#5308 X-ray crystallography reveals important role for residues 207, 216 and 221 in catalytic activity of murine glutathione transferases A1-1 and A2-2 for conjugation of benzo[a]pyrene-7,8-diol-9,10-epoxide with glutathione. Yijun Gu, Bing Xiao, Heather L. Wargo, Matthew H. Bucher, Shivendra V. Singh, and Xinhua Ji. *University of Pittsburgh Cancer Institute, Pittsburgh, PA and National Cancer Institute- Frederick, Frederick, MD.*

Murine glutathione (GSH) transferase isoform A1-1 (mGSTA1-1) is unique among Alpha class mammalian GSTs due to its exceptionally high catalytic activity for GSH conjugation of 7*R*,8*S*-dihydroxy-9*S*,10*R*-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene [(+)-anti-BPDE], which is the ultimate carcinogenic metabolite of widely spread environmental contaminant benzo[a]pyrene. The catalytic efficiency of mGSTA1-1 toward (+)-anti-BPDE is also significantly higher compared with mGSTA2-2, which is a close structural homologue of mGSTA1-1. Our previously solved structure of mGSTA1-1 in complex with GSH conjugate of (+)-anti-BPDE (mGSTA1-1•GSBpd) indicated that R216 and I221 in the last helix play important roles in mGSTA1-1 catalyzed GSH conjugation of (+)-anti-BPDE. To gain insights into the structural basis for catalytic differences between mGSTA1-1 and mGSTA2-2 for GSH conjugation of (+)-anti-BPDE, we have determined the crystal structure of mGSTA2-2 in complex with GSH conjugate of (+)-anti-BPDE (mGSTA2-2•GSBpd). The overall structure of mGSTA2-2•GSBpd is similar to that of mGSTA1-1•GSBpd. However, the conformation of GSBpd in mGSTA1-1 · GSBpd and mGSTA2-2 · GSBpd differs greatly, mainly because of the different conformation around the CB2-SG2 bond. In mGSTA2-2•GSBpd, the CB2-SG2 bond points away from the -OH of Y8, whereas in mGSTA1-1•GSBpd the CB2-SG2 bond is pointing toward the hydroxyl group of Y8 forming a hydrogen bond between SG2 and -OH. In the H-site of mGSTA1-1 and mGSTA2-2, two amino acid variations are present: M207/I221 in mGSTA1-1 and L207/F221 in mGSTA2-2, respectively. The structures reveal that residue 207 may be responsible for the different GSBpd-binding modes in the two isoenzymes. Our previous crystallographic studies of mGSTA1-1•GSH and mGSTA1-1•GSBpd indicated that R216 plays an important role in both substrate binding and catalysis by providing electrostatic assistance in the epoxide-ring opening reaction. The structure of mGSTA2-2•GSBpd, however, reveals a significantly different conformation of R216 such that its side chain is pointing away from the H-site and its guanidinium group does not interact with any hydroxyl group of GSBpd. Therefore, R216 is unable to provide any electrostatic assistance in the epoxide-ring opening reaction catalyzed by mGSTA2-2, which may account for its relatively lower catalytic efficiency in comparison with mGSTA1-1 (supported in part by NIH grants CA76348-05 and ES09140-05).

#5309 Analysis of benzo[*a*]pyrene-induced DNA adducts in MCF-7 breast cancer cells with different levels of HSP70 expression. Leon C. King, Linda D. Adams, Susan D. Hester, Ernest Winkfield, Jill A. Barnes, and James W. Allen. *U.S Environmental Protection Agency, Research Triangle Park, NC and North Carolina State University, Raleigh, NC.*

Heat shock proteins (HSPs) protect cells from damage by their ability to function as molecular chaperones. We have recently developed an MCF-7 breast cancer cell line with doxycycline regulation of HSP70 expression and demonstrated that overexpression of this protein is protective against heat cytotoxicity and arsenic genotoxicity. Preliminary microarray analyses in earlier studies have indicated that overexpression of HSP70 is associated with altered expression of other genes involved in growth, apoptosis and signal transduction. In the present study, we examined the formation of B[a]P DNA adducts following *in vitro* exposure of B[a]P to MCF-7 cells containing normal and different doxycycline-regulated expression levels of HSP70. Cytotoxicity experiments were performed in which MCF-7 (ATCC) cells, HSP70-On (overexpression) and HSP70-Off (doxycycline down-regulated expression) MCF-7 cells were exposed to B[a]P at 0, 2, 5, 10, 20, 30 and 40 μM for 24 h at 37°C. Subsequently, similar B[a]P exposure experiments were repeated for DNA adduct analysis using the ^{32}P-postlabeling assay and reverse-phase HPLC. Treatment of the MCF-7 cell systems with B[a]P (2 to 20 μM) showed no cytotoxicity as measured by total cell number. However at 30 and 40 μM B[a]P, the total cell number was reduced by a range of 32 to 78%. The maximum level of B[a]P DNA adducts occurred at 2 μM in all three MCF-7 cell systems. The ATCC cells, which showed lowest levels of HSP70 protein with Western blot analysis, had two to four-fold higher levels of B[a]P-induced DNA adducts at all doses when compared with Tet-On and Tet-Off cells. However, this inverse relationship between HSP70 expression level and B[a]P-induced DNA adduct formation was not observed for comparisons of Tet-On *vs.* Tet-Off MCF-7 cells. We conclude that the results do not present consistent evidence of HSP70 cytoprotection at the DNA damage level. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

