Report for 2003MS19B: Chemical Mixtures: Consequences of WNV Eradication on Water Quality

There are no reported publications resulting from this project.

Report Follows

RESEARCH PROPOSAL

(1) TITLE: Chemical Mixtures: Consequences of WNV Eradication on Water Quality

(2) Focus Categories: SED, TS, WQL

(3) Keywords: Ecosystems, Mixtures, Pesticides, Residues, Sediments, Toxic Substances, Water Quality

(4) Duration: March 1, 2004 to February 28, 2005

(5)	Federal Funds:	16,540 (Total)	(16,540) Direct	
(6)	Non-Federal Funds:	33,077 (Total)	(19,223) Direct	(13,854) Indirect

(7) Principal Investigator, University and City: Marc Slattery, University of Mississippi, University, MS

(8) Congressional District No: District No.1

(9) Water Problem, Need for Research:

Recent outbreaks of West Nile Virus (WNV) throughout the United States, and particularly in the Mississippi Valley States, have spurred plans to control the vector (= *Culex* mosquito). A probable phase in each plan requires using chemical agents that affect either adult or larval vector life stages. Chemical agents commonly used to control mosquito vectors are non-species specific pesticides that will potentially interact with non-target aquatic organisms. These compounds enter the aquatic environment via direct or indirect routes eventually becoming part of water and sediment matrices. Most of the WNV vector control compounds are hydrophobic. Upon entering the aquatic environment they readily partition from surface waters onto particulate organic matter in the water column or directly onto the sediment. Within aquatic matrices through direct contact, respiration or indirect ingestion non-target organisms are exposed to vector control compounds individually or as mixtures with persistent or transient anthropogenic compounds such as regional crop pesticides and metals. Individually or as mixtures, acting additively or synergistically, these compounds can potentially affect adult and juvenile life stages of non-target organisms. At the present time, there is limited knowledge regarding effects of WNV vector control compounds in mixtures. Evaluating water quality and aquatic habitat are critical to an overall assessment of vector eradication programs.

This proposal directly addresses Mississippi Water Research and South Atlantic-Gulf Region priorities related to water quality, particularly with respect to needs addressing protection of water and sediment from environmental degradation.

(10) Expected Results, Benefits, Information:

Water and sediment quality in aquatic environments are essential indicators of overall success of WNV vector control programs. Aquatic matrices are complex mixtures of natural and anthropogenic compounds. Our proposed research encompasses both individual compound and mixture exposure studies in both water and sediment matrices. We will be able to assess toxicological effects not predicted by individual compound toxicity studies. Our pre-stress exposure experiments will allow us to evaluate model organisms' responses to vector control compounds after pre-exposure to commonly occurring persistent anthropogenic compounds. We will compare critical body residue values determined from controlled laboratory studies to tissue residues from exposed organisms collected from areas during vector control application. By comparing residue levels we can more accurately evaluate risk to aquatic organisms during vector control application periods. During periods of environmental application of vector control compounds we will evaluate water and sediment samples for mixture concentrations of vector control and commonly occurring anthropogenic compounds. By mimicking environmental mixture concentrations in controlled exposure studies we can assess "real-world" chemical mixture toxicological effects in model organisms commonly found in water column and sediment habitats.

In summary, the proposed research utilizes a novel approach to address the issue of chemical mixture toxicity. The model chemicals were selected to assess the influence of WNV vector eradication compound effects in conjunction with two persistent and interacting compounds in the environment that have the potential for occurrence as mixtures. Results of the proposed investigation will contribute to our currently limited understanding of chemical-chemical interactions. Accordingly, this project is directly applicable to Mississippi and the South Atlantic-Gulf because of the importance of accurately assessing ecological risk.

(11) Nature, scope, and objectives of the research:

The rapid spread of WNV throughout the United States in 2002 resulted in 3231 laboratory-verified infections and 176 deaths (as of October 21st 2002); cases in Mississippi rank within the top 5 nationwide with 178 infections and 9 deaths. Public outcry resulted in hasty plans for eradication of the *Culex* spp. mosquito vectors via insecticide spraying; *these plans often were developed locally and without much consideration to environmental and/or economic consequences.* This proposal directly addresses Mississippi Water Research and South Atlantic-Gulf Region priorities related to water quality, particularly with respect to needs addressing protection of water and sediment from environmental degradation. The following is our three–year approach for assessing impacts of WNV vector control compounds on the aquatic environment.

Phase I - Single Chemical Exposures/Insecticide, Analytical Method Development. *H. azteca*, and *D. magna*, will be exposed to single chemicals to determine concentration threshold values at which adverse toxicological effects occur. In particular, we will focus on those compounds for which this information is not reported in the literature (Table 1). Long-term exposures will be conducted to evaluate the effects of individual chemicals on survival, growth and reproduction. Estimates of no observed effect concentrations (NOECs) and EC_{50}'s for individual compounds will be calculated. Whole body residue concentrations and toxicological effect levels will be used to calculate bioconcentration factors and critical body residues for each compound in both *H. azteca* and *D. Magna*. Targeted WNV vector eradication compounds will be spiked into water and sediment for liquid:liquid or liquid:solid extractions/recovery experiments. The extracts will be separated and quantified using LC-MS analysis, and the methods refined for future use in field matrices.

