Central Arkansas Summer Undergraduate Research Symposium
Sample abstracts (300 word limit)

"Inhibition of Rodent CYP2E1 by Butadiene Epoxide Metabolites"
Kaila Pianalto,[1]* Jessica H. Hartman,[2] and Grover P. Miller[2]
[1]University of Arkansas – Fayetteville, [2]University of Arkansas for Medical Sciences

Cytochrome P450 2E1 (CYP2E1) plays an important role in 1,3-butadiene metabolism to generate three genotoxic and possibly carcinogenic epoxides, 1,2-epoxy-3-butene, 1,2:3,4-diepoxybutane, and 1,2-epoxy-3,4-butanediol. The toxicological response associated with butadiene exposure in rodent models saturates at high exposure levels for rats, while toxicity does not attenuate in mice as a function of concentration. It has been hypothesized that butadiene metabolites inhibit CYP2E1 activity and thus suppress butadiene activation in rats but not mice. The goal of this study was to test the inhibitory potency of butadiene metabolites toward CYP2E1 activity present in Fisher 344 rat and B6C3F1 mouse liver microsomes. Steady state experiments for 4-nitrophenol, a CYP2E1 marker substrate, demonstrated that rat and mouse liver microsomes demonstrated similar parameters for substrate oxidation. Through IC50 studies, 1,2-epoxy-3-butene was determined to be the strongest inhibitor with IC50 values of 54 μM for mouse and 98 μM for rat. 3-Butene-1,2-diol was considerably weaker (IC50 values 1200 μM for mouse, 1000 μM for rat). 1,2:3,4-Diepoxybutane demonstrated minimal inhibition at 25 mM, so further studies were not performed with this metabolite. Through kinetic inhibition studies, the mechanisms of inhibition were determined for the butadiene metabolites studied. Both 1,2-epoxy-3-butene and 3-butene-1,2-diol inhibited 4-nitrophenol oxidation through complex two-site mechanisms in which the inhibition constants reflected trends observed in the IC50 studies. Our findings suggest that metabolites may alter metabolism by CYP2E1 through simple competition at the catalytic site as well as a second allosteric site. Ongoing studies will likely determine whether these mechanisms are significantly different between rat and mouse CYP2E1 and could provide a biochemical explanation for differences in toxicity resulting from butadiene metabolism between these rodent models.

"Preparation of oligonucleotide-linked gold nanoparticles for inhibition of Hepatitis C Virus"
Claire Desrochers,[1]* and Kevin D. Raney[2]
[1]University of Central Arkansas, [2]University of Arkansas for Medical Sciences

An RNA virus, Hepatitis C (HCV) affects about 200 million people worldwide, and it increases the risk for liver cirrhosis and cancer in infected individuals. Antisense inhibition in which complimentary oligonucleotides anneal to the internal ribosomal entry site (IRES) region of RNA thus preventing HCV replication has been suggested as a potential HCV treatment. Since cells do not readily take up oligonucleotides, various transfection agents such as cationic polymers, modified viruses, and liposomes are required for cellular uptake of oligonucleotides. Gold nanoparticles are widely employed as transfection agents for oligonucleotides because the oligo-gold conjugates increase cellular uptake and are resistant to DNase degradation. 17-mer oligonucleotides, complementary to the HCV IRES, and scrambled 17-mer oligonucleotides, a negative control, were coupled to gold nanoparticles to form DNA-gold conjugates. The results of a native PAGE gel and UV-Vis absorbance readings indicated that both DNA-gold conjugates were formed. The 17-mer-gold conjugates and the scrambled 17-mer-gold conjugates were introduced into EN5-3 cells containing the HCV replicon. Evidence of antisense inhibition of the HCV replicon was observed in cells treated with the 17-mer-gold conjugates.