accompanied by a loss of binding function. Supported by NIH grant S06 GM008092-33

WEDNESDAY MORNING

DNA Adducts and Human Health

Paul Henderson, Organizer

100 Chemical biology of aristolochic acid nephropathy, a global disease Arthur P. Grollman

The molecular mechanisms underlying aristolochic acid nephropathy (AAN) and urothelial cancer were explored in the context of Balkan endemic nephropathy (BEN), an environmental disease. Aristolactam- DNA adducts were used to establish the etiologic role of aristolochic acid in BEN. Using ³²P- post-labelling and mass spectroscopic techniques, dA- and dG-aristolactam adducts were identified in the renal cortex and urothelial cancer tissue of patients with BEN. Genotoxicity is manifested by A:T toT:A transversions in the p53 mutational spectrum of upper urothelial cancers uniquely associated with BEN. Aristolactam-DNA adducts also serve as biomarkers for AAN in exfoliated urothelial cells of patients ingesting Aristolochia herbs. Aristolochic acids exhibit structural specificity in their nephrotoxic effects; a genetic basis for this syndrome has been demonstrated in a mouse model of AAN.

101 DNA adducts involved in xeroderma pigmentosum neurologic disease and in alcohol-related carcinogenesis Phillip J Brooks

DNA damage is associated with an increased risk of certain types of cancers, but also cause neurologic disease. Carcinogenesis resulting from DNA damage is generally thought to result from mutations during DNA replication, whereas neurologic diseases affecting non-dividing cells is more likely due to the effects of DNA lesions on transcription. In this presentation, I will first discuss a class of oxidative DNA lesions called cyclopurines deoxynucleosides, which result from the hydroxyl radical but are specifically repaired by the nucleotide excision repair (NER) pathway, much like the pyrimidine dimers that result from UV light. I will discuss the effects of these lesions on transcription, and the evidence for their role in neurologic disease in NER-deficient XP patients. I will then discuss the formation of DNA adducts resulting from acetaldehyde, the first metabolite of ethanol, and the evidence for a role for the lesions in cancer resulting from alcohol consumption.

102 DNA-protein and DNA-DNA crosslinking by bis-electrophiles: Lesion identities and biological effects Natalia Tretyakova, Rachel L. Loeber, Erin Michaelson-Richie

Bifunctional alkylating agents, such antitumor nitrogen mustards, platinum compounds, and 1,2,3,4-diepoxybutane, are capable of reacting with two nucleophilic sites on cellular biomolecules to form DNA-DNA and DNA-protein cross-links. The purpose of this research was to structurally characterize the

resulting bifunctional lesions, to identify the proteins which become cross-linked to DNA in the presence of bis-electrophiles, and to evaluate their biological effects. A combination of mass spectrometric and immunological methods was used to analyze DNA-DNA and DNA-protein cross-links in vitro and in cultured HT1080 human fibrosarcoma cells. Over 50 human proteins were identified in the resulting DNA-protein lesions using proteomic analysis. HPLC-ESI+-MS/MS analysis of nucleobase-nucleobase and amino acid-nucleobase conjugates in total digests from drug treated cells was used to quantify the formation of covalent drug-induced DNA-DNA and DNA-protein cross-links. To examine the cytotoxicity of DNA-protein and DNA-peptide cross-links in vivo, recombinant proteins or synthetic peptides containing a monoepoxide "warhead" were introduced in human cells, followed by cytotoxicity analysis. Finally, nornitrogen mustard-induced DNA-DNA cross-links were quantified in blood of cancer patients undergoing cyclophosphamide therapy. Taken together, these studies provide insight into the prevalence and the biological relevance of DNA-DNA and DNA-protein cross-linking induced by chemotherapeutic bis-electrophiles.