Table 1. Ecotoxicological Information Relevant to Proposed Research

Compound	Percent Active Ingredient	*Daphnia* [b] µg/L		*Hyalella azteca* µg/L	
		LC_{50}	EC_{50}	LC_{50}	EC_{50}
Larvicides					
Temephos (Abate) [a,c]	5 - 43	0.011 - 0.54	----	----	----
Methoprene (Altosid) [a]	----	----	89 [d] - 360 [i]	----	----
Diflubenzuron [e]	25 - 97.6	----	7.1 - 16	----	----
Adulticide					
Malathion [a,f]	57 - 95	----	1 - 2.2	----	----
Naled [g]	58 - 91.6	----	0.3 - 1.55	----	----
Permethrin [i]	----	----	0.60	----	----
Resmethrin [i]	----	----	3.7	----	----
Model Compound					
Chlorpyrifos [a,h]	25.6 - 97.7	0.10 -115	----	0.119 - 0.219 [j]	----
Methylmercury [a]	97.0 [j] (CH_3HgCl)	----	----	3.8 - 23.5 [j]	3.2 - 10 [k]

a: Bioaccumulates or potential to bioaccumulate in aquatic organisms. b: Daphnia species not stated.
c-h: EPA's website, see references. i: Crop Protection Publications, 1994. j: Benson et al, 2000.
k: Borgmann et al., 1993

Phase II - Multiple Chemical Exposures, Pre-exposure Stress Responses. Mixture toxicity experiments evaluating binary and three ways chemical-chemical interactions of select vector control compounds (Table 2) with two anthropogenic compounds, chlorpyrifos and methylmercury (Table 3), will be conducted during the second year of our investigation. Each vector control/anthropogenic compound mixture study will consist of three binary and one three ways combination at selected concentrations and ratios. Additionally each mixture study will include single chemical concentrations and a control group. Fifteen replicates of each exposure level will be necessary to adequately meet the requirements of the statistical model. Juvenile *H. azteca* and adult *D. magna* will be exposed ten days and seven days, respectively, with survival, growth and reproduction as toxicological endpoints. We will also conduct these experiments in the manner of pre-exposure to a binary combination of chlorpyrifos and methylmercury, followed by addition of a vector control compound to assess the effects of pre-exposure stress on our model organisms' survival, growth, and reproduction.

Table 2. Physical-Chemical Properties of Mosquitocides Targeted for WNV Vector Eradication

Compound	Formula and Molecular Wt.	Solubility Water (@ 25^{o} C)	log K_{ow}	log K_{oc}	Mode of Action	Stability in Water Soil
Larvicides						
Temephos	$C_{16}H_{20}O_6P_2S_3$ 466.5	0.03 mg/L	4.91	5.0 (est.)	Cholinesterase Inhibitor	Low Persistence Low-Mod Persistence
Methoprene	$C_{19}H_{34}O_3$ 310.5	1.4 mg/L	5.21		Mimics Insect Growth Regulator	Degrades Rapidly Low Persistence
Diflubenzuron	$C_{14}H_9ClF_2N_2O_2$ 310.7	0.08 mg/L (pH 5.5, 20^{o} C)	3.89 (log P)	4.00	Chitin Synthesis Inhibitor	Low-Mod Persistence Low Persistence
Adulticide						
Malathion	$C_{10}H_{19}O_6PS_2$ 330.3	145 mg/L	2.75	3.26	Non-systemic Cholinesterase Inhibitor	Low-Mod Persistence Low Persistence
Naled	$C_4H_7Br_2Cl_2O_4P$ 380.8	practically insoluble		2.26	Non-systemic Cholinesterase Inhibitor	Rapidly hydrolyzed Rapidly Degrades
Permethrin	$C_{21}H_{20}Cl_2O_3$ 391.3	0.2 mg/L (20^{o} C)	6.10 (log P)	5.00	Non-systemic Insecticide	Low Persistence Low-Mod Persistence
Resmethrin	$C_{22}H_{26}O_3$ 338.4	37.9 ug/L	5.43	5.00	Non-systemic Insecticide	Low-Mod Persistence Low-Mod Persistence

Sources: Crop Protection Publications, 1994, and EXTOXNET (http://ace.ace.orst.edu/info/extoxnet/, 10/23/02).
K_{ow} = octanol/water partitioning coefficient. K_{oc} = organic carbon partitioning coefficient.

Phase III - Assessment of Bioaccumulation/Field concentrations. During the third year of investigation, concentrations of the WNV vector eradication compounds, chlorpyrifos and methylmercury in water and sediment from natural waterways throughout Mississippi will be assessed using our LC-MS methodology. Also, whole body residues of the compounds mentioned above will be assessed in field collected *H. azteca* and *D. magna* and respective bioconcentration factors calculated. Additional ten-day and seven-day experiments will be conducted using spiked formulated sediment or water at environmentally relevant concentrations. Bioconcentration of the chemical mixtures will be determined from body residue analysis and chemical concentrations in the water and sediment. Environmentally relevant critical body residues will be derived through correlation of toxicity data (if any) and bioconcentration data.

Table 3. Physical-Chemical Properties of Model Compounds

Compound	Formula and Molecular Wt.	Solubility Water (@ 25° C)	log K_{ow}	log K_{oc}	Mode of Action	Stability in Water Soil
Model Cmpds						
Chlorpyrifos [a]	$C_9H_{11}Cl_3NO_3PS$ 350.6	1.4 mg/L	4.70	3.78	Non-systemic Cholinesterase Inhibitor	Low-Mod Persistence Moderate Persistence
Methylmercury [b]	CH_3Hg 215.6				Many physiological systems effected	High Persistence

Sources: EXTOXNET, 1996. Kow = octanol/water partitioning coefficient. Koc = organic carbon partitioning coefficient.

Progress to Date on Original Objectives (Funding Received July 8, 2003 = 4 months)

After receipt of the award letter we have accomplished the following: 1) began characterizing the 82 Mississippi counties to determine locations of field sampling sites, 2) have increased our *Hyallela azteca* population to sustainable levels so we can begin performing single compound and mixture bioassays and 3) graduate student getting trained to use ArcView GIS software and undergraduate getting trained to care for the Hyallela and perform basic bioassays. These results are pertinent to phase III of the original proposal. Our current efforts will focus on phase I and II.

