



# News Flash

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## Abstracts

**Division of Chemical Toxicology ,  
236th ACS National Meeting  
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**Program Chair, Kent Gates**

**Sunday Morning: Structural Biology of DNA  
Damage and Repair**



**1. Interconversion of the cis-5R,6S- and trans-5R,6R-thymine glycol adducts in duplex DNA.** M. P. Stone, K. L. Brown, T. Adams, V. Jasti, A. K. Basu.

The 5R thymidine glycol (Tg; 5,6-dihydroxy-5,6-dihydrothymidine) lesion was incorporated into duplex oligodeoxynucleotides to form either a Tg•A base pair, corresponding to the oxidative damage of thymine in DNA, or a mismatched Tg•G base pair, corresponding to the formation of Tg following oxidative damage and deamination of 5-methylcytosine in DNA. Solution structural studies carried out by NMR revealed that at equilibrium, the ratio of cis:trans epimers was 7:3 for the Tg•A sample. In contrast, for the Tg•G sample, the cis:trans equilibrium favored the cis-5R,6S epimer; the level of the trans-5R,6R epimer remained below the level of detection by NMR. The structural differences between the two base pairing motifs will be compared. We conclude that the position of the equilibrium between the cis-5R,6S and trans-5R,6R thymine glycol epimers in duplex DNA is affected by the complementary base, which may modulate the repair of the Tg lesion. Funded by NIH grants CA-55678 (M.P.S.) and ES-013324 (A.B).

**2. Crystal structure of DNA repair protein AlkD reveals a new protein architecture for locating and excising alkylpurines from DNA.** E. H. Rubinson, Z. Wawrzak, A. H. Metz, J. O'Quin, B. F. Eichman

DNA glycosylases safeguard the genome by locating and excising chemically modified bases from DNA. AlkD is a recently discovered bacterial DNA glycosylase specific for positively charged alkylpurine nucleobases 3-methyladenine and 7-methylguanine. The crystal structure of *Bacillus cereus* AlkD revealed that the protein is composed exclusively of helical HEAT-like repeats, which form a solenoid perfectly shaped to accommodate an undistorted DNA duplex on the concave surface. Structural analysis of the variant HEAT repeats in AlkD provides a rationale for how this protein scaffolding motif has been modified to bind DNA. Comparison of AlkD to existing DNA glycosylase structures, along with mutational studies of 7mG excision and DNA binding activities, provides important insight into the requirements for alkylation repair within DNA. We suggest that AlkD utilizes a novel strategy to manipulate DNA in its search for alkylpurine bases. Funded by American Cancer Society grant RSG-07-063-01-GMC (B.F.E.).

**3. Interrogation, recognition and repair of damaged bases in DNA.** A. Banerjee, J. C. Fromme, S. Jiralerspong, C. T. Radom, S. D. Bruner, S. J. Chung, W. Yang, M. Karplus, **G. L. Verdine**

The bases in DNA are subject to attack by a wide variety of endogenous and exogenous agents. The resulting products cause mutations to occur during DNA replications and are therefore genotoxic. Repair of these lesions takes place by the base-excision DNA repair pathway, the central components of which are DNA glycosylases. These enzymes cleave the glycosidic bond linking the damaged base to the DNA backbone. Studies in our lab are focused on understanding how DNA glycosylases interrogate up to 10 million normal DNA bases in order to locate just one damaged base, and on understanding the mechanism by which DNA glycosylases catalyze the excision of damaged bases upon locating them. Recent progress on these fronts will be discussed.

**4. Enzymatic detection of uracil in DNA.** **J. T. Stivers**

The enzyme uracil DNA glycosylase (UNG) excises unwanted uracil bases in the genome using an extrahelical base recognition mechanism. Uracil removal is essential for prevention of C to U transition mutations and cytotoxic U/A pairs arising from misincorporation of dUTP in DNA. A central event in all of these UNG-mediated processes is the singling out of rare U/A or U/G base pairs in the human genome. Recent NMR dynamic measurements and crystallographic studies have revealed that discrimination of thymine and uracil is initiated by thermally induced opening of T/A and U/A base pairs and not by active participation of the enzyme. DNA base pair dynamics are sensed by the enzyme as it rapidly slides short distances along the contour of duplex DNA. Thus, base-pair dynamics and short range DNA sliding have critical roles in the genome-wide search for uracil, and these same mechanisms may be utilized by other DNA repair glycosylases

**5. DNA base excision repair initiation from the structural biochemistry of N-glycosylases and endonucleases that recognize and excise DNA base damage.** **J. A. Tainer**

We seek to define the structural biochemistry for initiation of the Base Excision Repair (BER) pathway by damage-specific DNA N-glycosylases plus DNA abasic site endonucleases, and by non-classical nuclease activities that cut the DNA backbone at damaged bases. BER overall features, conserved from microbes to humans, depend upon damage detection and committed repair initiation by enzymes that we have structurally and biochemically characterized as DNA substrate and product complexes. To characterize central facets of BER initiation, we are combining Small Angle X-ray Scattering (SAXS), for solution structures, and Macromolecular X-ray crystallography (MX), for high-resolution, with targeted genetic, mutational, and biochemical approaches. As these BER enzymes are conserved across all 3 domains of life, we use powerful cross-genomic comparisons and *E. coli* genetics to select proteins that optimally address key questions regarding activities and mechanisms. Our current results speak to base cleavage and backbone incision points pivotal to initiating BER pathways: DNA base damage detection and removal by N-glycosylase, or combined with backbone cleavage by N-glycosylase/AP-lyases; DNA backbone incision by AP-endonucleases (APE1/ExoIII, EndoIV), classically at abasic sites or alternatively at oxidized bases for the glycosylase-independent Nucleotide Incision Repair (NIR) pathway, and by endonuclease V (EndoV) at deaminated bases in Alternative Excision Repair (AER); and the coordinated handoff that avoids release of toxic and mutagenic DNA intermediates. These results help define the molecular basis for and regulation of BER initiation from damage detection to coordination of pathways. Overall, this work helps to characterize BER initiation complexes and to define their roles in genome fidelity plus potential vulnerabilities to toxins, degenerative diseases, and cancer.

**Sunday Afternoon: Founders Award Symposium**

**6. Mechanisms of furan toxicity and carcinogenicity.** **L. A. Peterson**

Furan is a liver toxicant and carcinogen in rodents. Based on these results and the large potential for human exposure, furan has been classified as a possible human carcinogen. The mechanism of tumor

induction by furan is unknown. The toxicity and carcinogenicity of furan is initiated by cytochrome P450 catalyzed oxidation of furan to an A,B-unsaturated dialdehyde, cis-2-butene-1,4-dial (BDA). This metabolite reacts readily with glutathione, amino acids and DNA and is a bacterial mutagen in Ames assay strain TA104. These results support the hypothesis that this metabolite may be responsible for the carcinogenic properties of furan. Characterization of urinary metabolites of furan indicated that BDA is formed in vivo. A number of these metabolic products appear to be degradation products of BDA-lysine protein adducts. These products are not only potential biomarkers for furan exposure and metabolism but they also provide insights into the molecular targets of BDA [Supported by ES-10577].

### **7. Lipid Affinity Tag Substrates: $\omega$ -Alkynes as Probes for Lipid-Derived Electrophile Protein Adducts.** K. A. Tallman, H -Y. Kim, R. Serwa, D. C. Liebler, **N. A. Porter**

$\omega$ -alkynyl affinity tagged lipids designed for use in the isolation of lipid-derived protein adducts have been synthesized and preliminary studies of these compounds carried out. The  $\omega$ -alkyne structural modification is a minimal perturbation of the natural substrate but it allows for an easy separation of the lipid-derived compounds from complex mixtures by means of an alkyne-azide dipolar cycloaddition (click cycloaddition) with a biotin azide and subsequent purification of lipid-derived compounds of interest by (strept)avidin complexation. Concise syntheses of  $\omega$ -alkyne analogs of the electrophile 4-hydroxynonenal (HNE) and the corresponding fatty acid precursors linoleic [(9Z,12Z)-octadeca-9,12-dien-17-ynoic] and arachidonic [(5Z,8Z,11Z,14Z)-eicosa-5,8,11,14-tetraen-19-ynoic] acid derivatives have been achieved. The alkynyl fatty acids undergo free radical oxidation to give products analogous to those formed from the natural substrates and experiments demonstrate that alkynyl lipid-derived electrophiles adduct to human plasma proteins. The protein-lipid adducts can be isolated from whole plasma by the affinity-tag strategy.

### **8. Mechanisms of protein tyrosine nitration in hydrophilic and hydrophobic biocompartments.** **R. Radi**

Protein tyrosine nitration is a post-translational modification found in vivo, secondary to excess formation and reactions of nitric oxide-derived oxidants. Formation of protein 3-nitrotyrosine depends on free radical mechanisms and nitration yields are responsive to increases of either superoxide/hydrogen peroxide or nitric oxide levels. Within a protein, site-specificity and yields of nitration are controlled by its structure including tyrosine solvent exposure, influence of neighboring amino acids, presence of transition metal centers and participation of intramolecular electron transfer processes. Recent studies using hydrophobic tyrosine analogs and tyrosine-containing peptides indicated that factors affecting nitration in hydrophobic biocompartments differ to those in aqueous phases and are associated to lipid oxidation processes. Tyrosine probes that compete with endogenous constituents for the nitrating intermediates currently serve to unravel nitration mechanisms in vitro and in vivo and to provide cyto- and tissue protective functions against the toxic effects of protein tyrosine nitration.

### **9. Design of DNA damaging agents that block DNA repair and disrupt cancer specific cellular signaling programs.** **J. M. Essigmann**

An approach to anticancer drug design is described in which a DNA damaging nitrogen mustard is tethered to a ligand for the estrogen or androgen receptors. DNA adducts formed by the mustard attract the receptor, which engages the adducts in a tight noncovalent complex. The androgen receptor is expressed in most prostate tumors, and the estrogen receptor is expressed in many breast and ovarian tumors. One consequence of the binding of a receptor to the DNA adduct is that the adduct becomes shielded from DNA repair enzymes. Thus, the adduct persists in vivo (in tumor cells) and shows enhanced toxicity in steroid receptor positive tumors. The new DNA damaging agents also disrupt signaling pathways in malignant cells. The androgen receptor, for example, is a transcription factor that controls pathways of growth as well as maintenance of secondary sexual characteristics. The compounds we designed form DNA adducts numbering in the tens of thousands per cell. These abundant decoy binding sites for the androgen receptor, we expect, severely disrupt signaling in cancer cells. The hijacking of steroid receptors with concomitant disruption of transcription, combined with

adduct persistence owed to the repair shielding phenomenon, are assumed to be the cause of the potent anticancer properties of the designed molecules.

#### **10. Lipid and DNA aldehydes as mediators of cellular responses to oxidative damage. L. J. Marnett**

Oxidative damage to cellular constituents is an important component of multiple diseases including inflammation, cardiovascular disease, and cancer. Hydroperoxides are the initial products of oxidative damage but these are rapidly converted to a variety of electrophilic species such as aldehydes and epoxides, which modify DNA and protein and induce cellular responses. Major challenges in chemical toxicology include the elucidation of the chemistry of macromolecular modifications and the linkage of these modifications to the ultimate cellular response. This presentation will focus on the chemistry of protein modifications by lipid aldehydes and DNA aldehydes and approaches for unraveling their biological consequences.

### **Monday Morning: Young Investigator Session**

#### **11. General theory of toxicity. E. P. Nwosu**

Sequel to the special theory of toxicity, the general theory of toxicity covers the toxicity of both organic and inorganic compounds. In this work, it has been shown that the number, position, arrangement of electrons in a given compound and element's position in the periodic table contributes to toxicity. This was achieved by; discovering new properties of electron, making two (2) assumptions, introducing three (3) toxicity postulates and a fourth (4th) postulate specific to organic compounds. This theory is sine qua non in predicting the toxic properties of any compound, and could be very useful in neutralizing toxic effects. The phenomenon, atoxopy, was also introduced to treat some special cases of toxicity. This work calls for some structural modifications and on proper application can predict the toxic properties of any known and yet-to be synthesized compounds.

#### **12. Developing single nanoparticle optics and in vivo assays for real-time characterization of transport and biocompatibility of nanomaterials . K. J. Lee, P. D. Nallathamby, L. M. Browning, X. N. Xu**

Real-time study of transport and biocompatibility of single nanoparticles in embryos will provide new insights into molecular transport mechanism and structure of developing embryos at nanometer (nm) spatial resolution. We have developed single silver (Ag) nanoparticle optical probes for probing their transport, biocompatibility and toxicity in early development of zebrafish embryos in real-time. We found that single Ag nanoparticles are photostable and show size-dependent localized-surface-plasmon-resonance spectra (LSPRS), allowing us to directly probe their diffusion, transport mechanism and biocompatibility in embryos at nm spatial resolution. Our results show that individual Ag nanoparticles can passively diffuse into developing embryos via chorion pore canals, create specific effects on embryonic development and selectively generate particular phenotypes in a dose-dependent manner. The early embryos are highly sensitive to the nanoparticles, showing the possibility of using zebrafish embryos as an in vivo assay to screen the biocompatibility and toxicity of nanomaterials

#### **13. Enhanced identification of biotin-derivatized peptides. P. G. Slade, M. V. Williams, J. S. Wishnok, S. R. Tannenbaum**

Biotinylation of post-translational modifications followed by affinity chromatography and LC-MS/MS is becoming a common technique to identify protein/peptide modifications. Automated database searches, e.g., Mascot, of biotin-tagged peptides demonstrate artificially lowered statistical scores, which greatly increases the identification of false positives. CytC and BSA were modified with DODE and the carbonyl was derivatized with biotin-tag ARP. Following LC-MS/MS, a number of biotin-tag-specific neutral losses and fragments were observed. Using these fragment ions as a fingerprint, a Python script was written to search MS/MS peak lists for these signals. With a set of selection rules and a scoring system, the

program successfully filtered the peak lists to highlight modified peptides. Mascot analysis of the filtered peak lists resulted in virtually no false positives. We are working to adapt the filter program for other biotinylated adducts as well as investigating a new database-searching algorithm designed for biotin-derivatized peptides.

**14. 1,N<sup>2</sup>-Ethenodeoxyguanosine adduct structure-function relationships: Comparing solution structure with polymerase structure.** G. Shanmugam, I. D. Kozekov, F. P. Guengerich, C. J. Rizzo, M. P. Stone

The structures of DNA duplexes adducted with 1,N<sup>2</sup>-ethenodeoxyguanosine (1,N<sup>2</sup>-dG) with complementary dC, dA, and a one base deletion (1BD) were determined. At neutral pH, a mixture of the syn and anti conformations were observed for 1,N<sup>2</sup>-dG placed opposite cytosine, while a stable anti conformation was observed when the lesion was placed opposite adenine or 1BD. The 1,N<sup>2</sup>-dG adduct adopted the syn conformation under acidic conditions, whereas, it adopted the anti conformation under basic conditions when placed opposite cytosine. The structures of each of these conformations and their effects upon the base pair arrangement at the adduct pair and its neighboring base pairs will be discussed. The structural results in duplex DNA will be compared with structural data from template-primer complexes site-specifically modified with the 1,N<sup>2</sup>-dG adduct in the presence of the *Sulfolobus solfataricus* DNA Polymerase Dpo4. Supported by NIH grants ES-05355 (M.P.S. and C.J.R.) and ES-010375 (F.P.G.).

