UNITED STATES GOVERNMENT

memorandum

DATE: June 2, 1992

REPLY TO ATTN OF: Leader, North Carolina Cooperative Fish and Wildlife Research Unit

SUBJECT: Study proposal on diagnosing and effects of cholinesterase inhibitors on freshwater mussels.

TO: Mussel Coordinator, Asheville Field Office

Attached is the study proposal that we discussed. We have a very short turn around time on this if you want to fund it. Our deadline is for all paperwork for new research work orders to be in Washington by June 10. If you wish to proceed with this, all that I will need is a memo from you, signed by you or someone that has the authority to commit the funds, stating that you want to fund the study. This will buy some time for securing the extra funding if it is not available now. Your memo must state the proposal title, name the North Carolina Unit as the recipient, and include an account number to charge. You mentioned that funding might be more available later in the year; therefore, we discussed the option of you initially obligating only $2000 of the $6000 project cost. If you do this, I trust that you will work with us to complete the project.

If you decide to go ahead and start this project, my responsibility, upon receiving the approval from you, is to write the research work order and to get the paperwork to Washington. To meet my deadline, I would like to have your memo by June 8. A fax is OK as long as it is followed by an original.

I think that the study has merit based on the fact that we have had mussel die-offs where cholinesterase depression has been found. Yet we cannot interpret this information fully nor do we know the full threat that cholinesterase inhibitors might pose to mussels. I have been doing some preliminary work on the assay development and dosing protocols using some of our station's operating funds. However, these funds are now gone and we will not be able to proceed further without outside funds.

The current study was designed as one component of a two part study. The second part is the study for biomonitoring proposed by the Raleigh Enhancement Office. Because summer is upon us, we will not be able to supply the Enhancement Office with information

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in time to implement it this year. It is our hope that the information we generate will lead to the implementation of the field study next year. Your help and support in finding support for the current proposed study and the biomonitoring study could contribute significantly to our understanding of the potential impact of agricultural chemicals on freshwater mussels.

Jim

Jim Fleming

STUDY TITLE: Diagnosing and assessing freshwater mussel exposure to agricultural pesticides.

BRIEF DESCRIPTION: Techniques will be developed and validated to determine if field populations of freshwater mussels are being exposed and potentially depressed by organophosphate and carbamate pesticides. This information will be provided to the Contaminants Specialist, Raleigh Enhancement Office to assist in the design, implementation, and conduct of a biomonitoring study of freshwater mussels in agricultural areas.

BACKGROUND: In August, 1990, a large die-off of the endangered Tar River spiny mussel occurred in Swift Creek, Nash County, NC. About 50% of the land in Nash County is in agricultural production. Mussels collected at the die-off site exhibited cholinesterase depression, indicating exposure to one of the more common groups of agricultural chemicals (see attachment 1). Freshwater mussels in most areas of North Carolina are experiencing population declines. The potential role of agricultural chemicals in this decline has not been previously investigated. The potential for agricultural chemicals to adversly affect mussels seems great. However, the first step in this assessment is to document whether or not mussels are being regularily exposed to agricultural pesticides. This proposal is designed to develop criteria to determine exposure of mussels to organophosphate and carbamate pesticides. With this information, a pilot mussel biomonitoring program will be jointly developed with the Raleigh Enhancement Office, which they will implement. Once the degree of exposure of wild populations of mussels has been established, we will know whether or not to proceed with investigations of biological effects on both adult and immature stages.

The North Carolina Cooperative Fish and Wildlife Research Unit has proceeded with the development of techniques for measuring cholinesterase activities in mussels and for experimentally dosing mussels to determine their sensitivity to agricultural chemicals. This preliminary work was conducted with aldicarb, a carbamate pesticide, and acephate, an organophosphate. Both of these chemicals are heavily used in North Carolina, especially in the eastern part of the state.

A recent, dramatic shift toward cotton acreage in eastern North Carolina could bring additional challenges to aquatic fauna. Cotton receives heavy pesticide use, including the use of some of the most toxic pesticides. The potential for mussels to be affected by pesticides would seem to be elevated in counties involved with cotton production.

OBJECTIVES:

1. Develop techniques and monitoring criteria for determining adult mussel exposure to organophosphorus and carbamate pesticides.

2. Determine the toxicity of commonly used organophosphate and carbamate pesticides to adult mussels.

3. Determine the sublethal effects of commonly used organophosphate and carbamate pesticides to adult mussels.

METHODS: Adult *E. complanata* will be collected from Swift Creek, Johnson County, NC. Within 4 days of collections, mussels will be placed in test chambers and exposed to multiple dose levels of acephate and aldicarb. Exposure will be for 96 hours. Mussels will be considered dead when the gape and do not respond to a touch stimulus. Dead mussels will be frozen when they are found during the study. All mussels will be sacrificed by freezing at the end of the 96 hour test period. Behaviors (siphoning, gaping) will be noted during the test.

