

Period Covered by the Report: October 1, 2001 – September 30, 2005

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Title: U.S. Environmental Protection Agency Experimental Program to Stimulate Competitive Research (EPSCoR) From the Commonwealth of Kentucky, SIP

Investigators: David S. White

Institution: Murray State University

Research Category: EPA EPSCoR

Project Period: October 1, 2001 – September 30, 2005

SIP

Objective: The objective of the SIP has been to enhance competitive research in environmental problems in the Commonwealth of Kentucky under the management of the EPA EPSCoR Subcommittee of the Kentucky Statewide EPSCoR Committee through information exchange activities, fellowships, and review processes to assist the investigators of the SEER Projects as they address research on environmental and human health biomarkers and use of biomarkers for pollution prevention in Kentucky.

Because of some time of year issues in beginning the research in the fall and logistics of the accounts for the SEER grants, SEER research was not fully underway until January 2002. Originally we had planned to hold a Special Conference on Biomarkers in May of 2002, but with the late start, the KY EPSCoR Committee agreed to move the first Conference to 2003 followed by a second conference in May 2004. This proved to work out very nicely. The 2003 Special Conference was held in conjunction with The Kentucky Statewide EPSCoR Conference at the Hyatt Regency in Lexington Kentucky May 11-12. Three internationally known biomarker researchers agreed to be invited speakers (with Hatcher also being a keynote speaker for the entire conference). Each of the 4 SEER Co-PIs presented results to date, which was followed by an open forum discussion. Special Conference speakers were

Patrick G. Hatcher, Professor and Director of the Ohio State EMSI, Department of Chemistry
Newman and Wolfrom Lab., 100 West 18th Avenue, Columbus, OH 43210: *Biomarkers
for PAH exposure, an example of the future of environmental research.*

Timothy R. Fennell, Center for Bioorganic Chemistry, Chemistry and Life Sciences, RTI
International, P.O. Box 12194, Research Triangle Park, NC 27709-2194

Margaret Whalen, Department of Chemistry, Tennessee State University, Nashville, TN 37209-
1561

Howard Whiteman, Department of Biological Sciences, Murray State University, Murray, KY
42071

Bommanna Loganathan, Department of Chemistry, Murray State University, Murray, KY 42071

Harrell E. Hurst, Department of Pharmacology & Toxicology, University of Louisville School of Medicine, Louisville, KY 40292

Steven Myers, Department of Pharmacology & Toxicology, University of Louisville School of Medicine, Louisville, KY 40292

The 2004 second Special Conference on Biomarkers was held on May 13, 2004, also in Lexington in conjunction with the Kentucky Statewide EPSCoR Conference where the following presentations were made:

Darrell Winner, US Environmental Protection Agency, National Center for Environmental Research, Washington, DC: *An overview of EPSCoR and other research programs at EPA*

Prasada Rao Kodavanti, US Environmental Protection Agency, Research Triangle Park NC: *Are PBDEs the New PCBs: A Neurotoxicology Perspective.*

Harrell E. Hurst and Steven Myers, Department of Pharmacology & Toxicology, University of Louisville School of Medicine, Louisville, KY: *Biomarkers for Air Pollutants: Development of Hemoglobin Adduct Methodology for Exposure Assessment.*

Howard Whiteman and Bommanna Loganathan Departments of Biological Sciences and Chemistry, Murray State University, Murray, KY: *Developmental Stability in Amphibians as a Biological Indicator of Chemical Contamination and Other Environmental Stressors.*

Russell A. Barnett, Kentucky Institute for Environmental and Sustainable Development (KIESD), University of Louisville, Louisville, KY: *An overview of the Biomarker Presentation to the Kentucky Environmental Quality Commission.*