**15. Atom site selectivity in DNA alkylation by primary diazonium ions .** X. Lu, J. C. Fishbein

Diazonium ions are reactive metabolic intermediates of carcinogenic nitrosamines. They can react with DNA at many heteroatoms and form different adducts, which might eventually lead to cancer. Compared to other classical alkylating agents, simple diazonium ions react more selectively at oxygen atoms of nucleobases. The resulting oxygen adducts have been shown to be correlated with the mutagenicity of the parent nitrosamines. In order to elucidate the factors that dictate the site selectivity of DNA alkylation by diazonium ions, alkylation of pyrimidine nucleobases in nucleosides and in dsDNA by 1-propanediazonium ion was carried out. Together with the previously reported results of purine alkylation (1), the difference in yield of nucleoside and dsDNA reactions allows analyses of the factors that contribute to the atom site selectivity. Reference: 1. Lu, X.; Heilman, J. M.; Blans, P.; and Fishbein, J. C. The structure of DNA dictates purine atom site selectivity in alkylation by primary diazonium ions. *Chem. Res. Toxicol.* 2005, 18, 1462.

**16. Examination of reactive oxygen species as general signaling agents of dithiolethiones and the biochemical repercussions of their action.** R. Holland, M. Velayutham, A. Hawkins, A. L. Egger, J. L. Zweier, D. Fabris, A. D. Mesecar, T. W. Kensler, J. C. Fishbein

The mechanism by which dithiolethiones elicit the induction of cytoprotective proteins remains unclear. Unlike other cytoprotective protein inducers, dithiolethione induction potency does not correlate with thiol reactivity. However, dithiolethiones have been shown to cause single strand breaks in DNA via the generation of oxidative stress. It is hypothesized that the parent structure or a down stream metabolite may generate oxidative stress, which can act as a secondary messenger in the induction of cytoprotective proteins.

Human Keap1, an inhibitor of cytoprotective protein induction, contains 27 reactive cysteine residues. Several of these cysteine residues have been implicated as the sensors of environmental stress through several electrophile adduction experiments. Their integrity is pivotal in the repression of protein induction. However, the effect of oxidative stress on Keap1 sulfhydryls remains unclear. This study examines the generation of oxidative stress by dithiolethione metabolites as well as the effects of oxidative stress on Keap1.

**17. S-nitrosation regulates signaling in breast cancer.** K. Pant, H. Saito-Benz, J. Li, F. M. White, S. R. Tannenbaum

The role of Nitric Oxide (NO) via the post-translational modification S-nitrosation in regulating breast cancer growth and survival when treated with the drug Tamoxifen was investigated. Drug resistant variants of the estrogen receptor positive breast cancer cell line MCF-7 were developed by long term low dose exposure to 4-OH Tamoxifen and the two cell lines were examined for NOS expression, S-nitrosation and their effects on downstream signaling. The NOS isoform nNOS was found to be expressed in both the MCF-7 cells and its drug resistant variant while the isoform iNOS was found to be expressed in drug resistant cells. Screening for inhibitors further demonstrated the efficacy of isoform specific inhibitors in reversing S-nitrosation of key phosphatases, regulating cell growth and effecting downstream signaling. This study shows that S-nitrosation plays an important role in cancer cell signaling and is a carefully regulated process.

**18. Influence of alkylating cytotoxins and chemotherapy drugs on cellular redox enzymes.** X. Liu, S. J. Sturla

Acyfulvenes (AFs) are a family of antitumor derivatives with improved therapeutic indices from Illudin S, the cytotoxic natural product from which the AFs are semisynthetically derived. AFs and illudin S are DNA-alkylating agents with similarities in cellular accumulation and DNA binding levels, suggesting that binding to other vital cellular macromolecules contributes to the increased efficacy of AFs. The glutathione-thioredoxin redox system is a major regulator of intracellular redox balance, offering protection against oxidative stress. Disrupting this system can affect cell viability and lead to apoptosis. Furthermore, overexpression of thioredoxin and thioredoxin reductase in certain tumors is associated with higher proliferation capacities and lower apoptosis rates. We will discuss actions of illudin S and AFs on these enzymes, and correlate enzyme activity with protein alkylation. Potential contributions to antitumor selectivity will be presented.

**19. In vitro studies of nimesulide idiosyncratic hepatotoxicity: Diiminoquinone formation and its conjugation.** F. Li, M. D. Chordia, T. Huang, T. L. Macdonald

Nimesulide is well-established non-steroidal anti-inflammatory drug (NSAIDs) marketed in 50 countries except the United States. Nimesulide caused rare but severe hepatotoxicity, generally regarded as idiosyncratic liver toxicity due to its unpredictability in occurrence and yet unknown reasons. 4-Amino-2-phenoxy-methanesulfonanilide (M1) is identified as one of the major metabolites from Nimesulide in human. We hypothesized that M1 be more prone to biological oxidation to form reactive metabolite. Indeed the experimental results in the lab demonstrated that M1 is susceptible to facile oxidation by P450 enzymes to form a very reactive diimino-quinone metabolite (M2), which was difficult to detect directly by LCMS. However, indirect detection by conjugation with biological nucleophiles such as NAC and/or GSH by LC-MS led us to delineate its formation. The detailed studies on identification and characterization of M2-conjugate with NAC, GSH and human serum albumin by LCMS and NMR spectroscopic methods will be discussed.

**20. Selective enrichment and analysis of nitrated proteins from iNOS activated mouse macrophages.** K. E. Schlicht, J. S. Wishnok, S. R. Tannenbaum

Activated macrophages can generate nitric oxide (NO $\cdot$ ) and superoxide (O $_2^{\cdot-}$ ). These reactive species can rapidly combine to form peroxynitrite anion (ONOO $^-$ ), a highly reactive and potent molecule capable of lipid oxidation, DNA damage, and protein modification. A major product of protein nitration by ONOO $^-$  is 3-nitrotyrosine, an important biomarker of nitric oxide/peroxynitrite-related damage that has been associated with inflammatory diseases, neurological disorders, and cancer. Identification of individual sites of nitration has proven difficult with current methods. Therefore, we developed an enrichment method to isolate nitrated proteins and determine sites of nitration on individual peptides in mouse macrophage RAW 264.7 cells. Nitrated macrophage proteins were selectively labeled with a biotin tag, isolated using a novel affinity-capture method, and identified. Using this selective proteomics-based approach, we can identify the specific protein targets of nitration from complex biological samples to better understand the link between nitric oxide/peroxynitrite damage and disease.

## 21. Solution Structures of Trans-4-Hydroxynonenal Derived 1,N<sup>2</sup>-dG Adduct in

**Oligodeoxynucleotides: Insights into Mutagenicity.** H. Huang, H. Wang, A. Kozekova, C. J. Rizzo, M. P. Stone

trans-4-Hydroxynonenal (4-HNE) reacts with dG through Michael addition to produce the cyclic 1,N<sup>2</sup>-gamma-hydroxypropano-dG (g-HOPdG). When placed opposite to dC in duplex DNA, the 6S,8R,11S g-HOPdG adduct opened to the N<sup>2</sup>-(3-oxo-propyl)-dG aldehyde, which further re-arranged to exist predominantly as a set of minor groove diastereomeric cyclic hemiacetals, with relatively little perturbation of DNA structure. When placed opposite to dA, the 6S,8R,11S g-HOPdG did not re-arrange. The conformation of the X•A mismatch was pH-dependent. At the glycosyl bond, the modified base adopted the syn conformation in acidic solution and the anti conformation in basic solution. In both cases, the DNA duplex was highly perturbed. The conformational differences of X•C and X•A basepairs suggest a basis to explain, in part, the low mutagenicity of HNE-dG adduct and the high frequency of G to T mutation. Funded by NIH grant ES-05355 (C.J.R. and M.P.S.).

## 22. The contribution of key enzyme-substrate interactions to the cytochrome P450 catalyzed dehydrogenation of tamoxifen metabolites and the role of P450 conformational dynamics during tamoxifen metabolism.

K. Shahrokh, G. S. Yost, T. E. Cheatham III

The conformational and electronic factors that contribute to the formation of reactive electrophilic intermediates during drug metabolism by hepatic cytochrome P450 (P450) enzymes are of great interest for the in silico prediction of drug metabolism. To gain insight into the contributions of substrate-enzyme interactions during P450 catalysis we are using a combination of theory and experiment to study the P450 isozyme-specific metabolism of a commonly used anti-cancer drug: tamoxifen. Here we present the results of HF/6-31G\* and B3LYP/6-31G\* level calculations for key tamoxifen metabolites that are formed during P450-catalyzed oxygenation and dehydrogenation reactions. We have also performed molecular dynamics simulations of major classes of hepatic P450 involved in tamoxifen metabolism to determine the contribution of predicted P450 conformational dynamics on docking studies of tamoxifen and its metabolites. These results provide encouraging insights into the role of electronic and conformational constraints in the selectivity for P450-catalyzed oxygenation versus dehydrogenation reaction mechanisms.

## Monday Afternoon: Chemical Biology of Epigenetics

### 23. DNA methylation can be monitored by isotope tracers and GC/MS.

L. Sowers, J. Herring

Reactivation of genes silenced by methylation is of great interest to the medical field for cancer treatment and prevention as well as for development of new medical therapeutics. Current research has focused on the area of epigenetic regulation via cytosine methylation. Our studies use stable isotopes to label newly incorporated pyrimidines incorporated into DNA and newly methylated cytosines independently. This technique allowed us to verify the presumption that methylation closely follows DNA replication and led to the discovery of a process where 5-methylcytosine residues on the parental strand are in dynamic flux. Furthermore, our technique opens the door for the monitoring of cellular toxicity via DNA replication and methylation perturbations resulting from methylation inhibitors and other drugs.

### 24. Endogenous cytosine methylation influences the formation and repair of DNA adducts at CpG dinucleotides.

N. Tretyakova, R. Guza, B. Matter

Lung tumors of smokers typically contain G to T mutations within endogenously methylated MeCG dinucleotides of the p53 tumor suppressor gene, e.g. codons 157, 158, 245, 248, and 273 (MeC = 5-methylcytosine). The presence of MeC at these sites may mediate the reactivity of neighboring guanine base towards tobacco carcinogens, leading to targeted binding of metabolically activated tobacco carcinogens to MeCG sequences. We have employed stable isotope labeling

HPLC-ESI+-MS/MS methodology to analyze the formation of guanine lesions of benzo[a]pyrene diolepoxide (BPDE), NNK, acetaldehyde, and reactive oxygen species within DNA duplexes representing p53 mutational "hot spots" and surrounding sequences. We found that all four N2-BPDE-dG diastereomers and oxidative dG lesions were formed preferentially at guanine bases within MeCG dinucleotides, including frequently mutated p53 codons 157, 158, 245, 248, and 273. MeC in the basepaired position was largely responsible for this effect. In contrast, MeC inhibited the formation of NNK-induced O6-Me-dG and O6-POB-dG adducts, leading to poor adduct yields at methylated CG dinucleotides in the p53 gene. The structural basis for these effects was investigated by conducting structure-activity studies with a series of MeC structural analogs.

## **25. Genetics and epigenetics of metal carcinogenesis. A. Zhitkovich**

Carcinogenic metals have long thought to act primarily as the indirect inducers of oxidative DNA damage. However, recent discoveries revealed the importance of active stress signaling and nonoxidative processes targeting genome structure. Cellular ascorbate appears to play a central role in epigenetic and genotoxic effects of more than one metal. High ascorbate levels are important for rapid Cr(VI) reduction and efficient removal of histone methyl groups by Fe(II)-dependent demethylases that require ascorbate as a cofactor. Depletion of ascorbate was found to be largely responsible for the ability of Ni<sup>2+</sup> and Co<sup>2+</sup> ions to activate HIF1 $\alpha$  transcriptional factor and the subsequent expression of hypoxia-inducible genes. Hypoxic signaling leads to chromatin condensation and low expression of DNA repair genes. Epigenetic inactivation of genome maintenance mechanisms promotes persistence of metal-induced DNA damage and its fixation in the form of mutations and gross chromosomal rearrangements.

## **26. Inflammation mediated DNA damage induces epigenetic changes . L. Sowers, V. V. Lao**

DNA damage, inflammation and alterations in cytosine methylation patterns have all been associated with the development of cancer in humans; however, no mechanistic link has yet been established. Recent studies on inflammation-mediated DNA damage may have provided important new clues. The neutrophil-derived DNA damage product, 5-chlorocytosine, has been shown to mimic 5-methylcytosine in in vitro biochemical studies. In this study, we demonstrate that the random incorporation of 5-chlorocytosine residues into the DNA of dividing vertebrate cells can result in heritable changes in cytosine methylation patterns and result in gene silencing. Gene reactivation studies with the methylase inhibitor 5-aza-2'-deoxycytidine and bisulfite sequencing confirm that the observed effect is epigenetic and not mutagenic. These studies further strengthen a mechanistic link between inflammation-mediated DNA damage, epigenetic changes and the development of human cancer.

## **27. Impact of carcinogen-DNA adducts on DNA methylation. N. E. Geacintov, E. S. Gromova**

The methylation of DNA at CpG dinucleotides by DNA methyltransferases (MTases) is an epigenetic alteration of the genome that plays an important role in the regulation of gene expression in eukaryotes. Abnormalities in the levels of methylation are one of the hallmarks of tumorigenesis. Polycyclic aromatic hydrocarbons such as benzo[a]pyrene are ubiquitous environmental pollutants that are metabolized in vivo to highly genotoxic and tumorigenic diol epoxides. The latter react with cellular DNA to form covalent adducts that can, if not removed by cellular DNA repair mechanisms, cause mutations and the initiation of tumorigenesis in animal models, and probably in humans. The potential impact of such lesions on DNA methylation has received relatively little attention. Utilizing an in vitro biochemical approach, it is shown that BPDE-derived DNA lesions can alter DNA methylation at CpG dinucleotide sites in a manner that depends on the conformational properties of the lesions and their positions within the DNA recognition sequence. The results of these studies suggest that PAH diol epoxides may initiate cancer not only by genotoxic mechanisms, but might also contribute to tumor development by epigenetic effects that involve changes in DNA methylation status.

## **28. DNA methylation inhibitors for the treatment of cancer . A. S. Yang**

The methylation of cytosine in DNA is an important part in the epigenetic regulation of gene expression. DNA methylation along with histone modifications have also been identified as important targets for

cancer therapy and possibly other diseases. Currently there are two nucleosides, azacitidine and decitabine, that are FDA approved for the treatment of myelodysplastic syndrome, a cancer of the blood. These drugs are believed to have their clinical effect through inhibition of DNA methylation that plays a role in control of gene expression. We will examine the mechanism by which these drugs inhibit DNA methylation, explore the mechanism of clinical effect in cancer, and discuss how these drugs may be improved.

## **Tuesday Morning: Drug Safety**

### **29. Prediction of cytochrome P450-based drug-drug interactions from in vitro information. R. S. Obach**

A frequent mechanism that underlies pharmacokinetic-based drug-drug interactions (DDI) is through inhibition of cytochrome P450 enzymes that are responsible for the metabolic clearance of a majority of drugs. With our understanding of the multiplicity of human P450 enzymes and their substrate and inhibitor specificities, we have been able to use in vitro data to predict the magnitude of DDI for new drugs that inhibit or inactivate these enzymes. Prediction of DDI from in vitro data requires a conceptual picture of drug disposition, with particular emphasis on the liver and intestine, such that in vitro inhibition or inactivation data can be used in equations that model these drug disposition properties for the inhibitor and the drug affected by the inhibitor. The process by which this is done will be reviewed in this presentation and the inherent assumptions and shortcomings will be described. This is important because the in vitro tools used to determine which P450 enzymes can be inhibited by a new drug have become commonplace, and such experiments have become an expectation for supporting the development and registration of new drugs.