<u>Field Sampling Site Locations</u>

To locate possible field sampling sites state area land use data will be assessed at two levels: county and local area. County level assessments will eliminate large geographic areas not suitable for this study or have a low probability of finding both pesticides and mosquitocides in environmental samples. Local area assessments will define specific locations where there is a high probability that pesticides and mosquitocides will co-occur in the aquatic environment. The two most useful county level characteristics are an active eradication program and data regarding land use devoted to crops. Local area characteristics will be more specific than county level but in general will include the following: 1) detailed information about mosquito control programs including compounds used, amounts applied and application frequencies and locations, 2) agricultural information regarding detailed crop and pesticide data and 3) watershed data, particularly related to streams receiving runoff from agricultural fields. Understanding the spatial and temporal scales related to pesticide and mosquitocide applications are essential to confidently predict locations where these compounds will co-occur and possibly affect the water quality in Mississippi aquatic habitats.

In Mississippi there is no state controlled mosquito eradication effort, cities and counties organize their own programs. Many local government agencies hire pest management services to control mosquitoes. Information regarding which counties or cities currently use a control program will continue to be difficult to obtain until the Department of Health, Division of Epidemiology, compiles data from a recent survey of eradication programs in the state. The database will provide information regarding active control programs and methods being used but will not include specific information such as application frequencies or locations. To date, a few local government agencies and mosquito control services have been contacted to get information regarding control methods being practiced. Though aerial or truck spraying is in use application of time released larvicides, particularly Altosid™ which contains the active ingredient methoprene, directly into aquatic systems is becoming more common. We have decided to use methoprene as our representative mosquitocide for this study.

Counties can be further characterized as potential field study areas by evaluating agricultural practices. According to the Mississippi Department of Agriculture and Commerce there are approximately 42,000 farms occupying 11 million acres in Mississippi. Commercial forests, which comprise 61% of the state's land area, will not be considered when evaluating counties. We evaluated counties based on their 2002 estimated planted acreage of six crops: corn, cotton, rice, sorghum, soybean and wheat (USDA, 2003). Of the 47 counties growing one or more of the above crops it was evident there is a wide range of acres planted: from 1,100 acres of corn planted in George County to 398,100 acres of all six crops planted in Bolivar County. The county land area devoted to crops ranged between 0 and 80%. Of the counties growing crops approximately 94%, 81%, 66%, 21%, 21%, 19% and 4% grew corn, soybean, cotton, rice, sorghum and/or wheat, respectively. Of the six crops planted approximately 13%, 28%, 26%, 11%, 4% and 19% of the counties grew either 1, 2, 3, 4, 5 or 6 crop types, respectively. Though most counties grew some corn, soybean acreage was greatest at 1,392,400 followed by cotton (1,077,300), corn (524,500), rice (244,600), wheat (204,100) and sorghum (68,700). Counties with large tracts of land devoted to crops and have active eradication programs will receive the greatest consideration when deciding on field study areas. Below is a table listing relevant crop information from selected Mississippi counties with active control programs (Table 4). Approximately 57% of the 82 counties can be eliminated because they do not grow any of the six common crops. Of the counties remaining an additional 41% can be eliminated by only

Tabel 4. Crop data of representative MS counties with mosquito eradication programs.

County	Land Area miles^2	Crops acres	% Land Use: Crops	Number Crops	Largest Single Crop, % Total Planted
Bolivar	876	398,100	71	6	Soybean, 46%
DeSoto	478	45,900	15	3	Soybean, 70%
Hinds	869	27,400	5	3	Corn, 49%
Jackson	727	0	0	---	---
Lee	450	45,500	16	2	Soybean, 86%
Leflore	592	235,500	62	6	Soybean, 38%
Pike	409	0	0	---	---
Rankin	775	9,000	2	2	Cotton, 59%
Washingon	707	326,900	72	6	Soybean, 38%

County land area: US Census, 2003. % land use- crops, was calculated: planted crops (mi^2) /county land area (mi^2) x 100. All other information from USDA, 2003; crop acreage based on 2002 estimates of planted corn, cotton, rice, sorgum, soybean and wheat.

considering those with three or more crop types planted, leaving about 19 counties. These remaining counties can be further reduced to 10 by selecting those with approximately 50% county land use devoted to agriculture. The remaining ten are all located in the Delta area in districts 1 and 4. Of these 10, three are know to have active mosquito control programs and once the Department of Health's eradication program database is complete we will be able to determine if the other 7 have active programs too. As counties are considered for field sampling local agencies or hired control services will be contacted to obtain specific information such as compounds used and application frequencies and locations.

After specific counties have been selected from the above mentioned process local areas within these counties will be evaluated as potential field sampling sites. To obtain the specific information regarding control programs and agricultural practices local governmental agencies and individual farmers will have to be contacted. Detailed data regarding pesticide and mosquitocide application needs to be obtain, e.g. compounds used, amount applied and application frequencies and locations. Mosquitocide information can be obtained from local government agencies or their hired mosquito control services while some crop and pesticide information can be obtained from county land managers individual farmers will need to be contacted regarding specific field conditions. Of the common organophosphate pesticide active ingredients listed by the US EPA's Office of Pesticide Programs only three are used on all six of the crops we have selected: malathion, disulfoton and chlorpyrifos. We have decided to use chlorpyrifos as our representative pesticide for this study. All of the pesticide and mosquitocide data as well as watershed information will be organized and evaluated using GIS technology.

Once co-occurring application areas of chlorpyrifos and methoprene are located they will be further assessed by their relationship with local watershed data. Drainage ditches or small watershed tributaries that receive agricultural runoff are likely aquatic locations where both pesticides and mosquitocides can co-occur. Detailed GIS watershed information can be obtained through state, MARIS (Mississippi Automated Resource Information System) and MDEQ (Mississippi Department of Environmental Quality), and federal, US EPA (Environmental Protection Agency) and US GS (Geological Survey), agencies. It will be helpful to choose local areas that are contained with in a single watershed. Many Mississippi counties have more than one watershed; some are associated with more than one river basin. Below is a table containing watershed information for the same counties selected for the crop data (Table 2).

