NCER Assistance Agreement Annual Report Summary

Period Covered by the Report: October 1, 2002 - September 30, 2003
Date of Report: October 10, 2003
EPA Agreement Number: R82941091-02
Title: Biomarkers for Air Pollutants: Development of Hemoglobin Adduct Methodology for Assessment of Exposure to Butadienes and Polycyclic Aromatic Hydrocarbons, SEER project of SIP: Experimental Program To Stimulate Competitive Research (EPSCoR) From The Commonwealth Of Kentucky
Investigators: Harrell E. Hurst and Steven R. Myers
Institution: University of Louisville
Research Category: EPSCoR
Project Period: October 1, 2001 - September 30, 2004

Objective of Research: This research has been conducted to develop methodology that can measure biomarkers of systemic exposures to chloroprene (2-chloro-1,3-butadiene; CAS-126-99-8) and selected polycyclic aromatic hydrocarbons (PAH: fluoranthene, CAS# 205-44-0; benzo(a)pyrene, CAS# 50-32-8).

Progress Summary: During the second year of this grant, research focused on development of working assays for measurement of hemoglobin (Hb) adducts formed from reactive metabolites of the toxics. As this protein is abundant in blood, persists for up to four months in humans, and exhibits nucleophilic sites that serve as surrogates for molecular sites of toxic actions, Hb adducts may serve as biomarkers of significant exposure to these compounds. Standards were synthesized by reaction of valine, ^{13}C-valine, or the tripeptide valine-tyrosine-valine with a synthetic epoxide metabolite of chloroprene, (1-chloroethenyl)oxirane (CEO). Model Hb adducts were produced using mouse erythrocytes exposed to CEO in vitro. An assay for chloroprene epoxide-Hb adducts was developed with selected ion monitoring gas chromatographic/mass spectrometry (SIM-GC/MS). Following in vitro CEO exposure, hemolysis, and precipitation of mouse globin, this method relies on sequential reactions of Edman cleavage of adducted N-terminal valine and trimethylsilylation of hydroxyl groups produced by epoxide opening during adduct formation. Adducts from PAH exposure have been analyzed after acid hydrolysis of labile PAH-Hb carboxylate adducts. Kinetics of in vitro reactions of CEO with N-terminal valine, and of PAH epoxides with various Hb sites, have been characterized in mouse red cell Hb. If sufficient assay sensitivity can be realized, these assays may enable assessment of exposure and formation of toxic metabolites of these air pollutants.

Publications/Presentations: No journal publication has been produced as yet from these efforts. Results of this work have been presented at the following:

Hurst, H.E., Biomarkers for air pollutants: Development of hemoglobin adduct methodology for exposure assessment, *9th Annual Kentucky EPSCoR Conference* Lexington, KY, May 12, 2003.

Ali, M.Y. and Hurst, H.E. Development of a GC/MS method to determine hemoglobin *N*-valine adducts from (1-chloroethenyl)oxirane, a chloroprene metabolite. *51st ASMS Conference on Mass Spectrometry and Allied Topics*, Montreal, Canada, June 11, 2003.

Ali, M.Y. and Hurst, H.E. Development of a GC/MS method to determine hemoglobin *N*-valine adducts from (1-chloroethenyl)oxirane, a chloroprene metabolite. *2nd Annual James Graham Brown Cancer Center Retreat*, Louisville, KY, September, 17, 2003.

Future Activities: Future efforts will attempt to determine the utility of the assays for assessment of exposure, which will require treatment of mice with the parent compounds to enable in vivo formation of electrophilic metabolites and assessment of the extent of epoxide formation. These studies are required as usefulness of the potential biomarkers depends on the sensitivity of the analytical methods, extent of adducts formed in vivo, and alternative detoxification reactions.

Supplemental Keywords: carcinogen, chemicals, EPA Region 4.