### **30. Grapefruit: A food that potentially impairs intestinal drug metabolism and uptake transport clinically. D. G. Bailey**

The 1991 report that grapefruit juice caused 3-fold higher oral bioavailability of the antihypertensive medication, felodipine, provided the primary important clinical example of food-mediated inactivation of first-pass drug metabolism. Many scientists subsequently significantly contributed to the understanding of this type of interaction. More than 40 medications, some of which are highly prescribed or essential medications, now appear to carry the risk of a grapefruit - induced adverse drug interaction. Recently, grapefruit juice was also shown to lower the oral bioavailability of other drugs through inhibition of a specific intestinal uptake transporter. For each type of interaction, the discovery, mechanism and active ingredient(s) will be reviewed. Clinically relevant issues (volume-effect relationships, individual variability and reproducibility, duration of effect, repeated juice consumption, age and affected drugs) will be discussed. The action of other fruit juices and the potential clinical usefulness of inclusion of the active ingredient(s) into drug formulations will be considered.

### **31. Pregnane X receptor: Structure and binding properties of xenobiotics . M. A. Sinz**

The pregnane X receptor (PXR) is a nuclear hormone receptor responsible for transcriptional regulation of drug metabolizing enzymes. Most importantly, PXR upregulates the expression of CYP3A4 which significantly increases the metabolic clearance of drugs ultimately resulting in therapeutic failure. PXR is unlike most nuclear hormone receptors in that a wide variety of agonist can bind to the ligand binding domain which is extremely large and hydrophobic with several polar residues distributed throughout. Pharmacophore models and ligand bound crystal structure data indicate that significant agonist binding requires the ligand to contain at least one hydrogen bond acceptor group along with 3-5 hydrophobic interactions. Chemical modifications are being elucidated that disrupt these interactions between agonists and ligand binding domain residues. These alterations in structure allow the medicinal chemist to develop new chemical entities which reduce the liability of PXR activation (as measured by ligand binding or transactivation assays) and drug-drug interaction potential.

### **32. Humanized mouse models to predict chemical safety and metabolism. F. J. Gonzalez, X. Ma,**

Q. Yang, J. R. Idle

Pregnane X receptor (PXR) and peroxisome proliferator-activated receptor alpha (PPARalpha) are members of the nuclear receptor superfamily that heterodimerize with the retinoid X receptor and activate target gene expression; they are widely considered as xenosensors since their ligands include a wide variety of structurally diverse chemicals including drugs and industrial agents. PXR is responsible for regulation of the cytochromes P450 3A (CYP3A) forms that metabolize many drugs while PPARalpha is involved in the hepatocarcinogenicity of the non-genotoxic peroxisome proliferators. Most importantly there are major species differences between the homologs of these receptors, especially between rodents and humans thus making animals inappropriate for accurately testing the safety of PXR and PPARalpha. To this end, mice humanized for PXR and PPARalpha were produced and characterized. Specific examples of the value of these new models, including the role of PXR in drug-drug interactions and PPARalpha in hepatocarcinogenesis, will be discussed.

## **Tuesday Afternoon: Cytochrome P450 Structure, Function, and Mechanism**

### **33. Recent advances in structural characterization of drug metabolizing P450 monooxygenases. E. F. Johnson, S. Sansen, C. D. Stout**

Crystal structures of the principal human drug metabolizing P450s reveal distinct active site architectures that underlie individual contributions to xenobiotic oxidations. The more conserved and rigid structural features support heme binding and redox partner interactions, whereas flexibility of the substrate-binding site contributes to broad substrate recognition as well as substrate access to the cavity. P450/substrate interactions are predominately hydrophobic leading to substrate selectivity based largely on size and fit. Selective polar interactions further modulate relative selectivity. The binding of two or more substrate molecules can lead to complex kinetic properties. Major challenges for the future are to better define the contributions of protein flexibility in substrate binding and to incorporate protein flexibility into methods for predicting substrate binding. Additionally, there is a need to develop methods to accurately predict the binding of substrate pairs that lead to complex pharmacokinetic properties. Supported by NIH grant GM031001 and Pfizer.

### **34. Mechanism-based inactivation of human cytochrome P450s . P. F. Hollenberg, H -L. Lin, U. M. Kent**

The cytochrome P450s catalyze the metabolic activation of a wide variety of xenobiotics to reactive intermediates that can react with cellular macromolecules leading to toxicity. These reactive intermediates can also react with moieties in the P450 active sites resulting in covalent adduct formation that leads to irreversible (mechanism-based) inactivation. Experimental approaches for characterizing mechanism-based inactivators, criteria for classification as a mechanism-based inactivator, the kinetic scheme for inactivation and the calculation of the relevant kinetic constants describing inactivation will be presented. Several examples of mechanism-based inactivation of human P450s will be presented where the site of modification, either heme moiety or apoprotein, and the identity of the reactive intermediate are known. Recent advances in trapping procedures and methods for the identification of reactive intermediates will be presented. The clinical significance of inactivating human P450s in drug safety will be discussed.

### **35. Structure and function of cytochrome P450 enzymes: dynamics of catalytic steps in oxidation of drugs and model substrates. F. P. Guengerich, C. D. Sohl, E. M. Isin, R. L. Eoff**

Several cytochrome P450 (P450) enzymes involved in the biotransformation of drugs have been examined in regard to kinetics of individual steps (esp. 1A2, 2A6, 3A4). The results indicate a mixture of rapid events (e.g., binding and release of substrate) and some that are unexpectedly slow (substrate rearrangement). The slower events, when they occur (in the P450s with the larger sites), are sequential as opposed to parallel with other events. The kinetic complexity of the P450substrate interactions is viewed as one contributor to the ligand cooperativity seen with some P450s. Another contribution is

structural, in that P450 1A2 does not show cooperativity with one substrate that fills most of the active site (beta-naphthoflavone) but does with smaller ligands. The nature of the ligands is an issue in that polycyclic aromatic hydrocarbons show positive cooperativity, possibly because of the potential to stack, but other small non-aromatic ligands show opposite cooperativity patterns.

### **36. What do industrial medicinal chemists need to know about cytochrome P450 R. E. White**

Metabolism by cytochrome P450 enzymes is the single most important type of clearance of drugs from the human body, and several important adverse clinical pharmacokinetic phenomena are corollaries of this fact. These include large individual variability, autoinduction, drug-drug interactions and covalent binding of P450-generated reactive metabolites to proteins and DNA. Therefore, design of practical clinical medicines must take account of these features of P450-mediated metabolism to create molecules for clinical development that are optimized with respect to pharmacokinetics. The chemical, biochemical and biological basis for these phenomena will be discussed and practical approaches to anticipating, detecting and remediating them during drug design presented. Special attention will be given to the generation of reactive intermediates and metabolites, which can be attributed to the rich oxidative reaction manifold of the P450 iron-oxo enzyme intermediate. Finally, the current strategies for avoiding their occurrence in clinical candidates will be critiqued.

## **Tuesday Evening: General Posters**

### **37. Inhibition of hepatobiliary transporters by a novel kinase inhibitor contributes to liver toxicity in nonclinical species. J. S. Daniels, Y. Lai, J. Davis, S. South, J. Stevens, R. Mourey, D. Anderson**

Following oral administration of a novel polycyclic kinase inhibitor, beagle dogs experienced an acute, reversible liver toxicity characterized by increases in biomarkers associated with hepatotoxicity; particularly noteworthy was a reversible elevation in bile salts and total bilirubin. Accompanying this observation was an ADME appraisal including interactions with key hepatobiliary transport proteins. Indeed, closer examination employing MDCK cells and membrane vesicles revealed potent compound-dependent inhibition of MRP2 (IC-50 38 micromolar) and BSEP (IC-50 10 micromolar), two crucial hepatobiliary transport proteins accountable for bilirubin and bile salt homeostasis, respectively. Introduction of pKa-altering modifications to a second generation compound proved successful in reducing its affinity for these key efflux transporters (MRP2 IC-50  $\gg$ 80  $\mu$ M; BSEP IC-50  $>$  70  $\mu$ M), consequently mitigating this overt organ toxicity in nonclinical species. Our results underscore the importance of transport inhibition screens as a means to predict potential hepatotoxicity inherent to new chemical entities.

### **38. Absolute quantification assay for measuring protein tyrosine kinase and phosphatase activity by stable isotope dilution liquid chromatography/ tandem mass spectrometry. E. Ciccimaro, I. A. Blair**

The generation of intracellular reactive oxygen species has been linked to aberrant cell signaling. Tyrosine kinases and phosphatases represent the two major enzyme families critical to the initiation and propagation of multiple cellular signaling pathways. The dynamic equilibrium between these counter-acting enzyme families is altered in response to various environmental toxins, chemicals, smoking and drugs, leading to a variety of patho-physiological conditions. In this study we have developed a unique stable isotope dilution liquid chromatography/ tandem mass spectrometry assay to absolutely quantify site-specific tyrosine phosphorylation on focal adhesion kinase (FAK), a critical regulatory kinase. Tryptic peptides of FAK containing the regulatory tyrosines were synthesized and phosphorylated to be utilized as internal standards. Standard curves were generated to quantitate site-specific phosphorylation in FAK to accurately predict the kinase-phosphatase activity status in biological samples and act as biomarker for exposure to toxins and chemicals. Supported by NIH grants 5R01CA095586, P30ES013508, and T32HL007954.

### **39. Absolute quantification of oxidative modifications on apoA-I protein by liquid**

**chromatography/tandem mass spectrometry.** K. Rangiah, E. Ciccimaro, I. Parastatidis, H. Ischiropoulos, M. P. Reilly, S. J. Shah, I. A. Blair

Apolipoprotein A-I (apoA-I), the major protein in High Density Lipoprotein (HDL) protects against atherosclerosis by removing cholesterol from arterial walls. Oxidative modifications mediated by myeloperoxidase (MPO) lead to nitration/chlorination on tyrosine residues at positions 192 or 166 in apoA-I to inhibit this ability during atherosclerosis progression. Our objective was to develop a quantitative assay for these oxidative modifications as a biomarker for atherosclerosis. Recombinant apoA-I protein was treated with peroxynitrite (ONOO), digested with trypsin to generate internal standard peptides with nitrated tyrosine residues, and analyzed by liquid chromatography/multiple-reaction-monitoring tandem mass spectrometry (LC-MRM/MS). Standard curves were prepared using these nitrated peptide standards. ApoA-I from fifty human plasma samples was immunopurified, spiked with these peptide standards to quantitate the oxidative modifications on apoA-I protein using these standard curves. This unique LC/MRM/MS assay to quantitate apoA-I modifications will be used as a prognostic predictor of atherosclerosis. Supported by NIH grant P30ES013508 and P50HL083799.

**40. Analysis of formaldehyde induced lysine-deoxyguanosine cross-links by mass spectrometry.** K. Lu, J. Swenberg

Formaldehyde is a known human carcinogen which can induce DNA-protein cross-links (DPC) as a primary genotoxic effect. DPC is formed through multiple-step reactions, with formaldehyde initiating a quick attack on the amino groups of protein followed by DPC formation. Lysine residue of protein was thought to be actively involved in the DPC formation, but the structures of DPC containing lysine have not been completely established yet. Here, we identified three distinct lysine-dG crosslinks induced by formaldehyde using model amino acids, peptides, oligos. These crosslinks were produced through either 1 methylene or 2 methylene bridges depending on the reaction conditions. The exocyclic N<sup>2</sup> of dG and the E-amino group of lysine were crosslinked for the adduct with 1 methylene group, while the endocyclic N1 and exocyclic N<sup>2</sup> positions were both covalently linked with the E-amino group of lysine for those crosslinks with two methylene bridges. Lysine-dG crosslinks are not stable in aqueous solutions, which does not allow further isolation and characterization of those adducts after the enzyme digestion of formaldehyde-treated DNA and protein. Therefore, we developed a reduction approach to distinguish those crosslinks and identify the crosslinking sites between histone and DNA.

**41. Analysis of gene expression changes of drug metabolizing enzymes in the livers of F344 rats following oral treatment with kava extract.** Q. Xia, L. Guo, S. Dial, Q. Li, P.-C. Chan, P. P. Fu

The association of use of kava products with liver-related risks has prompted regulatory decision in many countries. We studied the gene expression changes of drug metabolizing enzymes in the livers of F344 male rats administered kava extract by gavage for 14 weeks. Analysis of the entire 22,226 genes reveals that there are 14, 41, 110, 386, and 916 genes significantly expressed in the 0.125, 0.25, 0.5, 1.0, and 2.0 g/kg treatment groups, respectively. There are 16 drug metabolizing genes regulated in all the three high dose treatment groups, among which seven genes belong to CYP isozymes. As confirmed by real-time PCR analysis, while gene expression of Cyp1a1, Cyp1a2, Cyp2c6, Cyp3a1, and Cyp3a3 increased; Cyp 2c23 and Cyp2c40 decreased, all in a dose-dependent manner. Our results indicate that kava extract can significantly modulate metabolizing enzymes, particularly the CYP isozymes, which can cause herb-drug interactions and may potentially lead to hepatotoxicity.

**42. Analysis of hydroxybenzo[a]pyrenes in urine by liquid chromatography-tandem mass spectroscopy: Applications as a response biomarker for polycyclic aromatic hydrocarbon (PAH) exposure.** S. Sen, S. L. Gelhaus, I. A. Blair

Tobacco smoke, a leading cause of death in the US, contains a complex mixture of components including polycyclic aromatic hydrocarbons (PAHs). PAHs have been cited as causative agents in lung, skin and bladder cancer. Tobacco smoke exposure is currently measured by detection of the pyrene

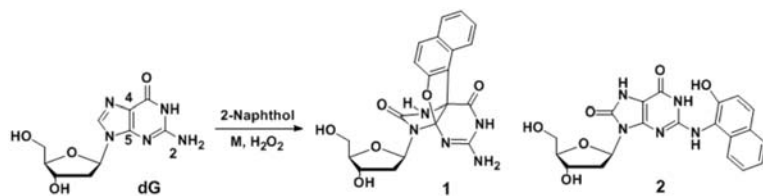
metabolite, 1-hydroxypyrene (1-OH-P) in urine and plasma. Unfortunately, 1-OH-P is not the best biomarker of tobacco carcinogen exposure because pyrene is non-carcinogenic. The current study focuses on the detection of benzo[a]pyrene (B[a]P), a model carcinogenic PAH, and its metabolites as biomarkers of tobacco exposure. These metabolites, 1-, 3-, 9-hydroxy-benzo[a]pyrene (OH-B[a]P) are found at very low levels in urine; therefore, extremely sensitive methods are required. Here we report the detection of the B[a]P metabolites, 1-OH-B[a]P, 3-OH-B[a]P and 9-OH-B[a]P, in urine utilizing the highly sensitive and highly specific stable isotope dilution LC-electron capture APCI/MRM/MS methodology based on the transition  $m/z$  267 (M-PFB) to  $m/z$  239 (M-PFB-CO) and relevant transitions from the C-13 internal standards.