On March 5, 2004, we presented results of the two SEER research projects to date to the Kentucky Environmental Quality Commission (KEQC). The Kentucky Environmental Quality Commission is a seven-member citizen advisory board that works to strengthen the public role in solving environmental problems in our communities and the state and that serves as an advisory board to the governor and other state officials on environmental matters. Presentations to the Commission usually are limited to 15 minutes including time for commissioner questions. Questions about the research and its implications wound up lasting for 45 minutes, and the Commission was extremely impressed by the research and the results to date. A summary of the meeting was presented at the 2004 Statewide EPSCoR conference (see above). Results of the meeting were made available statewide on the KEQC web site. In summary, the following recommendations were made to the EQC. "Environmental biomarkers should reflect the types (and potentially suites) of contaminants and/or problems likely to be encountered in Kentucky or that have been identified as regional or aerial concerns. In general biomarkers do not duplicate but complement monitoring efforts. It should be kept in mind that biomarkers can identify that there is a problem but, depending on the type, may not immediately pinpoint the exact cause. In the example of the salamander developmental stability, asymmetry is an exposure biomarker, indicating that a stressor is present, but not the stressor. Many molecular biomarkers, however,

are specific to the alteration of a biochemical pathway and demonstrate direct relationships between exposure and effect. While biomarkers for human health concerns have progressed dramatically over the past 5 years, biomarkers for ecological health are in their infancy. We would suggest the following as a starting point.

Potential biomarkers (aquatic or terrestrial systems) (invertebrates, fish, mammals) include

- Nitrogen contamination from agricultural/urban runoff
- Herbicide/pesticide contamination (particularly Atrazine)
- Endocrine disruptors
- Polycyclic Aromatic Hydrocarbons (PAHs):
- Polychlorinated biphenyls (PCBs)
- Heavy metals, particularly mercury, cadmium, and tin
- Petroleum byproducts (other than PAHs)

All investigators were again brought together at the 11th Annual EPSCoR Conference, May 13, 2005, at the Louisville Marriott Downtown in Louisville, KY. Four presentations were made followed by a group discussion.

Steven R. Myers, Harrell E. Hurst, and Md. Yeakub Ali, Department of Pharmacology and Toxicology, University of Louisville School of Medicine: *Kinetics of Binding of Polycyclic Aromatic Hydrocarbons to Hemoglobin (Hb) in the Mouse.*

Yeakub Ali and Harrell E. Hurst, Department of Pharmacology and Toxicology, University of Louisville: *GC/MS Identification of Enantiomeric Forms of Chloroprene Epoxide Metabolites and Their Enantiomeric Selectivity in Reactions with Red Cells.*

Beth A. Kobylarz, Howard H. Whiteman, Bommanna G. Loganathan, I-Lun Chien, and Prachya Mruetusatorn, Murray State University: *The Impact of Age and Sex on the Chemical Exposure and Developmental Stability in Rana catesbeiana.*

Bommanna G. Loganathan and Howard Whiteman, Departments of Chemistry and Biology and Center for Reservoir Research, Murray State University: *PCB Congeners and Chlorinated Pesticides Concentrations in Amphibian Samples Collected from Western Kentucky.*

Members of the KY EPA EPSCoR Committee continue to track publication of the results and new proposals being developed. A listing of presentations, publications, and theses is given at the end of the SIP section.

Other components have included a revamping of the web site, which is now connected to the Kentucky EPSCoR site and is searchable through the AERC web site. The site location has migrated to <http://www.murraystate.edu/qacd/cos/hbs/epaepscor-program.htm> and also can be accessed at http://www.kyepscor.org/ky_epscor_programs.htm. All reports will be maintained on the website including the final reports for the SIP and both SEER grants. Two members of the KY EPSCoR Committee (White, Farrell) were re-elected in 2005 to 3-year terms on the

Statewide Committee, which has provided a broader outlet for the importance of long-term funding for environmental research in the Commonwealth.

There have been no changes in key personnel with the exception of addition of new members of the KY EPA EPSCoR Committee in 2002 (see previous reports).

State matching funds through the Kentucky Statewide Committee for the 2001-2003 budget year were held up in the Kentucky legislature (no State budget passed) through April 2003 slowing some aspects of the research. All funds were released to us in April; however, we did not expend as much of the supplemental undergraduate/graduate student funding and travel funding as we had planned for both years. Thus we asked for and have received two, one-year no-cost extensions to the entire project. Both SEER grants have taken advantage of students and travel this during the final years.

Products:

Presentations:

Ali, M Y., Hurst, H.E., Myers, S.R., Characterization of epoxide adducts of polycyclic aromatic hydrocarbons (PAH) with hemoglobin (Hb). 44th Annual Meeting, Society of Toxicology, New Orleans, LA, March 6 - 11, 2005.