103 Envisioning alkylated and crosslinked DNA damaged responses controlling cell death, mutagenesis, and repair outcomes John A. Tainer

O⁶-alkylated guanine is mutagenic and cytotoxic: it mispairs during replication with thymine, resulting in G:C to A:T transition mutations. O⁶-Alkylguanine-DNA alkyltransferase (AGT, or O⁶-methylguanine-DNA methyltransferase (MGMT), repairs DNA mutagenic lesions, which provides protection but limits effectiveness of alkylating chemotherapies. To test AGT-mediated repair and the spectrum of repairable damage, we examined the ability of AGT to repair inter-strand cross-link DNA strands joined via guanine-O⁶. Alkyltransferase-like proteins (ATLs) also protect DNA from alkylation damage; yet, ATLs lack alkyltransferase activity and the reactive cysteine. To address this paradox, we combined structural and genetic analyses of *S. pombe* ATL. We determined ATL structures without and with damaged DNA substrates containing O⁶-methylguanine or O⁶-4-(3-pyridyl)-4-oxobutylguanine. Structures and genetic connections to XPG and ERCC1 in *S. pombe* homologs Rad13 and Swi10 suggest ATLs sculpt the stable, distorted ATL-DNA complex to create a novel intersection of base damage responses with nucleotide excision repair.

104 "CometChip": Application of microfabrication technologies for a DNA damage and repair assay Bevin P. Engelward, David Wood, David Weingeist, Yunji Wu, and Sangeeta N. Bhatia

DNA damage contributes to cancer, aging and heritable diseases. People are highly variable in their capacity to repair DNA damage, and deficiencies in DNA repair are associated with an increased risk of cancer. The comet assay is used for evaluating DNA strand breaks, sites of DNA modification and interstrand crosslinks. A limitation of the traditional assay is that each sample requires a separate glass slide and image analysis is laborious and data intensive, thus reducing throughput. We used microfabrication technologies to enable analysis of cells within a defined array, resulting in a >200 fold reduction in the area required per condition. The resulting 'CometChip' can be used to analyze dozens of conditions on a single chip. We developed automated image analysis software, thus greatly reducing analysis time. It is hoped that this platform will serve as a valuable tool in basic and applied research.

WEDNESDAY AFTERNOON

Platinum-based Chemotherapeutics: New Approaches for Cancer Treatment

Paul Henderson, Organizer

105 Construction and application of plasmids to evaluate site-specific Pt-DNA adducts in mammalian cells Stephen J. Lippard and Wee Han Ang.

The cytotoxic action of the anticancer drugs cisplatin, carboplatin, and oxaliplatin involves cell entry, drug activation, DNA binding, and transcription inhibition. In order to facilitate investigation of specific Pt-DNA lesion processing in live cells, we devised a strategy for constructing plasmids containing a single site-specific Pt-DNA adduct. We applied nicking restriction enzymes to create closely spaced tandem cuts on the plasmid and removed the doubly nicked DNA strand to create a short single-stranded gap. Synthetic platinumated oligonucleotides were incorporated into the gap to generate covalently closed circular platinumated plasmid in good yield. We discuss the application of this methodology to prepare plasmids containing a platinum 1,2-d(G*pG*) or 1,3-d(G*pTpG*) cross-link,

the major adducts formed by the three clinically approved drugs. Use of these probes to investigate transcription inhibition, repair, and the effects of the cell cycle will be described. This work was supported by a grant from the National Cancer Institute.

106 Quantitation of cisplatin 1,2-intrastrand guanine-guanine adducts James Swenberg, Irene Baskerville-Abraham, Gunnar Boysen, J. Mitchell Troutman, Esra Mutlu, Leonard B. Collins, Kathryn deKrafft, Wenbin Lin, Candace King, and Stephen Chaney.