**43. Analysis of RNA secondary modifications as biomarkers of exposure.** C. Chan, K. Taghizadeh, T. Begley, P. Dedon

Recent evidence suggests a role for translational contributions to the cellular responses to noxious exposures. To test the hypothesis that changes in RNA modifications play a role in this translational response, we have developed a coupled liquid chromatography-mass spectrometry approach to characterize and quantify changes in the spectrum of the >25 tRNA modifications occurring in cells. In *Saccharomyces cerevisiae* exposed to H<sub>2</sub>O<sub>2</sub>, ESI-QTOF analysis revealed increases in the relative abundance of methylated cytosines and guanines in tRNA, with decreases in other modifications. These and other results with a variety of chemical and physical agents will be discussed in terms of the chemical biology of RNA secondary modifications in states of both pathology and normal physiology. The unique changes in the spectrum of modified nucleosides from exposure to different toxins have a high potential to serve as biomarkers.

**44. Analysis of the products from the arylation of deoxyguanosine and DNA by phenols under oxidative conditions.** A. M. Fleming, X. Xu, A. Kannan, J. G. Muller, C. J. Burrows

Exposure to phenols through exogenous and endogenous sources shows deleterious effects through nucleobase modification occurring under oxidative conditions. Deoxyguanosine (dG) is the most electron rich of the four canonical bases, and has many nucleophilic sites, but is also susceptible to 2 and 4 electron oxidations. Studies were conducted where dG was allowed to react with 2-naphthol in the presence of di- and tri-valent metals with H<sub>2</sub>O<sub>2</sub> yielding two compounds. Analysis supports the assignments of 1, and 2 (tentatively). Both are the result of an overall 4 electron oxidation and consequently have the same masses, but drastically different structures, providing mechanistic insight into their formation. Comparisons of these structures will be made to reactions in which 2-naphthol is replaced with tyrosine, and its model p-cresol, to aid in elucidating DNA-protein crosslinks. Thus, modification of dG by phenols yields products whose geometry and base pairing properties are altered with potential mutagenic effects.



**45. Arylhydrocarbon receptor (AhR)-dependent DNA strand breaks by Benzo[a]pyrene-7,8-dione, a PAH metabolite of Aldo-Keto Reductase (AKR).** J. H. Park, A. J. Frey, T. M. Penning

PAH o-quinones are ligands involved in AhR activation and are able to produce oxidative DNA lesions in vivo. However, how the quinones gain entry into the nucleus and cause oxidative DNA damage is unknown. We used the single cell gel electrophoresis (comet) assay to detect DNA strand breaks in murine hepatoma Hepa1c1c7 cells and its AhR- and ARNT-deficient variants, Hepa1c1c12 and Hepa1c1c4 cells, following treatment with B[a]P-7,8-dione. Also, siRNA was used to achieve AhR knockdown in human lung bronchoalveolar H358 cells. B[a]P-7,8-dione produced less DNA strand breaks in AhR-deficient Hepa1c1c12 cells compared to Hepa1c1c7 and Hepa1c1c4 cells. A similar result

was also found in AhR knockdown H358 cells. The application of human 8-oxoguanine glycosylase (hOGG1) to the assay produced more B[a]P-7,8-dione-mediated DNA strand breaks in Hepa cells and H358 cells but the levels of hOGG1-dependent DNA strand breaks were lower in AhR-deficient Hepa1c1c12 cells and AhR knockdown H358 cells. These findings suggest that the AhR can enhance PAH o-quinone-mediated oxidative DNA damage (Supported by R01-CA39504, R01-ES015857 & P30-ES013508 awarded by T.M.P).

**46. Cellular nucleotide pools as targets for nitrosative deamination during inflammation. V. Dendroulakis, W. M. Deen, P. Dedon**

Epidemiological evidence points to a link between chronic inflammation and cancer. At sites of inflammation, phagocytic immune cells secrete reactive oxygen and nitrogen species to combat infection, with these chemicals causing collateral damage to surrounding host tissue. Among these reactive species, nitrous anhydride ( $N_2O_3$ ) arises from autooxidation of macrophage-derived nitric oxide and is the major nitrosating species responsible for deamination of DNA and RNA nucleobases. Our recent studies revealed modest DNA deamination in inflamed mouse tissues and higher levels of damage in RNA. To test the role of solvent exposure in the reactivity of nucleobases with chemical mediators of inflammation, we developed methods to quantify deamination products in the cellular nucleotide pool. We approached this problem using LC/MS-MS to resolve and quantify nucleotides in cellular extracts, and using model *E. coli* strains harboring mutations that cause overproduction of deaminated nucleotides. The results will be discussed in terms of studies in mammalian cells and the challenges facing rigorous quantification of components of the nucleotide pool.

**47. Characterization of furan metabolites and their potential role in furan-derived protein binding. D. Lu, M. M. Sullivan, L. A. Peterson**

The hepatotoxicity and carcinogenicity of furan observed in rodents is attributed to cytochrome P450 catalyzed formation of cis-butene-1,4-dial (BDA). However, the link between metabolites and toxic effects is not fully understood because of inadequate information on furan metabolism. We addressed this issue by studying furan metabolites from urinary samples of F344 rats treated with [ $^{12}C_4$ ]- and [ $^{13}C_4$ ]-furan. Structural information of the metabolites was revealed by LC/MS/MS analysis and confirmed by synthetic standards. The major urinary metabolites are derived from a pyrrole structure which is assembled by cross-linking cysteine to lysine via BDA. The regioselectivity of the reaction with lysine amino groups suggests these metabolites are degraded protein adducts. Characterization of metabolites from furan-treated hepatocytes indicates that the cysteine may be derived from glutathione (GSH). Consistently, GSH is covalently cross-linked to liver proteins in furan-treated rats, suggesting that GSH-BDA conjugates are responsible for protein adduct formation in furan-exposed tissues [Supported by ES-10577].

**48. Characterization of lipid peroxidation using a novel thiadiazabicyclo endogenous glutathione adduct in a paraquat-induced model of oxidative stress. S. S. Basu, V. V. Shuvaev, V. R. Muzykantov, I. A. Blair**

Paraquat is widely used as a model of intracellular oxidative stress. The mechanism of paraquat-induced toxicity involves redox cycling, ROS generation, and subsequent damage to intracellular macromolecules. While the number of techniques used to quantify biomarkers of oxidative DNA and protein damage is growing, many of the current techniques used to measure lipid peroxidation suffer from high background and unstable biomarkers. We have previously identified a novel bifunctional electrophile-derived thiadiazabicyclo-ONE-GSH adduct (TOG), which is formed by the reaction of glutathione and 4-oxo-2(E)-nonenal (ONE), a product of lipid peroxidation. We have also shown the intracellular formation of TOG in endothelial cells treated with ONE or  $Fe^{2+}$ . In this study, we used stable isotope dilution LC-MRM/MS to monitor intracellular TOG formation in REN cells treated with paraquat and  $Fe^{2+}$  to assess the stability of the molecule as well as the sensitivity and specificity of this

assay to monitor lipid peroxidation.

**49. CTP-347: A deuterated analog of paroxetine with greatly reduced CYP2D6 mechanism-based inactivation.** A. J. Morales, R. Gallegos, A. Jones, V. Uttamsingh, C. Cheng, G. Bridson, J. F. Liu, D. Wells, R. Tung, R. Zelle, **S. Harbeson**

Paroxetine, a frequently-prescribed serotonin reuptake inhibitor, is a potent CYP2D6 mechanism-based inactivator. As such, paroxetine is associated with significant drug-drug interactions due to the role of CYP2D6 in drug metabolism. We will present CTP-347, a new chemical entity that differs from paroxetine by specific deuterium incorporation at key positions of the molecule. CTP-347 possesses an in vitro pharmacology profile similar to paroxetine, but shows an increased rate of clearance in vitro and in vivo. Furthermore, we show that this difference in clearance is due to a significant decrease in the compound's ability to inactivate CYP2D6 relative to paroxetine. To our knowledge, this is the first report of deuterium isotope effects upon the mechanism-based inhibition of CYP2D6 by compounds containing a methylenedioxy phenyl moiety. These results suggest that the potential for drug-drug interaction for CTP-347 may be substantially reduced compared to paroxetine.

**50. Cu(II)/H<sub>2</sub>O<sub>2</sub>/Ascorbate-induced Formation and in-vitro Replication of Thymidine Glycol/8-Oxo-2'-deoxyguanosine Tandem Lesions.** **Y. Jiang**, Y. Wang

Exogenous and endogenous sources of ROS can induce DNA lesions, including intrastrand crosslink lesions and tandem single-nucleobase lesions. We successfully employed LC-MS/MS to identify and quantify the formation of a tandem lesion, Tg/8-oxodG, where a thymidine glycol (Tg) is adjacent to an 8-oxo-2'-deoxyguanosine (8-oxo-dG), induced by Fenton reagents, Cu(II)/H<sub>2</sub>O<sub>2</sub>/ascorbate. It turned out that the yield for the tandem lesion is dose-responsive. In-vitro replication results showed that, when compared with the substrates bearing only an 8-oxo-dG or a Tg, the synthesis of Klenow fragment of *E. coli* DNA polymerase I showed a marked drop in insertion efficiency. The synthesis stopped after bypassing the thymidine moiety of the tandem lesions. Although the lesions could be bypassed by a translesion synthesis polymerase, yeast polymerase  $\zeta$ , the efficiency is further reduced than that of both single-base lesions. We also found that the mutagenic property of 8-oxodG is not affected significantly by the presence of the neighboring Tg, and vice versa.

**51. Defining the molecular mechanisms of environmental carcinogens in lung carcinogenesis.** **S. L. Gelhaus**, T. Penning, I. A. Blair

Environmental carcinogens, such as polycyclic aromatic hydrocarbons (PAHs), are able to induce lung cancer through metabolic activation. Benzo[a]pyrene (B[a]P), a model PAH, was used to elucidate these activation pathways. Activation of PAHs by P450 is a widely accepted pathway of metabolism. P450 1A1/1B1 is known to oxidize B[a]P to 7, 8-dihydroxy-9, 10-epoxy, 7, 8, 9, 10-tetrahydrobenzo[a]pyrene (B[a]PDE). B[a]PDE forms stable, covalent adducts with DNA. H358, human bronchoalveolar, cells do not constitutively express P450s 1A1/1B1; however, they can be induced with pre-treatment of 2,3,7,8-tetrachlorobenzo-p-dioxin (TCDD). Unexpectedly, TCDD induction caused a decrease in B[a]PDE-DNA formation versus the untreated cells. Further investigation of B[a]P metabolites revealed that B[a]P-7,8-dione is formed in H358 cells despite the fact that no aldoketoreductases are present for catechol formation to take place. B[a]P-7,8-dione may be up-regulating and alternative pathway leading to increased B[a]PDE-DNA formation where as the TCDD pathway is protective. Funded by NIH 1F32ES016683 and 1-R25-CA-101871.

**52. Designing compounds capable of forming 3-methyladenine DNA adducts in estrogen receptor positive cells.** **S. Varadarajan**, H. Perry, L. Smith, T. Lynch, S. Miller, B. Kelly

This paper describes the design of new compounds that are capable of targeting cells that express the estrogen receptor, and capable of producing cytotoxic N3-methyladenine DNA adducts in those cells. These compounds contain a reactive methylsulfonate group connected to a unit that binds to DNA in the minor groove at adenine-thymine rich regions, and can therefore selectively methylate adenines in these

regions at the N3-position. This component is connected by a linking unit to the estrogen receptor targeting ligand, estradiol. The design, synthesis, and characterization of these new compounds will be presented.

### 53. Effect of N<sup>2</sup>-alkylguanine adducts on replication by Y-class *Sulfolobus solfataricus* DNA polymerase Dpo4. H. Zhang, R. L. Eoff, K. C. Angel, F. P. Guengerich

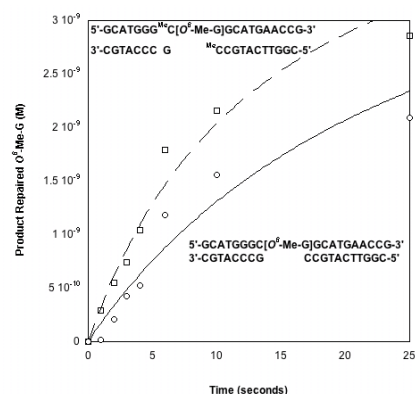
Genomic integrity and survival of organisms are subject to DNA damage, which may lead to base misincorporation, blockage of replication, cell death, aging, or cancer. The N<sup>2</sup> atom of guanine (G) is susceptible to attack. Some N<sup>2</sup>-G adducts are miscoding and have been detected in vivo and may be related to cancer. The Y-class *Sulfolobus solfataricus* DNA polymerase Dpo4 showed similar full length extension, steady-state incorporation efficiency, and fidelity for N<sup>2</sup>-G adducts with sizes from N<sup>2</sup>-methylG up to N<sup>2</sup>-CH<sub>2</sub>(2-naphthyl)G, compared with unmodified G (decrease 126-fold), but N<sup>2</sup>,N<sup>2</sup>-dimethylG severely blocked extension, and drastically decreased steady-state efficiency and fidelity by 4 orders of magnitude; misincorporation of dTTP over dCTP was preferred by 2.3-fold. Pre-steady-state kinetic analysis showed the burst amplitude decreased by 24-fold and dCTP binding affinity was weakened 24-fold for N<sup>2</sup>,N<sup>2</sup>-dimethylG compared with N<sup>2</sup>-methylG. (Supported by USPHS R01 ES010375 and P30 ES000267)

### 54. Effects of chemical structure of bulky DNA lesions and base sequence on the removal of DNA damage by nucleotide excision repair mechanisms in vitro. Y. Liu, K. Kropachev, D. Reeves, L. Zhang, J. Ren, M. Kolbanovskiy, N. E. Geacintov

The removal of bulky DNA lesions by nucleotide excision repair (NER) is an important line of defense against environmental carcinogenic chemicals. The capacity of the human NER system to remove different lesions can vary by two orders of magnitude, or more. However, the molecular origins of these effects are poorly understood. In order to better understand the parameters that elicit efficient NER response and those that do not, we have examined the effects of base sequence context on the conformation and nucleotide excision repair of a series of structurally different lesions derived from the binding of benzo[a]pyrene 7,8-diol 9,10-epoxide to N<sup>2</sup>-guanine in TG\*T, CG\*C, CG\*G, and CGG\* sequence contexts. Significant base sequence effects are observed on NER efficiencies catalyzed by both thermophilic UvrABC and human NER factors in Hela cell extracts. In other experiments, the effects of chemical structure of the DNA lesions were compared, and included DNA adducts derived from aromatic amines and from metabolites of equine estrogens that are important components of hormone replacement therapy formulations. Research supported by NIH/NCI grants CA099194 and CA112412 to N.E.G.

### 55. Effects of endogenous cytosine methylation on repair of O<sup>6</sup>-Me-dG adducts by O<sup>6</sup>-Alkylguanine DNA alkyltransferase. R. Guza, Q. Fang, A. E. Pegg, N. Tretyakova

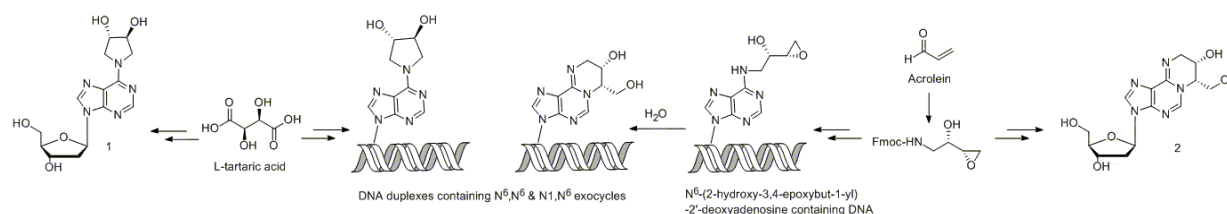
O<sup>6</sup>-alkyl-deoxyguanosine (O<sup>6</sup>-alkyl-dG) lesions are promutagenic DNA adducts that can be induced by a variety of alkylating agents. A specialized repair protein, O<sup>6</sup>-alkylguanine DNA alkyltransferase (AGT), recognizes and repairs these adducts by transferring the O<sup>6</sup>-alkyl group from the damaged base to a cysteine residue within the active site of the protein. This process may be influenced by local DNA sequence and endogenous modifications of DNA, e.g. the presence of neighboring 5-methylcytosine (MeC). We have developed an isotope dilution HPLC-ESI-MS/MS approach to examine the kinetics of AGT repair of O<sup>6</sup>-alkyl-dG lesions as a function of local DNA sequence context and cytosine methylation. Within p53 gene-derived sequences



our results reveal that the kinetics of AGT mediated repair of O<sup>6</sup>-Me-dG lesions is affected by the presence of neighboring MeC in a sequence-dependent manner. Slow repair of O<sup>6</sup>-alkyl-dG at methylated CG dinucleotides may be responsible for G → A transitions at these sites.