Ali, M. Y., Hurst, H.E., GC/MS identification of enantiomeric forms of chloroprene epoxide metabolites and their enantiomeric selectivity in reactions with red cell. 11th Annual Kentucky EPSCoR Conference Lexington, KY, presented May 13, 2005.

Ali, M. Y., Hurst, H.E., GC/MS identification of enantiomeric forms of chloroprene epoxide metabolites and their enantiomeric selectivity in reactions with red cell. 53rd ASMS Conference on Mass Spectrometry and Allied Topics, San Antonio, TX, presented June 9, 2005.

Ali, M. Y., Hurst, H.E., Myers, S.R., Characterization of epoxide adducts of polycyclic aromatic hydrocarbons (PAH) with hemoglobin (Hb). 44th Annual Meeting, Society of Toxicology, New Orleans, LA, March 6 - 11, 2005.

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. Research! Louisville2003 Postgraduate Research Day, November 4, 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.

- Boynton, J. and H. Whiteman. 2003. Using remote sensing and GIS to model habitat change and fragmentation in western Kentucky. Joint Meeting of Ichthyologists and Herpetologists, Manaus, Brazil, June 26-July 1.
- Boynton, J. and H. Whiteman. 2003. Utilization of remote sensing to model current and future threats to amphibian populations in western Kentucky. 10th Symposium on the Natural History of Lower Tennessee and Cumberland River Valleys, Land Between the Lakes, March 21-22.
- Cunningham, C.R., Wright, T., Hurst, H.E. and Myers, S.R., Characterization of epoxide adducts of polycyclic aromatic hydrocarbons with hemoglobin, American Association for Cancer Research, Orlando, FL, March 27-31, 2004.
- Cunningham, C.R., Wright, T., Hurst, H.E. and Myers, S.R., Characterization of epoxide adducts of polycyclic aromatic hydrocarbons with hemoglobin. 10th Annual Kentucky EPSCoR Conference Lexington, KY, May 13, 2004.
- Dunlap, K. Brown, A. and Loganathan, B.G. 2005. Preliminary studies on atrazine levels in selected ponds from westernmost Kentucky. MSU Sigma Xi Poster Competition, April 18-22, 2005.
- Hurst, H.E. and Ali, M.Y. Analysis of hemoglobin N-valine adducts from (1-chloroethyl)oxirane, a metabolite of chloroprene. 43rd Annual Meeting, Society of Toxicology, Baltimore, MD, March 23, 2004.
- Hurst, H.E. and Ali, M.Y., Analyses of hemoglobin N-valine adducts and headspace of (1-chloroethyl)oxirane in erythrocytes indicate selective detoxification of chloroprene epoxide enantiomers. International Symposium on Evaluation of Butadiene and Chloroprene Health Risks, Charleston, SC, to be presented September 21, 2005.
- 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.
- Hurst, H.E.: Analysis of hemoglobin n-valine adducts from (1-chloroethyl)oxirane, a metabolite of chloroprene, 10th Annual Kentucky EPSCoR Conference Lexington, KY, May 13, 2004.
- Kobylarz, B., H. H. Whiteman, B. G. Loganathan and L. Duobinis-Gray. 2005. Contaminants and asymmetry: effects of locality, gender, and age. Ninth Annual Symposium on the Natural History of the Tennessee and Cumberland River Valleys, Brandon Springs Group Camp, LBL.
- Kobylarz, B.A., Chien, I-L., Mruetusatorn, P. and Dunlap, K. 2005. The impact of age and sex on chemical exposure and developmental stability in North American Bullfrogs. Southeastern Ecology and Evolution Conference, University of Georgia, Athens, GA.