#5310 Altered gene expression patterns in MCF-7 cells induced by the diesel particulate complex mixture SRM 1650 monitored using DNA microarrays. Brinda Mahadevan, Channa Keshava, Tamara Musafia, Arta Pecaj, Ainsley Weston, and William M. Baird. *Environmental & Molecular Toxicology, Oregon State University, Corvallis, OR, Toxicology & Molecular Biology Branch, National Institute of Occupational Health and Safety, CDC, Morgantown, WV, and Oregon State University, Corvallis, OR.*

Human exposures to polycyclic aromatic hydrocarbons (PAHs) occur in complex mixtures. Standard Reference Material (SRM) 1650-diesel particulate matter, characterized by the National Institute of Standards and Technology, is a mixture of PAHs and nitro-substituted PAHs. In this study, gene expression patterns were investigated in MCF-7 cells exposed for 24h to SRM 1650 alone or SRM 1650 plus either benzo-[a]pyrene (BP) or dibenzo[a,l]pyrene (DBP). Gene expression was monitored using high density oligonucleotide arrays (Affymetrix U 133A) representing more than 22,000 human genes and expressed sequence tags. Duplicate treatments displayed a high degree of reproducibility. Gene expression was analyzed using Affymetrix GeneChip software. Global analyses of the gene expression data revealed alterations of at least 2 fold change [signal log ratio (SLR)$\leq$ -1 or $\geq$ 1] in 156 RNA transcripts in response to SRM 1650 exposure. Increase in expression of cytochrome P450 (CYP) genes was observed in response to BP exposure (CYP1A1 and CYP1B1; SLR of 6.5 and 2.8, respectively). An additive induction of CYP1A1 and CYP1B1 was observed with co-treatment of SRM 1650 and BP. In contrast, no change in expression of CYP1A1 and CYP1B1 was observed when the cells were exposed to DBP. To study the effect of complex PAH mixtures on the metabolic activation of carcinogenic PAH to DNA-binding derivatives and to correlate the results with gene expression studies, PAH-DNA adduct formation was determined in MCF-7 cells. 33P-postlabeling and reversed-phase high-performance liquid chromatography (HPLC) analysis of samples revealed that SRM 1650 decreased the total level of BP-DNA adducts in comparison with BP alone. No significant difference in adducts was observed in response to either DBP alone or in combination with SRM 1650. These results not only provide a transcriptional signature for chemical carcinogen exposure but also suggest a major factor in carcinogenic activity of PAH within complex mixtures is the ability of the complex mixture to promote or inhibit the activation of carcinogenic PAH by the induction of CYP metabolic enzymes. Supported in part by grant CA28825, NCI, DHHS

#R5311 Binding of (-)*anti* dibenzo*(a,l)*pyrene epoxide to short oligodeoxyribonucleotides. Tamara Musafia, Albrecht Seidel, Andreas Luch, and William M. Baird. *Oregon State University, Corvallis, OR, GenPharmTox BioTechAG, Martinsried, Germany, and Harvard Medical School, Boston, MA.*

Seven self-complementary DNA oligonucleotides were reacted overnight with - anti dibenzo(a,l)pyrene diol epoxide (DB(a,l)PDE). The DNA oligonucleotides were postlabeled with 33P, digested to mononucleotides and analyzed by HPLC. The reactions were carried on ice so that DNA oligonucletides were doublestranded; or at 37oC when some of the oligonucleotides were singlestranded. Adducts of DB(a,l)PDE can form on singlestranded or doublestranded DNA. There was not a significant difference in amount of adducts formed at 37oC or on ice. Dibenzo(a,l)pyrene diol epoxide binds with higher affinity to GC rich oligonucleotides then to AT rich oligonucleotides. We used reactions with AT or GC dinucleotides to identify adducts in each oligonucleotide. Our result[illegible]monstrate that short DNA oligonucleotides model DB(a,l-)PDE bin[illegible] Supported in part by NCI grant CA28825.