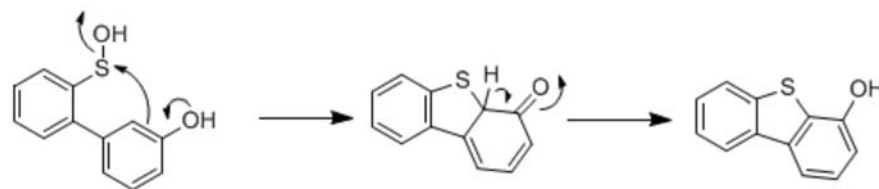
**56. Exocyclic 2'-deoxyadenosine adducts of 1,3-butadiene: synthesis, structure elucidation, and effects on DNA structure.** U. Seneviratne, T. D. Dissanayake, N. Tretyakova

Diepoxybutane (DEB) is a cytotoxic and mutagenic metabolite of 1,3-butadiene (BD). The potent genotoxic effects of DEB are attributed to its ability to form DNA-DNA cross-links and exocyclic nucleobase lesions by consecutively alkylating two nucleophilic sites within a DNA duplex. Recently, we isolated & characterized racemic N<sup>6</sup>,N<sup>6</sup>-DHB-dA (1) and N<sup>1</sup>,N<sup>6</sup>-HMP-dA (2) upon secondary reactions of N<sup>6</sup>-(2-hydroxy-3,4-epoxybut-1-yl)-dA. However, detailed structural and biological studies of these novel exocyclic lesions are dependent on the availability of DNA containing site specific lesions 1 and 2. In the present work optically pure S,S and R,R stereoisomers of 1 were prepared starting with tartaric acid isomers and incorporated in to DNA chains by a postoligomerization approach. Similarly, stereoisomers of 2 were prepared starting with acrolein following diastereomeric resolution.



**57. Formation of cysteine-tyrosine crosslinks via a sulfenic acid intermediate.** A. H. Cummings, K. Keerthi, K. 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. In the work presented here we investigated the intramolecular reaction between a sulfenic acid and a tyrosine mimic. The results provide chemical evidence that sulfenic acids have the potential to forge intrastrand protein crosslinks with tyrosine residues in proteins.



**58. Formation of RNA adduct with a novel endogenous mutagen and carcinogen, aminophenylnorharman.** Y. Totsuka, T. Takamura-Enya, T. Sugimura, K. Wakabayashi

Aminophenylnorharman (APNH) is produced from norharman and aniline in the presence of a metabolic activation system. APNH is metabolically activated by CYP1A2 and acetyltransferase to form a DNA adduct, 2'-deoxyguanosin-8-yl-aminophenylnorharman (dGuo-C8-APNH), and this adduct is detectable in various tissues of rats and mice after administration of APNH. Since APNH is detected in human urine samples collected from both healthy volunteers and inpatients, this compound could be considered to be an endogenous mutagen/carcinogen. In recent years, RNA modifications by mutagens/carcinogens have received attention as new biomarkers and for biological properties in carcinogenesis. To investigate this, we examined formation of APNH-RNA adducts and conducted a structural analysis using various spectrometric approaches. When a reaction mixture of an ultimate mutagenic form of APNH, N-acetoxy-APNH, and guanosine (Guo) was subjected to LC-ESI/MS analysis, one peak, with a similar

UV spectrum to dGuo-C8-APNH, exhibited a molecular ion peak at  $m/z$  541 along with a fragment ion peak at  $m/z$  409, consistent with loss of a ribose moiety. From  $^1\text{H-NMR}$  analysis, its chemical structure was concluded to be guanosin-8-yl-aminophenylnorharman (Guo-C8-APNH). The same adduct was yielded in yeast tRNA incubated with N-acetoxy-APNH. Additional analysis of *in vivo* adduct formation in the livers of rats administered APNH at a concentration of 100 mg/kg by the  $^{32}\text{P}$ -postlabeling method revealed that several adduct spots, including one that corresponded to Guo-C8-APNH, were observed. The total adduct levels of APNH-RNA were  $28 \pm 13.3$  adducts per  $10^6$  nucleotides. Comparisons demonstrated 6 times higher levels of total APNH-RNA than total APNH-DNA adducts in the same rat liver samples. These results indicate that the APNH-RNA adduct might be a useful biomarker for exposure to APNH.

**59. Genotoxicity and mutagenicity of 6-thioguanine and its oxidation/methylation products in vivo.** B. Yuan, Y. Wang

Thiopurine and 6-thioguanine are widely used as anti-cancer drugs for acute lymphoblastic leukemia and chronic myeloid leukemia. However, upon UVA exposure, 2-aminopurine-6-sulfonic acid (2-AP-6-SO<sub>3</sub>H) can be induced by the oxidation of 6-thioguanine. In addition, 6-thioguanine can be methylated by S-adenosyl-L-methionine (S-AdoMet) to afford S<sup>6</sup>-methylthio-2-aminopurine (2-AP-6-SCH<sub>3</sub>). Previous *in vitro* studies showed that 2-AP-6-SO<sub>3</sub>H and 2-AP-6-SCH<sub>3</sub> could miscode during DNA replication. To study the cytotoxic and mutagenic properties of 6-thioguanine and its oxidation/methylation products (2-AP-6-SO<sub>3</sub>H/2-AP-6-SCH<sub>3</sub>) *in vivo*, we constructed single-stranded M13 genomes containing a site-specifically inserted 6-thioguanine, 2-AP-6-SCH<sub>3</sub> or 2-AP-6-SO<sub>3</sub>H and allowed these genomes to propagate in wild-type as well as the isogenic AB1157 *E. coli* cells that are deficient in translesion synthesis DNA polymerases. We employed the REAP and CRAB assays developed by Essigmann and coworkers and employed mass spectrometry for interrogating the replication products. Our results revealed that both 2-AP-6-SCH<sub>3</sub> and 2-AP-6-SO<sub>3</sub>H are strongly mutagenic, and they could result in significant G to A mutation in wild-type and bypass polymerase deficient *E. coli* cells. In addition, 2-AP-6-SO<sub>3</sub>H can result in considerable -1 frameshift mutation. Moreover, both pol IV and pol V are involved in bypassing the lesion *in vivo*.

**60. Human AKRs display quinone reductase activity with PAH quinones and equilenin-o-quinone**. C. A. Shultz, J. L. Bolton, R. G. Harvey, T. M. Penning

Human aldo-keto reductases (AKRs) AKR1A1, 1B1, 1B10, 1C1-4, and 7A2 display substantial quinone reductase (QR) activity with non-K-region polycyclic aromatic hydrocarbon (PAH) o-quinones. AKRs reduce o-quinones to catechols, which auto-oxidize back to o-quinones, generating reactive oxygen species. We compared benzo[a]pyrene-7,8-dione, benzo[a]pyrene-1,6-dione, and benzo[a]pyrene-3,6-dione as QR substrates. The extended benzo[a]pyrene-quinones gave depressed rates of QR activity suggesting that AKRs display preferential activity with o-quinones produced from PAH-trans-dihydrodiols. We examined the HRT metabolite equilenin-o-quinone (3,4-EQ) as a QR substrate. AKR1B1, 1C1-3, and 7A2 catalyzed the reduction of 3,4-EQ at rates 14-500 fold greater than the non-enzymatic rate depending on the isoform. In endometrial cancer AKR1C1 and AKR1C3 expression is elevated (Mol. Cell. Endocrinol. 248 (2006) 126) and may contribute to a depressed Progesterone : 17 $\beta$ -Estradiol ratio. QR of AKRs may increase oxidative stress inflicted by PAH quinones and 3,4-EQ, which can contribute to chemical and hormonal carcinogenesis. (Supported by RO1 CA39504, P30-ES013508).

**61. Hydrogen sulfide, an endogenous cellular signaling agent, generates reactive oxygen species under physiologically-relevant conditions.** M. A. Hoffman, S. Sivaramakrishnan, K. S. Gates

Hydrogen sulfide has recently emerged as an endogenous cellular signaling agent. The molecular mechanisms by which hydrogen sulfide transmits signals remains unclear. Here we report that hydrogen sulfide undergoes autoxidation under physiologically-relevant conditions to generate reactive oxygen species (O<sub>2</sub><sup>-</sup>, H<sub>2</sub>O<sub>2</sub>, and HO $\cdot$ ). This property has the potential to explain many of the biological

properties of hydrogen sulfide, including its ability to serve as a signaling agent.

**62. Identification and quantification of 15-oxo-eicosatetraenoic acid, a novel 15-lipoxygenase-derived arachidonic acid metabolite in an atherosclerosis cell model.** C. Wei, P. Zhu, S. J. Shah, I. A. Blair

15-lipoxygenase (15-LOX-1) is up-regulated and localized at macrophage accumulation sites during atherosclerosis. We have previously identified 15(S)-hydroperoxyeicosatetraenoic acid [15(S)-HPETE] and 15(S)-hydroxyeicosatetraenoic acid [15(S)-HETE] as 15-LOX-derived bioactive lipids from arachidonic acid (AA) metabolism; however, their significance in atherosclerosis is not established. Using stable-isotope-dilution chiral-liquid-chromatography coupled with electron-capture-atmospheric-pressure-chemical-ionization mass spectrometry (LC-ECAPCI)/MS we have identified and quantified 15-oxo-eicosatetraenoic acid (15-oxo-EETE), a novel downstream metabolite in 15-LOX-transfected RAW 267.4 cells (R15L), in response to AA, given exogenously or produced endogenously following treatment with a calcium ionophore A-23187 (CI). Pretreatment with LOX inhibitor, cinnamyl-3,4-dihydroxycyanocinnamate (CDC), significantly inhibited 15-oxo-EETE production upon AA or CI treatment in R15L cells. Furthermore, 15-oxo-EETE treatment reduced R15L cell viability (MTT assay) and induced caspase-3 activation in human umbilical vein endothelial cells (HUVEC). This is the first report that 15-oxo-EETE, a novel 15-lipoxygenase-derived lipid-metabolite, is formed in cells relevant to progression of atherosclerosis. Supported by NIH grants RO1CA095586, 5P30ES013508, and P50HL08379.

**63. Identification and quantification of candidate protein biomarkers of preterm birth by liquid chromatography/tandem mass spectrometry.** S. J. Shah, E. Ciccimaro, K. Yu, S. I. Parry, I. A. Blair

Preterm birth (PTB), a leading contributor of perinatal mortality with poorly defined etiology, is influenced by complex genetic-environmental interactions, including race, toxins, smoking and diet. Early identification of at-risk women can alleviate this problem, and hence the need for novel biomarkers. Our study utilizes stable isotope labeling by amino acids in cell culture (SILAC) to develop labeled proteome standard which models human cervical-vaginal fluid (CVF) proteins. Secreted proteins from human endocervical (End1) and vaginal (VK2) cells labeled with [ $^{13}\text{C}_6^{15}\text{N}_2$ ]-Lysine and [ $^{13}\text{C}_6^{15}\text{N}_1$ ]-Leucine were characterized by three-dimensional liquid chromatography/tandem mass spectrometry (3D-LC-MS/MS). An LC-multiple reaction monitoring/MS (LC-MRM/MS) assay was developed to quantitate twenty promising biomarker candidates for PTB. This unique method provides for relative quantification of twenty proteins simultaneously. This assay has been applied to investigate human CVF samples for PTB, and study the effect of specific environmental toxins and smoking on PTB to identify novel biomarkers. Supported by NIH Grant P30ES013508.

**64. Identification of Artifacts in Cyanide Trapping of Reactive Intermediates in N-methyl Piperazine Analogs.** C. M. Resuello, Z. Minli, C. S. Elmore, M. E. Powell

During a reactive metabolite screen on methyl substituted piperazine compounds using potassium cyanide (KCN) as trapping agent, significant formation of cyanide adducts (CN) on the piperazine in many compounds tested was observed. However, it is not clear if the CN adducts were formed through bioactivation or through an artifact mechanism. From literature, two possible pathways of CN-adduct formation can occur. The first pathway occurs through bioactivation from a nitrinium ion intermediate. The second pathway is likely an artifact formed during the liver microsome incubation. In this second pathway, the dealkylation occurs in the N-methyl group in the piperazine. The demethylated piperazine reacts with naturally occurring formaldehyde to produce the nitrinium ion, which reacts with KCN. In this study, a group of piperazines with different N-substitutions and  $^{13}\text{C}$  stable isotope labeled substituted groups were used to determine which pathway is predominant and whether the formation of the CN adduct was proportional to N-demethylation. It was observed that both pathways occurred and that the contribution of the second pathway coincided with the extent of N-demethylation.

**65. Identification of DODE-modified serum proteins from mouse models of inflammation.** M. V.

**Williams**, A. Chiang, J. S. Wishnok, S. R. Tannenbaum

Lipid peroxidation has been implicated in the etiology of a wide variety of age-associated diseases, including cardiovascular disease, neurodegenerative diseases, and cancer. Lipid peroxidation forms reactive electrophiles that can cause aldehyde and ketone-containing damaged proteins, which can potentially serve as important biomarkers. We previously showed that 9,12-dioxo-10(E)-dodecenoic acid (DODE) was the most reactive electrophile in the presence of cytochrome C in vitro. Therefore, an antibody to DODE-modified proteins was made. The antibody was found to be highly specific. Western blot analysis of serum from mouse models of inflammation showed a finite number of DODE- damaged proteins which arose in a time dependent manner. We are currently identifying the proteins by LC-MS/MS.

**66. Isoprostanes profiling by liquid chromatography electron-capture atmospheric pressure chemical ionization mass spectrometry (LC-ECAPCI/MS). C. Mesaros, S -H. Lee, I. A. Blair**

Isoprostanes (iPFs) are a family of prostaglandin (PG) isomers formed in an enzyme-independent manner by the oxidation of polyunsaturated fatty acids (PUFA) in membrane phospholipids. These iPFs may be subsequently released by the action of phospholipase (PLA2) to circulate in plasma undergo metabolism and/or be excreted in urine. Quantitation of urinary iPFs produced from arachidonic acid has been used as an index of oxidative status. Cigarette smoke is known as a major inducer of oxidative stress. We have developed a stable isotope dilution LC-ECAPCI/MS for the quantitation of PGF2 $\alpha$ , 11 $\beta$ -PGF2 $\alpha$ , 8-iso-PGF2 $\alpha$ , 2,3-dinor-PGF2 $\alpha$ , iPF2 $\alpha$ -VI, 5-epi-iPF2 $\alpha$ -VI, 8,12-iso-iPF2 $\alpha$ -VI, 5-epi-8,12-iso-iPF2 $\alpha$ -VI. We tested the method for tissues and biological fluids, and we looked at the iPFs levels of smokers versus non-smokers in urine. A liquid-liquid extraction method was used to reduce sample preparation to a minimum, followed by pentafluorobenzyl bromide (PFB) derivatization.