Much of Mississippi's land area has been eliminated as possible study sites based on county level mosquito management and agricultural practice criterion. Once it has been determined which remaining counties have active mosquito control programs local area characteristics will be assessed. GIS models of chlorpyrifos and methoprene applications, agricultural drainage and tributaries that receive runoff will be used to define a smaller geographic location within counties. Much of the pertinent information can only be obtained from local sources.

Hyallela azteca maintenance and bioassays

The population of Hyallela maintained in the Environmental Toxicology Research Program needed to be increased before bioassays can be performed. The population has recently reached a sustainable level to supply enough individuals for the duration of this study

.

Table 5. Watershed data of representative MS counties with mosquito eradication programs.

County	Basin*	Watershed (State)**	US EPA Hydrolic Unit Code (HUC)**
Bolivar	Yazoo River	Lower Mississippi-Helena (AR, MS)	8020100
		Lower Arkansas (AR, MS)	8020401
		Lower Mississippi-Greenville (AR, LA, MS)	8030100
		Big Sunflower (AR, MS)	8030207
		Deer-Steele (AR, LA, MS)	8030209
DeSoto	North Independent	Lower Mississippi-Memphis	8010100
	Streams	(AR, IL, KY, MO, MS, TN)	
	Yazoo River	Horn Lake-Nonconnah	8010211
		Lower Mississippi-Helena (AR, MS)	8020100
		Coldwater (MS)	8030204
Hinds	Black River	Middle Pearl-Strong (MS)	3180002
	Pearl River	Lower Big Black (MS)	8060202
	South Independent	Bayou Pierre (MS)	8060203
	Streams		
Jackson	Coastal Streams	Pascagoula (MS)	3170006
	Pascagoula River	Black (MS)	3170007
	Escatawpa River	Escatawpa (AL, MS)	3170008
		Mississippi Coastal (AL, LA, MS)	3170009
Lee	Tennessee River	Upper Tombigbee (AL, MS)	3160101
	Tombigbee River	Town (MS)	3160102
Leflore	Yazoo River	Tallahatchie (MS)	8030202
		Yalobusha (MS)	8030205
		Upper Yazoo (MS)	8030206
		Big Sunflower (AR, MS)	8030207
Pike	South Independent	Bogue Chitto (LA, MS)	3180005
	Streams	Tangipahoa (LA, MS)	8070205
	Pearl River		
Rankin	Pearl River	Middle Pearl-Strong (MS)	3180002
Washington	Yazoo River	Lower Mississippi-Greenville (AR, LA, MS)	8030100
		Big Sunflower (AR, MS)	8030207
		Deer-Steele (AR, LA, MS)	8030209
		Bayou Macon (AR, LA, MS)	8050002

*Mississippi Department of Environmental Quality, 2003. **US EPA, 2003

Training

The graduate student working on this project has received or is in the process of receiving the following training:

- Mini-course ArcView GIS, August 13-14, 2003
- Taking a graduate level, Geology 500, ArcView GIS
- Enrolled in graduate level Remote Sensing class

ArcView GIS software is going to be loaded onto the graduate student's desktop computer. This software and support is being provided by the University of Mississippi's Geoinformatics Center (UMGC) at no charge to our project (>$10,000 value).

An undergraduate student is being trained to maintain our Hyallela population and on LC_{50} and EC_{50} bioassays using these crustaceans.

(12) Methods, procedures, and facilities: (refer to original proposal for detailed methods and procedures)

Year 2 Goals: Additions and Modifications

Our current year's goals (phase I) were modified after receiving our award notification. We planned to perform a series of single compound bioassays and develop detection/quantification methods appropriate for our analytical equipment. Instead efforts were spent addressing part of our final year's objectives (phase III), particularly assessing likely field sampling locations in the state. This adjustment has proved useful in allowing us enough lead time to obtain the necessary detailed information regarding counties we have been able to designate as having characteristics suitable for field sample sites. We are now satisfied with our progress in assessing field sampling sites to return to our original phase I objectives. So, for the remainder of this year we will focus on completing the necessary single compound bioassays, especially methoprene and chlorpyrifos exposures, and devote more time developing the required analytical methods.

Year two (phase II) will be basically unchanged from the original proposal, crustacean responses to multiple compounds and pre-exposure stress will be evaluated. In addition, if necessary, completion of any phase I objectives will be included into next year's goals. We have determined that our representative compounds will include chlorpyrifos, methoprene and methylmercury. These compounds will be used in binary, three-way mixture and pre-stress studies. By the end of the 2nd year we will be back on the schedule outlined in the original proposal.

Facilities

The facilities in the School of Pharmacy's Environmental Toxicology Research Program at The University of Mississippi that are currently available for this investigation can be divided into four major areas: (1) laboratories for basic toxicological research, (2) a Pharmacogenetics Core Facility, (3) an Aquatic Toxicology Laboratory and (4) an Environmental Toxicology Analytical Laboratory.

Basic laboratories are equipped with analytical and microbalances, scintillation counter, centrifuges, refrigerators, water baths, and an ultra-cold freezer. In addition, microscopes (Olympus B-Max 40; Olympus MEIJI), a cryostat (Leica CM1850), a rotary microtome (Olympus HM 315), and paraffin embedding station (Reichert-Jung Histembedder) are available for histological examination of tissues. A digital image analyzer system (Kodak Catseye DKC-5000 with Image Pro Plus version 3.03 software) is available for histological analysis and quantifying the size of adult, larvae, and eggs of aquatic vertebrate and invertebrate species. A TECAN SLT Rainbow UV-VIS scanning microplate spectrophotometer with WinSelect version 2.0 software is utilized for biochemical measurements. Field analysis of water quality is performed with a Hydrolab Quanta water quality monitoring system. There are several desktop and notebook computers available for word processing and data handling and analysis. Recently, the PI equipped these laboratories with an Agilent GC/MS , a Waters LC/MS, and a JOEL SEM to provide greater toxicological identification abilities.