Platinum chemotherapeutic agents have been widely used in the treatment of cancer. An ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) assay for quantification of 1,2 guanine-guanine intrastrand cisplatin adducts [CP-d(GpG)], using ¹⁵N10 CP-d(GpG) as an internal standard, was developed. Samples containing CP-d(GpG) in DNA were purified by enzyme hydrolysis, centrifugal filtration and HPLC prior to quantification by UPLC-MS/MS in the selective reaction monitoring (SRM) mode (m/z 412.5 to 248.1 for CP-d(GpG); m/z 417.5 to 253.1 for [¹⁵N10] CP-d(GpG)). The limit of quantification was 3 fmol or 3.7 adducts per 108 nucleotides when injecting 25 µg of DNA. The method was suitable for in vitro and in vivo studies as demonstrated with ovarian carcinoma cell lines and mice. The development of biomarkers to determine tissue-specific molecular dosimetry during treatment should lead to a more complete understanding of both therapeutic and adverse effects of cisplatin and carboplatin.

107 The effect of conformational dynamics on the protein recognition of platinum DNA adducts Stephen Chaney, Srinivas Ramachandran, Nikolay V Dokholyan, and Brenda Temple. (

Oxaliplatin (OX) and cisplatin (CP) form the same types of adducts at the same sites in DNA, yet OX adducts are generally more cytotoxic than CP adducts and OX is often effective in CP-resistant tumors. The differences in biological effectiveness of OX- and CP-DNA adducts may derive from the ability of proteins such as HMGB1a to bind to CP-DNA adducts more strongly than OX-DNA adducts. We have postulated that differences in the conformational dynamics of OX- and CP-DNA adducts are responsible for the differences in protein affinity and have performed molecular dynamic simulations of OX- and CP-DNA adducts ±HMGB1a. Our data show that the amines associated with CP are more able to form hydrogen-bonds with adjacent bases on the DNA because of constraints imposed by the cyclohexane ring of OX, and that formation of those hydrogen-bonds is associated with DNA conformations that are favorable for binding of HMGB1a.

108 Toward personalized chemotherapeutics: Correlation of carboplatin chemoresistance to cellular uptake, drug inactivation, DNA damage, and repair using nontoxic drug doses Paul T. Henderson, Tao Li, Miaoling He, Teesta Jain, Michael A. Malfatti³, Kenneth W Turteltaub, and Chong-Xian Pan

Accelerator mass spectrometry (AMS) is an ultrasensitive method for detection of rare isotopes that is increasingly used for drug metabolism and pharmacokinetics studies. We report progress towards using AMS for personalized chemotherapeutics with the cancer drug carboplatin. A variety of human cancer cell lines of known sensitivity to carboplatin were exposed to therapeutic or microdose (1/100th of therapeutic dose) concentrations of [¹⁴C]carboplatin. Carboplatin influx and efflux, drug-DNA damage and nucleotide excision repair activities of the cells were assayed by AMS and liquid scintillation counting. The resulting data set provided a definition of the magnitude of resistance independent of IC₅₀ measurement and a mechanistic determination of the major resistance factors for each cell line. The data were essentially identical, when corrected for dose, for the microdose and the therapeutic dose for the majority of cell lines. AMS may someday guide carboplatin treatment decisions in a clinical setting.

109 Preclinical oxaliplatin microdosing studies for personalized medicine applications Chong-Xian Pan, Tao Li, Miaoling He, Teesta Jain, Michael A. Malfatti, Kenneth W Turteltaub, and Paul T. Henderson.

The platinum chemotherapeutic drug oxaliplatin exerts its cytotoxic effects through drug-induced DNA damage (adducts). We hypothesize that cellular responses to low levels of oxaliplatin-DNA adducts can predict chemoresistance. In this project, we used ultrasensitive accelerator mass spectrometer (AMS) to measure [¹⁴C]oxaliplatin in a variety of cancer cell lines and tumors. With AMS, we were able to measure oxaliplatin-induced DNA adducts when cells and animals were exposed to subtoxic microdoses (1/100th the therapeutic dose) of [¹⁴C]oxaliplatin. DNA adduct levels over time induced by the microdose corresponded to chemoresistance as measured by MTT assay. Furthermore, some of the underlying chemoresistant mechanisms, such as drug uptake and DNA repair rates were determined. A phase 0 clinical trial has been designed to identify chemoresistance before patients receive toxic chemotherapy, and to determine the underlying chemoresistant mechanisms in order to enable personalized therapy.