**67. Lipid-derived biomarkers of cigarette smoke exposure in exhaled breath condensates. S. H. Bhat, S. L. Galhauz, I. A. Blair**

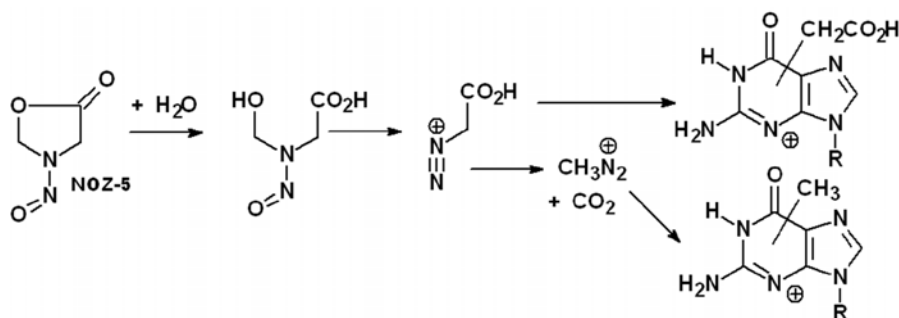
Exposure to cigarette smoke is a leading cause of death in United States and is associated with 90% of lung cancer. There is increasing evidence that show increased levels of several oxidized lipid metabolites in Exhaled Breath Condensates (EBCs) of smokers, resulting at least in part from the inflammatory response in lungs. Our current objective lies in the identification, validation and absolute quantitation by stable isotope dilution method to characterize oxidized lipids as biomarkers of tobacco smoke exposure. Using organic extraction, lipids are extracted from smokers EBCs which showed elevated levels of isoprostanes and hydroxy fatty acids. All samples as well as standard lipid mixtures for calibration are spiked with appropriate quantities of deuteriated internal standards. High sensitivity detection as well as quantification is achieved using derivatization with pentafluorobenzyl moiety and employing electron capture atmospheric pressure chemical ionization, followed by analysis using selected reaction monitoring on a triple quadrupole mass spectrometer.

**68. Mass spectrometric analysis of histone phosphorylation induced by ionizing radiation and other DNA damaging agents. M. S. DeMott, K. T. Taghizadeh, P. C. Dedon**

The posttranslational modifications of histone proteins in chromatin play a significant role in the control of gene expression. As one example, the phosphorylation of serine 129 in histone H2A of the yeast *Saccharomyces cerevisiae* (139 in mammals), has gained prominence with a putative direct link to DNA damage and repair. Other histone phosphorylation responses may exist, but use of antibodies, while extremely sensitive, is limited to availability and is often hindered by cross-reactivity and a phenomenon known as epitope occlusion in which some nearby amino acid modifications could interfere with proper detection. To more directly identify and monitor different phosphorylation events within histones, we have developed an LC-MS/MS based method that analyzes the extent of phosphorylation of histone-derived tryptic peptides isolated from cells treated with a variety of DNA damaging agents, including ionizing radiation. This sensitive and specific approach should provide novel insights into the relationship between different histone phosphorylation events and the cellular response to DNA damage.

**69. Methylating and carboxymethylating agents from N-nitroso-1,3-oxazolidin-5-one (NOZ-5), a possible product of endogenous nitrosation.** R. N. Loeppky, Y. Li, S. Rajagopal

N-Nitroso-1,3-oxazolidin-5-one (NOZ-5) could form from the reaction of dietary components glycine and formaldehyde with an endogenous nitrosating agent under human gastric conditions. Hydrolysis products from NOZ-5s are consistent with the sequential formation of reactive  $\alpha$ -hydroxynitrosamine and diazonium ion intermediates. Because NOZ-5 is genotoxic, while 2-, or 4-methyl NOZ-5s so far show only marginal activity, we have proceeded to demonstrate the nature of the NOZ-5 hydrolysis products, and to explore this substrate's guanosine alkylating capacity. The hydrolysis (pH 7, 37 °C,  $t_{1/2}$  =19 h) of NOZ-5 was followed kinetically by UV and its hydrolysis products were determined by both NMR and GC/MS analysis in comparison with standards. Formaldehyde, glycolic acid, and methanol are the major products, and arise from the carboxymethyl- and methyl-diazonium ions, respectively. The decomposition of NOZ-5 in the presence of guanosine led to the formation of carboxymethyl-, and methyl-guanine adducts (HPLC/MS/MS), the percentage of the former being significantly higher.



**70. N<sup>2</sup>-(1-Carboxyethyl)deoxyguanosine: Potential mutagenic and prognostic implications of glycated DNA.** D. Tamae, J. Termini

Methylglyoxal is an electrophilic  $\alpha$ -oxoaldehyde that is formed by the enzymatic and non-enzymatic conversion of triose and triose-phosphates. Under certain pathological conditions such as diabetes, aging, and cancer, the increased cellular concentration of MG can lead to reactions with protein, lipid, and DNA, resulting in advanced glycation end-products (AGEs). The exquisitely sensitive and highly specific technique of HPLC-ESI-MS/MS was used to both identify and quantitate both stereoisomers of the DNA adduct, N<sup>2</sup>-(1-carboxyethyl)-deoxyguanosine (CEdG). Utilizing a stable isotope dilution method, this study represents the first time this DNA lesion has been quantitated in biological samples. Levels of CEdG were measured in normal and cancerous human breast tissue (12 and 4 adducts per 107 dG, respectively) and free CEdG from the urine of non-diabetic and diabetic rats (76.8 and 277.2 pg/ml/24hr, respectively). Separately, the chemical synthesis of CEdG in the triphosphate form and in an oligonucleotide has allowed for steady state kinetic studies using model replicative polymerases. Using Klenow fragment, the level of misincorporation of dATP opposite the lesion was favored by an order of magnitude relative to the "correct" insertion of dCTP.

**71. Oxidative stress mediated DNA damage on exposure of cigarette smoke.** D. Mangal, A. Y. Wehr, S. H. Lee, C. Mesaros, T. grosser, S. Fries, T. M. Penning, I. A. Blair

Oxidative stress is one of the major consequences of exposure to environmental pollutants such as cigarette smoke. These environmental pollutants have a major amount of polycyclic aromatic hydrocarbons (PAHs), their metabolic pathway generate the reactive oxygen species that in turn cause the DNA damage. Of all DNA adducts formed, 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-oxo-dGuo) adduct is of a particular significance and it has been proposed as a biomarker of DNA damage. DNA repair enzymes are able to excise the DNA-adducts which may be excreted in the urine. We have developed a stable isotope dilution liquid chromatography-multiple reaction monitoring/mass spectrometry (LC-MRM/MS) method coupled with immunoaffinity purification for the quantification of

urinary 8-oxo-dGuo. This method was validated over the range of 0.4-20.0ng/mL above endogenous levels with precision and accuracy values within 4 to 14%. This method reproducibly detects endogenous adduct formation at levels 2.39 ng/mL. Supported by NIH grants 1R01CA130038 and 5P30ES013508.

**72. Photosensitized oxidative DNA damage followed from hole injection to chemical product formation and strand cleavage.** B. H. Yun, Y. A. Lee, A. Kolbanovskiy, P. C. Dedon, N. E. Geacintov, V. Y. Shafirovich

Oxidatively generated damage to DNA induced by a pyrenyl photosensitizer residue (Py) covalently attached to a guanine base in the DNA sequence context 5'-d(CAT[G1Py]CG2TCCTAC) (I) in aerated solutions was monitored from the initial one-electron transfer, or hole injection step, to the formation of chemical end-products monitored by HPLC, mass spectrometry, and high-resolution gel electrophoresis. Hole injection into the DNA was initiated by two-photon excitation of the Py residue with 355 nm laser pulses, thus producing the radical cation Py<sup>+</sup> that caused predominantly the oxidation of the guanine residue G1 to which it is attached, and to a lesser extent to the more distant guanine G2. The major product formed is the alkali-labile 2,5-diamino-4H-imidazolone product (Iz1Py). Product formation in the modified strand I is smaller by a factor of 2.4 in the double-stranded form I.Ic than in the single-stranded DNA form (Ic is the complementary strand of I). In double-stranded DNA, hot piperidine-mediated cleavage at G2 occurs only after G1Py, an efficient hole trap, is oxidized, thus generating tandem lesions. The formation of Iz products in the unmodified complementary strand Ic in I.Ic duplexes is ~ 10 times smaller than in the modified strand I. The formation of tandem lesions is observed even at low levels of irradiation corresponding to "single-hit" conditions when less than ~10% of the oligonucleotide strands are damaged. A plausible mechanism for this observation will be discussed. This research was supported by NIH CA R01 CA110261 and CA26735 (P.C.D., PI).

**73. Quantification of N6-formyl lysine: A pathological secondary modification of histone proteins.** B. Edrissi, B. Pang, K. T. Taghizadeh, P. Dedon

DNA oxidation by endogenous and exogenous agents affects both base and sugar moieties of DNA, with the latter leading to formation of reactive electrophiles capable of forming adducts with neighboring nucleobases and proteins. We recently discovered an abundant endogenous modification of histones, N6-formylation of lysine, that arises by transfer of the formyl moiety from a 3'-formylphosphate residue (a product of 5'-oxidation of deoxyribose in DNA). This adduct is a chemical analog of lysine N-acetylation that has important regulatory roles in gene expression. We now report an improved LC-MS/MS based method for quantifying N6-formyl lysine residues and its application for measuring the adduct in different classes of histones and in response to various DNA oxidizing agents. The results will be discussed in terms of potential epigenetic effects of lysine N-formylation on gene expression.

**74. Quantification of the spectrum of RNA damage products in a mouse model of inflammation.** J. L. McFaline, B. Pang, M. R. Sullivan, E. G. Prestwich, K. T. Taghizadeh, P. Dedon

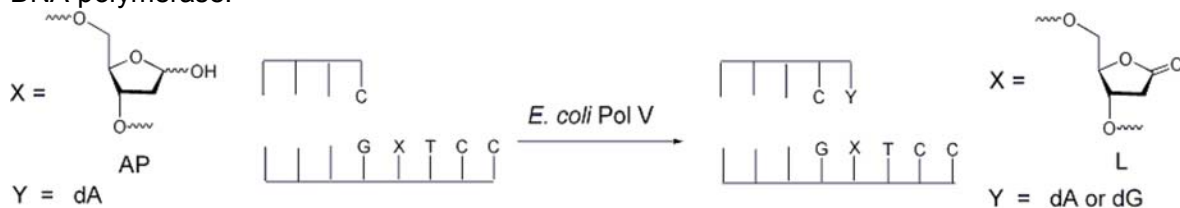
The link between chronic inflammation and cancer involves infiltration of macrophages and neutrophils into tissues with subsequent production of reactive oxygen and nitrogen species that cause damage in surrounding cells. Our previous analysis of DNA damage in the SJL mouse model of inflammation revealed modest increases in etheno adducts that arise from lipid peroxidation. To assess the potential for RNA lesions as biomarkers of inflammation, we have developed sensitive LC-MS/MS methods to quantify RNA lesions representative of the deamination, oxidation, and halogenation chemistries associated with inflammation in SJL mice. We observed time-dependent increases in all types of RNA damage chemistry to levels significantly higher than steady-state levels of DNA damage. Further, all but the halogenation products were found to be nitric oxide-dependent. The results will be discussed regarding the utility of RNA damage products as biomarkers of inflammation and the potential roles for RNA damage in cellular responses to inflammation.

**75. Quantitative NMR metabolic profiling for drug safety assessments.** H. Vu, Q. Xu, E. Xu, B. Schaefer

Withdrawn

**76. Replication of an Oxidized Abasic Site: The Structural Basis for Breaking the A-Rule.** H. Huang, M. M. Greenberg

Genotoxic abasic sites (AP, L) are produced in DNA by a variety of toxic agents. Recent studies in *E. coli* showed that bypass of 2-deoxyribonolactone (L) resulted in high levels of incorporation of dG, which was contrary to expectations based upon the "A-rule." A series of structural analogues of AP and L was designed and their mutagenicity in *E. coli* was studied under SOS-induced conditions. Bypass of the analogues that have a carbonyl group at the C1 position resulted in high levels of dG incorporation opposite the lesion. In contrast, other lesions followed the A-rule. Pol V was shown to be responsible for translesion synthesis opposite abasic lesions in *E. coli*. These experiments suggest that despite lacking a Watson-Crick base, the oxidized abasic site (L) utilizes hydrogen-bonding to direct the DNA-polymerase.



**77. Reversibility of covalent electrophile-protein adducts and chemical toxicity.** D. Lin, D. C. Liebler

The biotin-tagged electrophiles 1-biotinamido-4-(4'-[maleimidoethylcyclohexane]-carboxamido)butane (BMCC) and N-iodoacetyl-N-biotinylhexylenediamine (IAB) are model electrophile probes used to identify protein targets associated with chemical toxicity. Whereas IAB activates stress signaling and apoptosis in HEK293 cells, BMCC does not. Cysteine Michael adducts formed from BMCC and non-biotinylated analogs rapidly disappeared in the intact cells, whereas the adducts were stable in BMCC-treated subcellular fractions. In contrast, cysteine thioether adducts formed from IAB and its non-biotinylated analogs were stable in intact cells. Studies with a glutathione-BMCC conjugate indicated rapid hydrolysis of the adduct imide ring, but neither the conjugate nor its hydrolysis product dissociated to release the electrophile in neutral aqueous buffer. Loss of the BMCC adduct in cells was reduced at 4 °C, which suggests the involvement of a metabolic process in adduct removal. The results suggest that low BMCC toxicity reflects facile repair that results in transient adduction, which fails to trigger damage signaling pathways.

**78. Role of aldo-keto reductase (AKR) 1B10 in human lung carcinogenesis.** A. M. Quinn, R. G. Harvey, T. M. Penning

AKR1B10 is a candidate tobacco smoke exposure and response gene. Homogenous recombinant AKR1B10 was found to oxidize a wide range of polycyclic aromatic hydrocarbon (PAH) trans-dihydrodiol substrates in vitro to o-quinones. Catalytic efficiencies were comparable to or less than those measured for other human AKR isoforms implicated in these reactions. AKR1B10 displayed reasonable activity in the oxidation of both the (-)-R,R and (+)-S,S stereoisomers of benzo[g]chrysene-11,12-diol and the minor (+)-benz[a]anthracene-3S,4S-diol metabolite, as determined by circular dichroism spectroscopy. AKR1B10 retinal reductase activity was 5- to 150-fold greater than oxidation of PAH trans-dihydrodiols. AKR1B10 was highly expressed at the mRNA and functional protein levels in A549 adenocarcinoma cells. Robust retinal reductase activity was measured in cell lysates. These data show that, in addition to its role in PAH activation, AKR1B10 contributes to the loss of retinoic acid signaling often seen in lung cancer. (Supported by P30-ES013508, R01-ES015857, and PA-PDOH4100038714 awarded to T.M.P.)