The Pharmacogenetic Core Facility located within ETRP's suite of laboratories has recently been outfitted with state of the art molecular analysis equipment. At the heart of the facility are a Beckman Coulter CEQ 8000 Genetic Analysis System, an Agilent 2100 Bioanalyzer and a BioRad VersaDoc 3000 image analyzer. A technician is on staff to run samples. High quality water is provided by a Millipore Milli-Q system.

The Aquatic Toxicology Laboratory is equipped for specialized research with aquatic invertebrate and vertebrate species. The Laboratory is made up of nine rooms that have individual temperature and lighting controls and Gast Regenair Blowers to provide tank aeration. Ultra pure water is supplied by a Barnstead NANOpure Infinity system. Dechlorinated water is provided by Model 2952 organic bed service exchange carbon for chlorine and chloramine removal (U.S. Filter Systems). Individual Model 2952 systems have been installed in each wet lab. There are numerous exposure systems (30- and 80-L aquaria and Frigid Unit Living Streams). For incubation of eggs, Precision Refrigerated Dual-Program Illuminated Incubators are available.

The Environmental Toxicology Analytical Laboratory occupies approximately 2,000 square feet within a 8,000 sq. ft. facility. Analytical equipment consists of a Hewlett-Packard Model 8452A diode array UV-VIS spectrophotometer with auto-sampler and kinetics software, two Hewlett-Packard Model 5890 Series II gas chromatographs (GCs) with dual electron-capture detectors, a Hewlett-Packard Model 5890 Series II GC with flame photometric and flame ionization detectors, a Hewlett-Packard Model 6890 GC with flame ionization and nitrogen-phosphorous detectors. The GCs are linked with a Hewlett-Packard Vectra 25 GC data station with Hewlett Packard Chemstation software. Also included is a Waters Model 600E HPLC system with Model 484 UV Absorbance Detector, Model 717 autosampler, a fraction collector and Millenium 2010 chromatography software. The laboratory is also equipped with an Ohmicron RPA1 Analyzer for analysis of chemicals using enzyme linked immunosorbent assays. For analysis of metals, a CEM Model MDS-2100 Microwave Digestion System as well as Varian SpectrAA-20 and SpectrAA 400 Zeeman atomic absorption spectrometers are available. A Brucker BioApex 30es High Resolution Fourier Transform Mass Spectrometer is maintained in the School of Pharmacy and is available for use in this project. Through the 1997 National Research Council of Canada/National Oceanic and Atmospheric Administration Intercomparison Studies (NOAA/10) the analytical laboratory has earned a rating of Very Good for accuracy evaluation of sediments and Superior for accuracy evaluation of biological tissues.

(13) <u>Related Research:</u>

Chemical Mixture Toxicity. Chemicals in the environment rarely occur alone, however, most toxicological studies are conducted using single chemical exposures. Therefore, it is necessary to characterize the toxicological hazards and risks associated with multiple chemical exposures (Parrott and Sprague, 1993; Feron *et al*, 1995). Chemicals occurring in complex mixtures have the potential for chemical-to-chemical, toxicokinetic and toxicodynamic, interactions affecting the resulting toxicological response. Chemical mixtures are characterized as having additive, synergistic, or antagonistic interactions and effects on the measured toxicological endpoint (Calabrese, 1991). Additivity is the summation of toxic responses from multiple chemicals in a mixture. Synergism is the interaction of multiple chemicals in which the toxic response is greater than would be predicted by simple summation. Antagonism is the interaction in which the toxic response is less than would be predicted by summation. The deviation of chemical mixture toxicity from traditional individual toxicological testing makes it necessary to evaluate mixture interactions further so that the hazards and risks associated with multiple chemical exposure may be assessed (Sexton *et al.*, 1995).

To date, aquatic toxicology studies have typically evaluated the interaction of chemicals having similar mechanisms of toxicity. Kraak *et al.* (1994) studied the effects of a mixture of cadmium, copper, and zinc in the Zebra Mussel (*Dreissena polymorpha*) and determined the mixtures to be additive. Similarly, zinc and copper were found to interact additively in the Rainbow Trout (Lloyd, 1961). Spehar and Fiandt (1986) observed mixtures of metals at concentrations acceptable by the individual water quality criteria were not protective of daphnids and fish due to additivity interaction. However, Hoagland et al. (1993) found that atrazine and bifenthrin, having dissimilar mechanisms of toxicity, were additive. Several studies in which chemicals having independent or dissimilar mechanisms of action have demonstrated non-additive interactions, and in some cases found synergistic and antagonistic effects (Marinovich *et al.*, 1996). Classical studies by Triolo and Coon (1966) demonstrated that aldrin antagonized the effects of parathion, paraoxon, as well as several other organophosphates. It is apparent that there have been a variety of conclusions drawn from chemical mixture interaction studies. Chemical interactions are more complex than the assumption of additivity presently utilized to assess the risks associated with multiple chemical contaminants in sediment. Therefore, there is a need to more fully understand the underlying mechanisms of chemical mixtures responsible for deviations from additive interactions.