- Kobylarz, B.A., Chien, I-L., Mruetusatorn, P. and Dunlap, K. 2005. The impact of age and sex on chemical exposure and developmental stability in North American Bullfrogs. MSU Sigma Xi Poster Competition, April 18-22, 2005.
- Loganathan, B.G. and Whiteman, H. 2002. PCB congeners and chlorinated pesticide concentrations in amphibians collected from western Kentucky. Poster presented at 8th Annual Kentucky EPSCoR Conference. October 20-21, 2002. Poster #: 14.
- Loganathan, B.G., Metzger, C., Kobylarz, B. and Whiteman, H. 2004. PCB Congeners and Chlorinated Pesticides Concentrations in Amphibian Samples Collected from Western Kentucky. A poster presented at the Fourth World Congress and 25th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Portland, OR. Nov. 14-18, 2004.
- Meredith, C. and H. Whiteman. 2003. Response of amphibian embryos and larvae to increasing nitrate concentrations: sublethal and lethal effects at levels found in agricultural run-off. Society for Conservation Biology, University of Minnesota-Duluth, June 29-July 2.
- Myers, S., C. Cunningham, T Wright, H. Hurst., Polycyclic Aromatic Hydrocarbon Adducts with Hemoglobin. American Association for Cancer Research, March, Orlando, FL, 2004.
- Myers, S.R. Hemoglobin as a biomarker tool in the assessment of polycyclic aromatic exposure assessment. University of Louisville, Dept. of Pharmacology and Toxicology. 19th International Meeting of the International Society for Polycyclic Aromatic Compounds, Amsterdam, Netherlands, Abstract #75, Sept 21- 25, 2004.
- Myers, S.R., Cunningham, C, Wright, T. and Hurst, H.E. Kinetics of reaction of epoxide metabolites of polycyclic aromatic hydrocarbons with human and mouse hemoglobin. 43rd Annual Meeting, Society of Toxicology, Baltimore, MD, March 22, 2004.
- Myers, S.R., Hurst, H.E. and Ali, M Y., Kinetics of binding of polycyclic aromatic hydrocarbons to hemoglobin (Hb) in the mouse. 11th Annual Kentucky EPSCoR Conference, Louisville, KY, May 13, 2005.
- Whalen, M.M. and Loganathan, B.G. 2002. Immunomodulation of human natural killer cell cytotoxic function by triazine and carbamate pesticides. Poster presented at 8th Annual Kentucky EPSCoR Conference. October 20-21, 2002. Poster #: 15.
- Whiteman, H. and Loganathan, B.G. 2002. EPA-EPSCoR Project Status. Presented at 8th Annual Kentucky EPSCoR Conference. October 20-21, 2002.
- Whiteman, H. and Loganathan, B.G. 2004. EPA-EPSCoR Project Status. Presented at 9th Annual Kentucky EPSCoR Conference.
- Whiteman, H. H. 2004. Developmental Stability in Amphibians as a Biological Indicator of Chemical Contamination and Other Environmental Stressors. EPA Environmental Research Seminar, Atlanta, GA, Sept 28-29.

Publications submitted or in progress:

Ali, M.Y. and Hurst, H.E. Chiral separation of chloroprene epoxide enantiomers by GC/MS and their kinetics in vitro with red blood cells. *J. Chromatography B*, manuscript in preparation 2005.

Benson, A. R., Whiteman, H. H., J. B. Boynton, M. Dotson, and R. Cates. Developmental stability as an indicator of amphibian population health. In preparation for *Conservation Biology*.

Hurst, H.E. and Ali, M.Y., Analyses of hemoglobin N-valine adducts and headspace of (1-chloroethenyl)oxirane in erythrocytes indicate selective detoxification of chloroprene epoxide enantiomers. *Proceedings of the International Symposium on Evaluation of Butadiene and Chloroprene Health Risks*, Charleston, SC, September 20-22, 2005, *Chemico-Biol. Interact.*, manuscript ready for submission.

Kobylarz, B., H. H. Whiteman, B. G. Loganathan and L. Duobinis-Gray. Contaminants and asymmetry: effects of locality, gender, and age. In preparation for *Ecological Applications*.

Loganathan, B.G., Chien, I-L., Kobylarz, B. and Whiteman, H. Polychlorinated biphenyls (PCBs), Chlorinated Hydrocarbon Pesticides in Amphibian Samples Collected from Western Kentucky. Intended for publication in *Environmental Toxicology and Chemistry*. SETAC Press.

Meredith, C. S. and H. H. Whiteman. Lethal and sublethal effects of nitrate on amphibian embryos and larvae. In preparation for *Ecological Applications*.

MS Theses:

Jessica Boynton: "Utilization of remote sensing to model current and future threats to amphibian populations". M.S. 2004.

I-Lun Chien: "Polychlorinated biphenyls (PCBs), Chlorinated Hydrocarbon Pesticides in Amphibian Samples Collected from Western Kentucky. M.S. 2005.

Beth Kobylarz: "Effects of age on stress bioindicators and chemical contamination in bullfrogs". M.S. 2005.