**79. SILAC based approach for the analysis of quiescent and activated pancreatic stellate cells.** A. Y. Wehr, K. H. Yu, I. A. Blair

Pancreatic cancer is believed to be caused by exposure to environmental toxicants. Pancreatic stellate cells (PSCs) may promote carcinogenesis since they increase the growth of pancreatic cancer cells (PCCs). Stable-isotope-labeling-by-amino-acids-in-cell-culture (SILAC) technique was employed to generate labeled proteome standard from PCC and PSC. An immortalized PSC line was used to study the effect of conditioned media (CM) taken from PCCs on the secretome of quiescent stellate cells and their activation. Activation markers were confirmed and [<sup>13</sup>C<sup>15</sup>N]-labeled proteins were harvested from the CM of PCCs, quiescent PSCs, and activated PSCs. Secreted proteins were characterized by two-dimensional liquid chromatography/tandem mass spectrometry (2D-LC-MS/MS) methodology. This unique approach has enabled identification of specific proteins in the PCC CM that induce the activation of PSCs. Analysis of CM collected from PSCs revealed a five-fold increase in proteins following activation, including growth factors capable of enhancing tumor growth. Supported by NIH Grant R25-CA-101871.

**80. Simultaneous quantitation of cellular cysteine, homocysteine, and glutathione by stable isotope dilution LC-MS.** S. Khartulyari, C. Wei, A. S. Whitehead, I. A. Blair

We describe a stable isotope dilution LC-MS method for the quantitation of free cellular homocysteine, cysteine, and glutathione using prior derivatization with the thiol-specific reagent 4-fluoro-7-sulfamoylbenzofurazan (ABD-F). The thiol-containing amino acids homocysteine and cysteine play significant roles in regulated various biochemical pathways, whereas the tripeptide glutathione is a determinant of the redox status in cells. The ability to quantitate each of these thiols is critical for understanding their role in pathophysiological processes. This is complicated by relatively low intercellular concentrations of free homocysteine and cysteine and the ease with which they undergo redox cycling with cellular glutathione disulfide and protein disulfides. Stable isotope dilution methodology makes it possible to analyze and quantitate these derivatized thiols with high precision and accuracy.

**81. Site-specific identification of 3-nitrotyrosine in proteins.** M. R. Martinez, I. A. Blair, H. Ischiropoulos

3-Nitrotyrosine (3-NT) is a post-translational modification that alters protein functionality and is implicated in pathological conditions that involve oxidative stress-inducing toxicants. Due to its low abundance, site-specific identification of 3-NT has posed many challenges. Thus, we compared four different methods for 3-NT identification. Whole lysates from human platelets were treated with peroxynitrite in the presence of carbon dioxide to induce tyrosine-nitration. Of the four methods, three utilized enrichment strategies. Two enrichment approaches employed anti-nitrotyrosine antibody capture of either nitrated proteins or nitrated peptides respectively. The third enrichment used N-Succinimidyl S-Acetylthioacetate (SATA) chemical derivitization of peptides enriched by sulfhydryl capturing. The fourth method fractionated tryptic peptides by strong cation exchange chromatography (SCX) without prior enrichment. Peptides derived by these methods were further separated and analyzed by microflow-LC-coupled-ESI-linear ion trap (LTQ) mass spectrometer. Preliminary results indicate that SCX fractionation most efficiently identifies 3-NT-containing proteins. Supported by NIH grants T32HL007971 and P30ES013508.

**82. Structure of a DNA-Peptide Conjugate Arising from the Acrolein-Induced 1,N<sup>2</sup>-gamma-Hydroxypropano-dG Adduct: Formation of a Carbinolamine Linkage and Minor Groove Orientation of the Peptide.** H. Huang, I. D. Kozekov, H. Wang, A. Kozekova, C. J. Rizzo, M. P. Stone

When placed complementary to dC in duplex DNA, the 1,N<sup>2</sup>-gamma-hydroxypropano-dG adduct arising from acrolein re-arranges to N<sup>2</sup>-(3-oxo-propyl)-dG, which can undergo further reaction to form DNA-protein conjugates. These may be repaired in vivo by a mechanism in which the protein conjugate is firstly proteolytically degraded to a DNA-peptide conjugate, which is then a substrate for nucleotide excision repair (NER). The 1,N<sup>2</sup>-gamma-hydroxypropano-dG adduct was introduced into an

oligodeoxynucleotide and conjugated with the peptide H<sub>2</sub>N-KWKK-CO<sub>2</sub>H. The peptide was conjugated through a carbinolamine linkage. Modeling studies suggested that the carbinolamine linkage allowed formation of a hydrogen bond between the peptide and the DNA. The solution structure of the fully reduced cross-link revealed that the peptide oriented in the minor groove of the DNA. Supported by NIH grants ES-05355 (C.J.R. and M.P.S.) and the Vanderbilt Center in Molecular Toxicology, ES-00267.

**83. Sub-picogram per milliliter determination of the tobacco-specific carcinogen metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in human urine using liquid chromatography - tandem mass spectrometry: Application to studies of secondhand tobacco smoke exposure.** P. Jacob III, C. Havel, D -H. Lee, L. Yu, N. L. Benowitz

Exposure to secondhand tobacco smoke (SHS) has been linked to increased risk for a number of diseases, including lung cancer. The tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is of particular interest due to its potency and its specificity in producing lung tumors in animals. The NNK metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in urine is frequently used as a biomarker for exposure. We developed a highly sensitive liquid chromatography – tandem mass spectrometry (LC-MS/MS) method for determination of NNAL in human urine. The method involves liquid-liquid extraction followed by conversion to the hexanoate ester derivative. This derivative facilitates separation from interfering urinary constituents by extraction and chromatography, and enhances detection with electrospray ionization mass spectrometry. The lower limit of quantitation is 0.25 pg/mL for 5 mL urine specimens. Applications to studies of people with a range of different SHS exposure levels will be presented. Supported by the Flight Attendant Medical Research Institute.

**84. Systematic study of the potential arylating capabilities of a series of benzoquinones, naphthoquinones, and anthraquinones.** R. E. Shaw, P. A. Mazzer, M. D. Backstrom

Aromatic quinones are toxic compounds which are highly significant biologically. These compounds are not only environmental contaminants, but also have found use as anthracycline anti-tumor agents. The chemical mechanism underlying the toxicity of the arylating quinones involves two competing hypotheses: either redox cycling and the production of reactive oxygen species, or quinones adducting to intracellular nucleophiles. To clarify the relative importance of these two mechanisms, we studied a series of one, two, and three-ring quinones with different electronic and arylating properties. We investigated the cytotoxicity of each of these quinones in rat alveolar macrophages and human HL-60 leukemia cells. Correlation of the cytotoxicities and mode of toxic action with physical properties of the quinones provided information on the relative importance of the two competing mechanisms. These investigations can help improve efforts in drug therapy development of the next generation of anthracycline anti-tumor agents.

**85. Toxicity study of hydroxyapatite nanomaterials with different shape using osteoblast cell.** L. Chen, J. C. -MLee, H. Li

Previous study showed that hydroxyapatite (HAP, Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub>, primary mineral of human bone) nanoparticles can cause cell damage in vitro. In the present study, we investigated the influence HAP nanomaterials, in the shape of nanoparticle or nanofiber, on cell adhesion (1 day test) and cell proliferation (3 days and 7 days test) using an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay with MC3T3-E1 cell lines. MTT assay revealed that the cell viability decreases significantly after being exposed to large dose of HAP nanoparticles while the cell viability is not much influenced with HAP nanofibers, indicating that HAP nanofibers cause less cell damage compared to HAP nanoparticles. Cell adhesion test results suggest that there is no significant difference between HAP nanofibers and HAP nanoparticles samples. Based on the MTT assay, greater cell viability and proliferation were observed with HAP nanofibers. It is suggested that the HAP nanofibers can exhibit favorable cell proliferation to optimize biological functionality, in which the materials size and shape are believed to play a key role. These in vitro findings are of great significance to understanding of cytocompatibility and biological activity of calcium phosphate based nanomaterials.

**86. Variability in levels of urinary hydroxylated polycyclic aromatic hydrocarbon metabolites.** E. N. Porter, Z. Li, L. C. Romanoff, D. A. Trinidad, A. Sjodin

Polycyclic aromatic hydrocarbons (PAHs) are formed during incomplete combustion and preparation of grilled and fried food among others. Urinary hydroxylated PAHs (OH-PAHs) have been used as biomarkers in humans and have been included in biomonitoring studies such as the National Health and Nutrition Examination Survey. Determined levels of urinary OH-PAHs are strongly influenced by recent exposure and time of sampling since PAHs have a relatively short half-life in the body, in the range of hours. The current study was aimed at determining the variability of OH-PAHs over a seven-day period for eight non-occupationally exposed subjects. The subjects recorded daily activities such as food intake as well as time and volume of each urine excretion. The determined concentrations were expressed on a fresh weight (ng/mL urine) and per creatinine weight (ng/g creatinine) basis. The two methods of reporting urinary concentrations are contrasted and compared with a calculated 24 hour void concentration.

**87. Translesion synthesis, blockage, and base sequence context effects with a bulky carcinogen N<sup>2</sup>-dG adduct: Comparing A- and Y-family DNA polymerases.** P. Xu, L. Oum, N. E. Geacintov, S. Broyde

It is now widely appreciated that high-fidelity polymerases that are stalled by DNA lesions are replaced by translesion bypass polymerases which are capable of transiting the lesions. The structural differences between high-fidelity and translesion bypass polymerases that determine error-prone or error-free bypass of the lesions is of fundamental interest since mutations can lead to cancer and other diseases. In order to gain insights into the effects of polymerase structure on translesion bypass on a molecular level, we have utilized a model system employing a bulky mutagenic DNA lesion derived from benzo[a]pyrene (BP), a widely studied environmental contaminant, specifically, the major 10S(+)-trans-anti-[BP]-N<sup>2</sup>-dG ([BP]G<sup>\*</sup>) adduct. As representative high-fidelity and bypass polymerases, we have adopted the high-fidelity A-family polymerase from the bacterium *Bacillus stearothermophilus*, specifically the fragment called BF, and Dpo4, a model Y-family bypass polymerase from the archaeon bacterium *Sulfolobus solfataricus*. Experiments have revealed stark differences in processing the [BP]G<sup>\*</sup> adduct by the two polymerases. While nucleotide incorporation opposite the adduct is allowed in both polymerases, further extension beyond the lesion is relatively facile in Dpo4, but is strongly inhibited in BF. In Dpo4 but not BF, a significant sequence context effect is observed in single dNTP insertion assays. We have used molecular modeling and dynamics simulations to investigate how Dpo4 and BF differentially process the [BP]G<sup>\*</sup> adduct through an entire replication cycle. We considered both binary and ternary complexes and all four partners opposite the [BP]G<sup>\*</sup> adduct during the dNTP insertion and extension steps. Our modeling studies demonstrate how the bulky [BP]G<sup>\*</sup> adduct is differentially processed in the tight active site of BF and the more spacious active site of Dpo4. In addition, we propose an unusual 5'-slippage mechanism to explain sequence context effects that are only observed in Dpo4. Supported by NIH grant CA28038 (S.B.) and CA099194 (N.E.G.).

**88. Trainable QSAR model of Ames genotoxicity.** R. Didziapetris, K. Lanevskij, P. Japertas

This study presents a predictive model of Ames genotoxicity built on a data set of more than 8000 compounds. A baseline fragmental model was developed using binomial PLS regression with multiple bootstrapping. This was followed by an automatic correction of predicted values based on similarity analysis of experimental data for similar compounds in the dataset. The final model produces highly accurate results (less than 5% of mispredictions in the validation set). This novel approach also allows assessing the quality of predictions by means of estimated Reliability Index values. A major feature of the model is trainability: user-defined experimental data may be added to extend its Applicability Domain without rebuilding the baseline model. The model is supplemented with a knowledge-based expert system that allows identification and visualization of hazardous substructures. The compiled set of 27 'genotoxicophores' covers 90% of genotoxic compounds from the data set used in Ames test model development.

## **Wednesday Morning: Environmental and Human Health Impacts of Nanomaterials**

### **8:30 -89. Environmental and human health impacts of nanomaterials. A. B. Kane**

It is now recognized that the recent world-wide investment in new nanomaterials should be accompanied by parallel efforts to explore and understand their potential impacts on human health and the environment. By virtue of their small size, nanomaterials may penetrate biological membranes, enter cells, carry high concentrations of absorbed molecules, or show elevated surface reactivity relative to their macroscopic counterparts. This symposium introduces the emerging field of nanotoxicology from an interdisciplinary perspective. The ultimate goal is to identify the specific material features (size, shape, surface chemistry, purity) that are the underlying causes of toxicity. The long-term promise of this approach is to learn how to modify synthesis or purification procedures to fabricate “green” nanomaterials in order to exploit their unique properties for applications in environmental sensing and remediation and in nanomedicine.

### **8:45 -90. Biological activity of mineral fibers and carbon particulates: Implications for nanoparticle toxicity and the role of surface chemistry. P. K. Dutta**

In this presentation, we will summarize our work on the correlations between biological activity and physicochemical characteristics of minerals and particulates, including the biological response (oxidative burst), mutagenicity and the chemical reactivity (Fenton reaction) of zeolite minerals and oxidative stress and inflammatory responses of carbon particulates. Zeolites, with well defined crystal structures serve as model systems for asbestos and other toxic minerals. For assessment of biological response, phagocytosis as well as the oxidative burst has been studied. For determining chemical reactivity, we have focused on the ability of the iron-exchanged forms of the zeolites to produce hydroxyl radicals from H<sub>2</sub>O<sub>2</sub> (Fenton reaction). Mutagenic potential of erionite and mordenite and how this mutagenic potential is modulated by iron has been examined. The impact of carbon-based particulate physicochemical characteristics upon their ability to induce oxidative stress and inflammatory responses has been studied. Internalization of particulates by freshly isolated and differentiated human monocyte-derived macrophages (MDM) is being examined. To determine the impact of particulate physicochemical characteristics upon their inflammatory potential, inflammatory endothelial adhesion molecule expression by immunofluorescence flow cytometry is being examined. Fenton activity of particulates is being assayed by measurement of their ability to catalyze the decomposition of hydrogen peroxide to hydroxyl radicals by spin trapping with 5,5-dimethylpyrroline-N-oxide (DMPO).

### **9:30 -91. Cellular and subcellular interactions with nanoparticles. G. Orr**

Widespread commercial applications of nanotechnology will increase potential human exposure to submicron and nanoscale particles by inhalation into the lungs. The mechanisms driving cellular and subcellular interactions of nanoparticles with target cells in the lung are unknown. We have developed novel imaging techniques to study the internalization pathway of individual amorphous silica particles following one particle at a time. This internalization pathway depends on particle surface charge and integrity of the cytoskeleton of lung alveolar epithelial cells. These studies have identified a novel retrograde transport pathway leading to particle recruitment, internalization, and toxicity in polarized epithelial cells with surface microvilli. This research was supported by a U.S. Environmental Protection Agency STAR grant RD 833338 and the Environmental Biomarkers Initiative at the Pacific Northwest National Laboratory.

### **10:30 -92. Potential adverse human health impacts of nanomaterials. G. Oberdörster**

Numerous epidemiological studies have shown that acute adverse health effects are associated with exposures to ambient airborne particles. These effects occur mostly in sensitive parts of the population such as the elderly with a compromised cardiorespiratory system. We hypothesize that ultrafine particles (particle size below 0.1  $\mu\text{m}$ ) are one potential source causing these effects. In addition, a new source of

exposure to particles below 100 nm in size engineered nanoparticles has become a cause for concern, giving rise to the emerging field of nanotoxicology. The propensity of nanoparticles of different shapes (e.g., spheres, tubes, rods), different chemistries (e.g., metals, semiconductors, carbon) and different surface characteristics (coating, charge, porosity) to translocate from the site of deposition in the respiratory tract to extrapulmonary organs such as heart, liver, bone marrow and brain is being studied. Examination of the influence of physicochemical properties of nanoparticles on their effects and biokinetics is the ultimate objective of these studies. Effects and underlying mechanisms of translocated nanoparticles (e.g., cellular oxidative stress) are evaluated in a multidisciplinary team approach. This research is supported by a U.S. Department of Defense MURI grant FA9550-04-1-0430 and U.S. Environmental Protection Agency Particulate Matter Center grant RD 83241501.