Bioaccumulation. Contaminated sediments have become an increasingly important issue for human and ecological health. Presently, 15 percent of the nations lakes, 4 percent of the nation's rivers, and 100 percent of the Great Lakes have fish consumption advisories associated with them (U.S. EPA, 1996). Of the fish consumption advisories, greater than 95 percent are due to bioconcentration of chemicals including mercury, PCB's, organochlorine pesticides, and dioxin. Nationally, a reported estimate of at least 29 percent of the benthic community in fresh and marine water is impacted by contaminated sediments (Veith, 1996). Long-term exposure to contaminants in the sediment can result in bioaccumulation of the chemical contaminant reaching concentrations capable of eliciting adverse toxicological effects (Borgmann et al., 1991). Toxicity, bioaccumulation and bioconcentration data can be utilized to further characterize the dose-effect relationship of a chemical. The critical body residue is the whole body concentration in an organism associated with a measured adverse toxicological effect. It accounts for variability in chemical bioavailability in the exposure media, metabolism, and uptake and depuration kinetics. The use of critical body residues in aquatic organisms has been proposed as a method to assess sediment contamination and the potential toxicological effects in aquatic organisms. McCarty and Mackay (1993) suggested the use of critical body residues and corresponding biological responses be studied to validate laboratory and field-based assessments of sediments. Currently, the assessment of sediment contamination is based on measured sediment concentrations of individual chemicals and toxicity to laboratory organisms. Safe sediment concentrations of chemical contaminants in sediment could be determined from the amount of that chemical accumulated and the corresponding measured toxicological effects. Due to site-specific differences in chemical bioavailability and metabolism, the use of critical body residues may be a better predictor of the degree of ecological risk associated with contaminated sediments than sediment concentrations alone (Landrum *et al.*, 1992; Borgmann *et al.*, 1993).

Electronic databases used to review the literature discussed above include: Environmental Sciences and Pollution Management Abstracts (Cambridge Scientific), Life Sciences Periodical Abstracts (Cambridge Scientific), Biological and Agricultural Index (H.W. Wilson), and Biological Abstracts Inc.

(15) Training potential:

ESTIMATED STUDENTS RECEIVING TRAINING

Currently a single Ph.D. graduate student (Biology- Environmental Toxicology emphasis) has been targeted for salary support and training under this WRRI program. Jim Weston has been a research scientist in our Environmental Signals & Sensors research program, and he recently decided to return to grad school to complete a Ph.D. This grant will provide the support for him to complete this degree, while performing work closely related to his own interests (the environmental consequences of mixtures of pharmaceuticals in aquatic systems). However our work, and techniques, is multi-disciplinary and it is likely that several other graduate students in my lab and within the ETRP and Biology programs will assist, and be trained, in various aspects of the project. My graduate students include Ph.D. (2 in Pharmacognosy) and M.S. (1 in Biology) candidates and I currently fund a 4th yr undergraduate as a laboratory technician in environmental toxicology. In addition, environmental toxicology collaborations with Kristie Willett (Pharmacology/ETRP), John Rimoldi (Medicinal Chemistry/ETRP) and Stephen Threlkeld (Biology) suggests that some of their students will also be involved in either field or laboratory-based training associated with this project.

INFORMATION TRANSFER PLAN

A critical issue that has been overlooked in the recent WNV eradication discussions is the impact of spraying on environmental health. While all of the proposed mosquito control agents have been tested utilizing standard EPA protocols, *these have largely focused on single chemical dosing regimes and aquatic systems typically are comprised of chemical mixtures.* These mixtures have the potential to work additively or synergistically, and the stress of exposure to one class of compound may exacerbate the effects of another compound, even if it is applied only transiently. **Thus our goal is to assess the effects of WNV vector eradication agents in two model populations of aquatic invertebrates under conditions of single chemical doses following exposure to a mixture of persistent pesticides.**

This research program targets several important user groups: 1) the health of Mississippi residents who fish our waterways for subsistence or recreation is potentially impacted by bioaccumulation of pesticides and metals, 2) several commercial fishery markets in Mississippi (most notably Crayfish) have the potential to be either directly or indirectly impacted by mosquito adulticides and larvacides, and 3) it goes without saying that the recreation and/or tourism potential of Mississippi aquatic systems might be adversely impacted by changes in environmental health.

Our strategy for dissemination of our data will follow two closely allied approaches. First we intend to provide our results to the scientific community via presentations (budgeted regional & national mtgs) and publications in as timely a manner as possible. We also believe it is important to open a forum for discussion of problem with the lay public and the regional health councils who are developing these eradication plans. We intend to give seminars to regional user groups and develop a link/listserv to the UM ETRP page focused on this issue.

We expect to reach our target audiences via existing collaborations between ETRP and the Field Station Extension Service. And through announcements provided to the WRRI (i.e., LORE newsletter, etc).

LITERATURE CITED

Allgood, J., J. Steevens, and W. Benson. 1997. Development and validation of an extraction procedure for atrazine and chlorpyrifos in tissue for analysis by immunoassay. Poster Presented at Society of Environmental Toxicology and Chemistry 18th Annual Meeting. San Francisco, CA. November, 1997.

American Society of Testing and Materials. 1994. Standard guide for conducting sediment toxicity tests with freshwater invertebrates. designing biological tests with sediments. *In: Annual Book of Standards.* Vol. 11.04, E1383-94. Philadelphia, PA.

Bass, P., Chief of Field Services Division, Mississippi Department of Environmental Quality. Personal Communication.

Benoit, J.M., Fitzgerald, W.F., and A.W.H. Damman. 1994. Historical atmospheric mercury deposition in the mid-continental U.S. as recorded in an ombrotrophic peat bog. In: *Mercury Pollution: Integration and Synthesis*. C.J. Watras and J.W. Huckabee, Eds. Lewis Publishers, Boca Raton, Fl.

Benson, W.H., Block, D.S., Steevens, J.A., Allgood, J.C. and Slattery M. 2000, Chemical mixtures: consequences for water quality. Technical Completion Report, GR-02679-18. Water Resources Research Institute Mississippi State University, Mississippi State, MS.

Berenbaum, M.C. 1989. What is synergy? *Pharmacol. Rev.* 41: 93-141.

Borgmann, U., Norwood, W.P., and C. Clarke. 1993. Accumulation, regulation, and toxicity of copper, zinc, lead, and mercury in *Hyalella azteca*. *Hydrobiologia.* 259: 79-89.

Borgmann, U., Norwood W.P., and I.M. Babirad. 1991. Relationship between chronic toxicity and bioaccumulation of cadmium in *Hyalella azteca*. *Can. J. Fish. Aquat. Sci.* 48: 1055-1060.

Calabrese, E.J. 1991. *Multiple Chemical Interactions*. Lewis Publishers, Inc. Chelsea, MI.

Canfield, T.J., N.E. Kemble, W.G. Brumbaugh, F.J. Dwyeer, C.G. Ingersoll, and J.F. Fairchild. 1994. Use of benthic invertebrate community structure and the sediment quality triad to evaluate metal-contaminated sediment in the upper Clark Fork River, Montana. *Environ. Toxicol. Chem.* 13:1999-2012.

Crop Protection Publications. 1994. A World Compendium The Pesticide Manual: Incorporating The Agrochemicals Handbook, Tenth Edition. Clive Tomlin ed. Crop Protection Publications, Surrey, UK

Dee, J.E. 2000. Larvicide may be factor in lobster die-offs. Ctnow.com April 20, 2000. http://www.seagrant.sunysb.edu/LILobsters/LobsterMedia/HC-Lobster042000.htm, (cited 10/28/2002).

ECOTOXNET, 1995. Pesticide Information profile, methoprene. http://pmep.cce.cornell.edu/profiles/extoxnet/haloxyfop-methylparathion/methoprene-ext.html, (cited 10/23/02).

ECOTOXNET, 1996. Pesticide Information profile, chlorpyrifos. http://pmep.cce.cornell.edu/profiles/extoxnet/carbaryl-dicrotophos/chlorpyrifos-ext.html, cited 10/23/02.

Feron, V.J., Groten, J.P., Jonker, D., Cassee, F.R., and P.J. van Bladeren. 1995. Toxicology of chemical mixtures: challenges for today and the future. *Toxicology*. 105: 415-427.

Filippelli, M. 1987. Deermination of trace amounts of organic and inorganic mercury in biological materials by graphite furnace atomic absorption spectrometry and organic mercury speciation by gas chromatography. *Anal. Chem.* 59: 116-118.

Gardiner, M.S. 1972. *The biology of invertebrates.* McGraw-Hill, New York, NY.

Gessner, P.K. 1995. Isobolographic analysis of interactions: an update on applications and utility. *Toxicology.* 105: 161-179.

Hewlett, P.S. 1969. Measurement of the potencies of drug mixtures. *Biometrices.* 22: 477-487.

Hoagland, K.D., Drenner, R.W., Smith, J.D., and D.R. Cross. 1993. Freshwater community responses to mixtures of agricultural pesticides: effects of atrazine and bifenthrin. *Environ. Toxicol. Chem.* 12: 627-637.

Ingersoll, C.G., W.G. Brumbaugh, F.J. Dwyer, and N.E. Kemble. 1994. Bioaccumulation of metals by *Hyalella azteca* exposed to contaminated sediments from the upper Clark Fork River, Montana. *Environ. Toxicol. Chem.* 13: 2013-2020.

Kraak, M.H.S., Daphna, L., Schoon, H., Toussaint, M., Peeters, W.H.M., and N.M. van Straalen. 1994. Ecotoxicity of mixtures of metals to the zebra mussel *Dreissena polymorpha*. *Environ. Toxicol. Chem.* 13: 109-114.

Landrum, P.F., Lee, H. II., and M.J. Lydy. 1992. Toxicokinetics in aquatic systems: model comparisons and use in hazard assessment. *Environ. Toxic. Chem.* 11: 1709-1725.

Lindquist, O.K., M.a. Johansson, A. Anderson, L. Bringmark, G. Hovsenius, A. Iverfeld, M. Meili, and B. Timm. 1991. Mercury in the Swedish environment. *Water Air Soil Pollut.* 55:193-216.

Lloyd, R. 1961. The toxicity of mixtures of zinc and copper sulphates to rainbow trout. *Ann. Appl. Biol.* 49: 535-538.

Lund, B.O., D.M. Miller, and J.S. Woods. 1991. Mercury induced H2O2 production and lipid peroxidation in vitro in rat kidney mitochondria. *Biochemical Pharmacology*. 42:S181-S187.

Marinovich, M., Ghilardi, F., and C.L. Galli. 1996. Effect of pesticide mixtures on in vitro nervous cells: comparison with single pesticides. *Toxicology.* 108: 201-206.

McCarty, L.S. and D. Mackay. 1993. Enhancing ecotoxicological modeling and assessment. *Environ. Sci. Technol.* 27: 1719-1727.

Mississippi Department of Environmental Quality. 2003. Environmental Data. http://deq.state.ms.us/MDEQ.nsf/page/Main_EnvironmentalData?OpenDocument, viewed 7/3/03.

Mokrzan, E.M., Kerper, L.E., Ballatori, N., and T.W. Clarkson. 1995. Methylmercury-thiol uptake into cultured brain capillary endothelial cells on amino acid system L. *Journ. Pharm. Exp. Therap.* 272: 1277-1284.

Montgomery, J.H. 1993. *Agrochemicals desk reference, environmental data.* Lewis Publishers, Chelsea, MI.

Parott, J. L., and J. B. Sprague. 1993. Patterns in toxicity of sublethal mixtures of metals and organic chemicals by Microtox and by DNA, RNA, and Protein Content of Fathead Minnows. *Can. J. Fish. Aquat. Sci.* 50: 2245-2253.

Pennak, R.W. 1989. *Fresh-water invertebrates of the United States*, Third Ed. John Wiley & Sons. New York, NY.