#### **11:15 -93. Designing nanomaterials for environmental health and safety. R. Hurt**

Nanotechnology has been given a unique “window of opportunity” to develop methods for managing EHS concerns before its products become truly widespread in the marketplace. In contrast to many traditional pollutants, nanomaterials are high-technology products under continual development and evolution. Their synthesis, surface modification, processing, release and environmental transformation, and biological impacts form a cause-effect continuum that offers many opportunities for intelligent intervention on behalf of safety. Short term risks may be reduced through materials and formulation-based strategies that do not require complete quantitative understanding of the relationship between exposure and adverse health impacts. These strategies typically target exposure or bioavailability of known toxicants and are devised through analogy with existing pollutants. Longer term risk management requires parametric and mechanistic studies on material libraries that can guide the development of current and future nanomaterials toward intrinsic safety. This talk uses carbon nanotubes as an example material family to discuss the role of individual material features (hydrophobic graphenic surface, functional groups, amorphous carbon, metals, and aggregate state), our current understanding of environmental transformations and biopersistence, and opportunities for safer formulation. This research was supported by grants from the National Science Foundation (NSF DMI-05066) and the National Institutes of Health (R01 ES016178 and P42 ES 013550).

### **Wednesday Afternoon: General Papers**

#### **94. Are we focusing on the right toxicants for assessing the toxicological properties of smokeless tobacco products J. H. Lauterbach**

At the October 2007 meeting of the Life Sciences Research Organization's expert panel on Differentiating the Health Risks of Categories of Tobacco Products, several tobacco industry presenters suggested that products that met the so-called GothiaTek® Standard (limits on TSNAs, VNAs, heavy metals, B[a]P, nitrite, agrochemicals) presented lower health risks than have been attributed to other smokeless tobacco products (STP) reported to contain higher levels of those analytes. One presenter suggested additional analytes, microbiological assays, and in vitro assays for mutagenicity and cytotoxicity. However, there are STP toxicants besides nicotine that are not covered by the GothiaTek® Standard and potentially nonresponsive to the proposed in vitro assays. This presentation will focus on such other toxicants including those reported to cause oxidative stress.

#### **95. Characterization of precursors to trihalomethanes formation in the north china source water . Y. Lin, J. Yin**

Resin adsorption techniques using three types of resin (DAX-8, AG-MP-50, and WA-10) were employed to characterize the raw water (RW) from the North-China reservoir :Xinlicheng, Shitoukoumen, Erlongshan. The dissolved organic carbon (DOC) mass distribution sequences of the six organic fractions in raw water, from high to low, were hydrophobic neutral (HPON), hydrophilic acid (HPIA), hydrophobic acid (HPIA), hydrophilic neutral (HPON), hydrophobic base (HPOB), and hydrophilic base (HPIB). HPOB, HPIB were the main precursors for trihalomethanes formation (THMFP) in the three water source following chlorination. HPIA is the least. The chlorination of

HPON and HPIN fractions only led to the formation of mostly chloroform and bromodichloromethane while HPOA, HPOB, HPIA, HPIB organic fractions formed chloroform, bromodichloromethane and dibromochloromethane. HPON corresponding with the character of SUVA (which is  $100 \times \text{UV}_{254} / \text{DOC}$ ). But the HPON fraction had a special character that its THMs yield ability was super than that of UV absorption, so SUVA couldn't be surrogate indicator for THMFPA absolutely. So it was needed to pay more attention.

**96. Computational evaluation of mechanisms for the formation of guanidinohydantoin from spiroiminodihydantoin . B. H. Munk, Y. Ye, C. J. Burrows, H. B. Schlege**

Oxidative damage of DNA nucleobases may produce biochemical changes leading to mutagenesis and contribute to aging, carcinogenesis and neurological disease. One of the most prevalent of these oxidation products, 8-oxo-7,8-dihydroguanine, can undergo further oxidation to form a variety of secondary oxidation products including guanidinohydantoin and spiroiminodihydantoin. Experimentally, spiroiminodihydantoin has been observed to undergo hydration and decarboxylation to form guanidinohydantoin under acidic conditions. In this paper, we describe our efforts to explore the energetics of possible mechanisms for the latter reaction. These studies have been conducted using B3LYP density functional theory and the IEF-PCM solvation model employing 6-31+G(d,p) and aug-cc-pVTZ basis sets. This level of theory has been shown to be computationally efficient for the study of the relative potential energy surfaces of small biomolecules

**7. DNA Adduct formation in F-344 rats treated chronically with the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and enantiomers of its metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL). S. S. Hecht, S. Zhang, Y. Lao, P. W. Villalta, P. Upadhyaya**

NNK and its metabolite NNAL, lung carcinogens and likely causes of lung cancer in smokers, are metabolized to diazonium ions which alkylate DNA. We compared adduct formation by NNK, (S)-NNAL, and (R)-NNAL in rats (10 ppm in drinking water, 1-20 weeks). Using LC-ESI-MS/MS-SRM, we quantified pyridyloxobutyl (POB) and pyridylhydroxybutyl (PHB) adducts in liver, lung, nasal mucosa, oral mucosa, and pancreas. Conclusions include: 1) adduct formation in lung exceeded that in other tissues; 2) O<sup>2</sup>-POB or PHB-thymidine adducts predominated; 3) mutagenic O6-POB-dGua was found mainly in lung and nasal mucosa; 3) adducts from NNK and (S)-NNAL were remarkably similar; 4) adducts were detected in all extrahepatic tissues; 5) PHB-DNA adduct formation from (R)-NNAL exceeded NNK and (S)-NNAL. The results of these studies support a mechanism in which NNK is converted to (S)-NNAL which is sequestered in the lung, gradually released, and re-oxidized to NNK which forms POB-DNA adducts leading to tumor development.

**98. Efficient and accurate bypass of a minor-groove DNA adduct by DinB DNA polymerase in vitro and in vivo. Y. Wang, B. YUAN, H. Cao, Y. Jiang, J. Wang**

DinB, a Y-family DNA polymerase, is conserved among all domains of life; however, its endogenous substrates have not yet been identified. DinB is known to synthesize accurately across a number of N<sup>2</sup>-dG lesions. Methylglyoxal is a common byproduct of the ubiquitous glycolysis pathway and it induces the formation of N<sup>2</sup>-(1-carboxyethyl)-2'-deoxyguanosine (N<sup>2</sup>-CEdG) as the major stable DNA adduct. Here, we constructed single-stranded M13 shuttle vectors harboring individual diastereomers of N<sup>2</sup>-CEdG at a specific site and assessed the cytotoxic and mutagenic properties of the lesion in wild-type and bypass polymerase-deficient E. coli cells. Our results revealed that DinB is the major DNA polymerase responsible for bypassing the lesion in vivo. Moreover, steady-state kinetic measurements showed that nucleotide insertion, by E. coli or human DinB, opposite the N<sup>2</sup>-CEdG is both accurate and efficient. Taken together, our data support that N<sup>2</sup>-CEdG is an important endogenous substrate for DinB DNA polymerase.

**99. Identification of binding hot spots of cytochrome P450 3A4 by computational solvent**

**mapping.** G -Y. Chuang, D. Kozakov, R. Brenke, **S. Vajda**

Computational solvent mapping places molecular probes - small organic molecules containing various functional groups – around the protein surface, finds favorable positions, clusters the conformations, ranks the clusters, and determines their “consensus” sites. The method provides accurate characterization in the shape and affinity of the various regions in protein binding sites. We have mapped the unbound and ligand-bound structures of CYP3A4 (with progesterone, metyrapone, ketoconazole and erythromycin). As reported for other mammalian P450s [1], a consensus site near the heme iron occurs only in the bound structures, indicating that it is induced by ligand binding. Two sub-sites, the first lined by Arg-372, Arg-106 and Glu-374, and the second containing Ser-119, are present in unbound and all four bound conformations, providing the opportunity for creating a structure-based pharmacophore. Sub-pockets induced by ligand binding, such as the subsite containing Ile-301 at the ketoconazole chlorobenzyl ring, are also identified. [1] Clodfelter KH, Waxman D J, Vajda S. *Biochemistry*, 45: 9393, 2006.

**100. Oxidation of DNA by carbonate radical anions in DNA results in the formation of novel intrastrand cross-links.** C. Crean, N. E. Geacintov, **V. Shafirovich**

The carbonate radical is a decomposition product of nitrosoperoxycarbonate, a molecule that is known to contribute to oxidative stress in vivo. We have been investigating the one-electron abstraction from guanine in DNA model systems such as 5'-GCT and the different oxidation products that are formed from the subsequent reactions of the guanine radical thus formed. We found that the reaction of the carbonate radical with this trimer sequence results in the formation of novel type of intrastrand cross-links between C8-guanine and N3-thymine. These cross-links were excised from the oxidized oligonucleotides by enzymatic digestion with nuclease P1 and alkaline phosphatase, and identified by LC-MS/MS as the covalently linked dG\*-dT dimer with a mass smaller by 2Da than the combined mass of dG and dT nucleosides. The effect of numbers of bridging C bases on cross-link formation was investigated in the series of 5'-d(GpCnpT) and 5'-d(TpCnpG) sequences with n = 0, 1, 2, and 3. The formation of the G\*-T\* cross-links is most efficient in the case of n = 1 (in 5'-d(GpCpT)). Cross-link formation (n = 0) was also observed in double-stranded DNA containing 5'-...GT... sequences. Supported by NIEHS Grant 5R01 ES011589-06.

**101. Oxidatively generated sugar fragments and DNA adduct formation.** **A. C. Bryant-Friedrich**, A. Boghici

Oxidative damage to nucleic acids instigates the formation of a variety of reactive intermediates capable of further reaction with cellular constituents. When the sugar phosphate backbone is the site of radical generation, the addition of oxygen to these reactive species ultimately leads to highly oxygenated electrophilic molecules. This laboratory has identified several of these fragments as the products of the abstraction of the major groove accessible C-3'-hydrogen in DNA under aerobic conditions. We have investigated the reactivity of a select group of these fragments towards several biological molecules that are nucleophilic in nature. Using model compounds we have elucidated the structure of several sugar-derived DNA adducts.

**102. Spontaneous DNA alkylation with a quinolinyl quinone methide adduct under aqueous conditions.** **Q. Zhou**

Quinone methide (QM) is a reactive intermediate and forms covalent adducts with biomolecules such as proteins and DNA. The reactive QM can be generated in situ via photo-irradiation, reduction, oxidation, enzyme-catalyzed or chemical-induced deprotection/elimination and many other processes. Most recently, Rokita's group reported that a DNA adduct transferred the QM between DNA strands through hybridization process. We report here a new quinolinyl QM-amine system, which spontaneously alkylated both single strand and duplex DNA under aqueous conditions at 37 degree yet remained stable in organic solutions. The resulting DNA adducts were found surprisingly stable after piperidine treatment at 90 degree. In addition, the extent of DNA alkylation was influenced by the presence of NaCl

or intercalator ethidium bromide. The covalent nature of DNA modification with the quinoliny-QM amine system was confirmed by ESI-MS analysis, and up to three quinoliny QMs were observed to be added on the ssDNA target.

**103. Structural studies of butadiene-derived adducts.** W. Xu, S. K. Musser, T. Nielsen, L. V. Nechev, R. P. Hodge, T. M. Harris, C. M. Harris, R. S. Lloyd, M. Egli, M. P. Stone

Complexes of R or S N3-(2-hydroxy-3-buten-2-yl)-2'-dU (N3-HBdU) adducts with the *Sulfolobus solfataricus* DNA Polymerase Dpo4 were examined, and compared with the replication of these adducts by the Dpo4 polymerase. For binary complexes, either stereoisomer of N3-HBdU paired with a 3'-terminal primer T. The incoming d(d)NTP inserted above the thymine in ternary complexes. Unusual complexes in which the 5'-terminal template T paired with the N3-HBdU were observed. The 1,4-bis(2'-deoxyadenosin-N6-yl)-2S,3S-butanediol intrastrand cross-link oriented in the major groove and existed in two conformations. The first featured a hydrogen bond between the beta-OH and the 5' base pair T16 O4, whereas the second featured a hydrogen bond between the beta-OH and the 3' base pair T17 O4. Base pairing was perturbed at the 5'-base pair of the cross-link, but was intact at the 3' base pair. Supported by NIH grants ES-05355 (T.M.H. and R.S.L.) and ES-05509 (M.P.S. and M.E.).

**104. Structure of duplex DNA containing (1,N<sup>2</sup>)- $\alpha$ -OH-PdG, the minor acrolein-derived lesion.** T. Zaliznyak, R. R. Bonala, M. Lukin, F. Johnson, C. de los Santos

Acrolein or propenal is a prevalent genotoxic compound. Worldwide, the chemical industry uses thousand of tons of acrolein per year for the synthesis of polymers and simple organic compounds. The incomplete combustion of organic materials, including wood, food, fuels and tobacco, is an additional source. In addition to environmental acrolein, the metabolic oxidation of polyamines and products of lipid peroxidation continuously generates small amounts of propenal inside mammalian cells. Acrolein is a highly reactive bi-functional alkylating compound that damages DNA and proteins without the need of metabolic activation. Acrolein reacts with double stranded DNA producing two isomeric 2'-deoxyguanosine adducts  $\gamma$ - and  $\alpha$ -OH-propano-dG, depending on whether the Michael addition occurs at the peripheral amino group or the N1 imino nitrogen of dG. We will present the NMR solution structure of two oligomeric DNA duplexes containing the  $\alpha$ -OH-PdG lesion opposite dC or dA and discuss their implications for DNA repair and mutagenesis.

**105. Synthesis and characterization of amino acid adducts from the HIV reverse transcriptase inhibitor nevirapine .** M. M. Marques, I. Martins, A. M. Antunes, P. P. Santos, G. Gamboa da Costa, F. A. Beland

Nevirapine (NVP) is a non-nucleoside reverse transcriptase inhibitor used against HIV-1, mostly to prevent mother-to-child transmission of the virus. However, reports of severe NVP-induced hepatotoxicity and serious adverse cutaneous effects have raised concerns about its use. NVP metabolism involves oxidation of the 4-methyl substituent to 4-hydroxymethyl-NVP (12-hydroxy-NVP), and the formation of phenolic derivatives. Further metabolism, either through oxidation to quinoid derivatives or Phase II esterification, may produce electrophilic derivatives capable of reacting with bionucleophiles to yield covalent adducts. These adducts could potentially be involved in the initiation of toxic responses. We synthesized 12-O-mesyl-NVP as a model electrophile derived from 12-hydroxy-NVP and investigated its reactivity towards glutathione and the nucleophilic amino acids arginine, cysteine, histidine, and tryptophan. Multiple covalent adducts, typically formed in significant yields, were isolated and fully characterized. Our results suggest that NVP metabolism to 12-hydroxy-NVP and subsequent esterification could potentially be factors in NVP toxicity. Supported by FCT(Portugal) and FEDER (POCI/QUI/56582/2004) and the NTP (IAG # 224-07-007).

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