Sexton, K., Beck, B.D., Bingham, E., Brain, J.D., Demarini, D.M., Hertzberg, R.C., O'Flaherty, E.J., and J.G. Pounds. 1995. Chemical mixtures from a public health perspective: the importance of research for informed decision making. *Toxicology*. 105: 429-441.

Slemr, F., and E. Langer. 1992. Increase in global atmospheric concentrations of mercury inferred from measurements over the atlantic ocean. *Nature*. 355: 434-437.

Smith, S. Jr., Cullum, R.F., and J.D. Schreiber. 1994. Pesticides in runoff and shallow ground water from upland corn production in north Mississippi, USA. Proceedings of the second international conference on ground water ecology. 249-258.

Sorensen, E.M.. 1991. *Metal Poisoning in Fish*, 312-323. CRC Press, Bocan Raton, FL.

Spehar, R.L., and J.T. Fiandt. 1986. Acute and chronic effects of water quality criteria based metal mixtures on three aquatic species. *Environ. Toxicol. Chem.* 5: 917-931.

Steevens, J.A., S.S. Vansal, K.W. Kallies, S.S. Knight, C.M. Cooper and W.H. Benson. 1998. Toxicological evaluation of constructed wetland habitat sediments utilizing *Hyalella azteca* 10-day sediment toxicity test and bacterial bioluminescence. *Chemosphere.* In press.

Steevens, J.A. and W.H. Benson. 1998. *Hyalella azteca* 10-day sediment toxicity test: Comparison of growth measurement endpoints. *Environ. Toxicol. Water Qual.* Accepted

Suedel, B.C., Boraczek, J.A., Peddicord, R.K., Clifford, P.A., and T.M. Dillon. 1994. Trophic transfer and biomagnification potential of contaminants in aquatic ecosystems. *Rev. Environ. Contam. Toxicol.* 136: 21-89.

Suedel B.C. and J.H. Rodgers, Jr. 1994. Development of formulated reference sediments for freshwater and estuarine sediment toxicity testing. *Environ. Toxicol. Chem.* 13: 1163-1175.

Svendsgaard, D.J. and R.C. Hertzberg. 1994. Statistical methods for the toxicological evaluation of the additivity assumption as used in the Environmental Protection Agency Chemical Mixture Risk Assessment Guidelines. In: R.S.H. Yang (Ed.), *Toxicology of Chemical Mixtures: Case Studies, Mechanisms, and Novel Approaches*. Academic Press, New York, pp. 599-642.

Tomasovic, M.J., Dwyer, F.J., Greer, I.E. and C.G. Ingersoll. 1995. Recovery of known-age *Hyalella azteca* (Amphipoda) from sediment toxicity tests, *Environ. Toxicol. Chem.* 14:1177 1180.

Tomlin, C. 1994. *The Pesticide Manual, 10th Edition.* British Crop Protection Council and The Royal Society of Chemistry, U.K.

Triolo, A.J. and J.M. Coon. 1966. The protective action of aldrin against the toxicity of organophosphate anticholinesterases. *J. Pharmacol. Exp. Therap.* 154: 613.

US Census. 2003. State and County Quick Facts. http://quickfacts.census.gov/qfd/states/28000.html, viewed 6/24/03.

US Department of Agriculture. 2003. National Agricultural Statistics Service Data Base. http://www.nass.usda.gov:81/ipedb/, viewed 6/24/03

U.S. Environmental Protection Agency. 1982. Guidance for the registration of pesticide products containing methoprene as the active ingredient. Office of Pesticide Programs, Washington, DC.

U.S. Environmental Protection Agency. 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, 2nd Edition. EPA 600/4-89-001. Environmental Monitoring Systems Laboratory, Cincinnati, OH.

U.S. Environmental Protection Agency. 1991. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, Fourth Ed. EPA/600/4-90/027. Office of Research and Development, EPA. Washington, D.C.

U.S. Environmental Protection Agency. 1994. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA/600/R 94/024. Office of Research and Development, Washington, D.C.

U.S. Environmental Protection Agency. 1996. National listing of fish and wildlife consumption advisories. EPA-823-F-96-006. Office of Water, Washington, D.C.

U.S. Environmental Protection Agency website, table 2 references.
c-http://www.epa.gov/oppsrrd1/REDs /temephos_red.htm#IIIB3, viewed 10/23/02.
d-http://www.epa.gov/oppsrrd1/REDs/old_reds/methoprene.pdf, viewed 10/23/02.
e-http://www.epa.gov/oppsrrd1/REDs/0144red.pdf, viewed 10/23/02.
f-http://www.epa.gov/oppsrrd1/op/malathion/efedrra.pdf, viewed 10/23/02
g-http://www.epa.gov/pesticides/op/naled/efed_rev.pdf, viewed 10/23/02

h-http://www.epa.gov/pesticides/op/chlorpyrifos/efedrra1.pdf, viewed 10/23/02

US Environmental Protection Agency. 2003. Surf Your Watershed. http://cfpub.epa.gov/surf/locate/index.cfm, viewed 7/3/03

U.S. Geological Survey. 1997. Pesticides in surface and groundwater of the United States: Preliminary results of the national water quality assessment program (NAWQA).

Veith, G.D. 1996. Presentation at the National sediment bioaccumulation conference. Bethesda, Maryland. September, 11-13, 1996. Office of Research and Development, Washington, D.C.

Weston, J., Huggett, D.B., Rimoldi, J, Foran, C.M., Slattery, M. 2001. Determination of fluoxetine (Prozac) and norfluoxetine in aquatic environments. Society of Toxicology and Chemistry 22nd Annual Meeting in North America, Baltimore, MD. November 11 –15, 2001.

Zumwalt, D.C., Dwyer, F.J., Greer, I.E. and C.G. Ingersoll. 1994. A water-renewal system that accurately delivers small volumes of water to exposure chambers, *Environ. Toxicol. Chem.* 13:1311-1314.