Cholinesterase activities will be determined on the adductor mussel dissected from each animal. Discrimination of organophosphate- and carbamate-induced cholinesterase poisoning will be attempted by cholinesterase reactivation techniques.

Lethal concentrations of acephate and aldicarb will be calculated and compared to published values for other aquatic species. Once lethal concentrations have been determined, at least one of the two chemicals will be used in a study to determine the effect of water temperature on pesticide toxicity. For this temperature study, the LC50 for one chemical will be administered to four groups of mussels each held in a different water temperature. Water temperatures will vary between test chambers by 5 degrees C. The maximum temperature used will approximate the maximum water temperature expected in eastern North Carolina rivers.

PROJECT DURATION: 1 year beginning with receipt of funds

FUNDING REQUIREMENTS $6000

COOPERATORS: North Carolina Wildlife Resources Administration, USFWS, Raleigh Enhancement Office

ANTICIPATED USERS OF INFORMATION: State and local regulatory officials, diagnosticians involved with investigations of mussel die-offs, Raleigh Enhancement Office for development of a mussel biomonitoring program.

Attachment 1

Subject: Preliminary analysis of cholinesterase activities from Tar River Mussels collected at the Swift Creek die-off, Nash County, NC

Jenny Hoeppner and I completed the preliminary analysis of the mussel samples from the August 1990, mussel die-off in Swift Creek. The results show that live mussels collected in the kill site and those collected downstream from the kill site had cholinesterase activities in the adductor mussel that were 27 and 35% (73 to 65% depression) of those in a reference site. Depression of cholinesterase activities are commonly seen in birds and mammals exposed to organophosphate and carbamate pesticides. Based on the available data, I believe that it is likely a one of these pesticides was responsible for the depressed cholinesterase activities in the mussels. Although I did not test any dead mussels from the site, I suspect that they would also have exhibited depressed cholinesterase activity. I also believe that an organophosphate or carbamate pesticide was responsible for the kill.

The analytical chemistry data on the mussels from the kill indicated no organophosphorus pesticides on carbamates in the mussel tissues. This not surprising because these pesticides generally do no accumulate in animal tissues. Confirmation of bird kills due to cholinesterase inhibitors is usually done by analysis of gut contents, ie. food samples. Analysis of gut contents were not performed on the mussels from the die-off. Even if this was practical to do, I suspect that the mussels were exposed to the toxicants via the water column, not by way of the food they consumed. Therefore, cholinesterase inhibition is likely to be our only real clue to exposure to these chemicals in mussels. Water samples may have revealed the presence of an organophosphate or carbamate pesticide, but we would have had to be lucky to have caught the chemical on site, before it was transported downsteam in the water column.

The finding of cholinesterase inhibition 3.15 miles (my estimate) downstream from the kill site suggests that mussels were exposed, and possibly affected, by pesticides over a much larger area than only the kill area. The small difference (a difference which was not significant) in cholinesterase depression between the kill site and the highway 48 bridge indicates that exposure probably continued for a considerable distance below the highway 48 bridge, perhaps 10 or more miles. In short, we do not know the full extent of the exposure or the biological impact to these downstream areas.

Diagnosis of cholinesterase inhibitors as a cause of death in avian and mammalian species is based on the the finding of cholinesterase inhibition of at least 50% in the brain and confirmation of a cholinesterase inhibiting pesticide in the gut. Diagnostic criteria for evaluating cholinesterase inhibition in mussels have not been established. Our assays were run on muscle, not brain. Dose-response relationships of mussels to cholinesterase inhibitors have not been shown.

We will be pursuing additional studies with the mussel samples we have from the die-off. I think that we have a good chance of determining whether the cholinesterase inhibitor was a carbamate or an organophosphate. I also want to run a variety of tissues through the cholinesterase assay to determine if there is a better tissue (than adductor muscle) to use for the assay. We have also completed a literature search on cholinesterase inhibitors in freshwater mussels. I found no reports of other die-offs of freshwater mussels related to cholinesterase inhibitors, but there are a number of papers that examine the effects of organophosphates on mussels. I think that these papers are aimed at use of organophosphates to kill unwanted bivalves. I have not seen any of these papers yet, many I have to order through interlibrary loan.

Our attempts in adapting cholinesterase assay procedures show great hope for developing diagnostic criteria and perhaps the development of a monitoring program for mussels. I would appreciate the opportunity to continue to work with your office on mussels, and perhaps to write this work up as an RIB and possibly a research note.

For your planned news release, I feel comfortable with the information contained in the following paragraph:

"Cholinesterase is an essential enzyme that is involved with nerve impulse transmission. Organophosphate and carbamate pesticides inhibit cholinesterase activity. Measurement of cholinesterase activity is used to detect exposure of animals to these chemicals. Determinations of cholinesterase activity in live mussels collected at the time of the die-off showed responses that would indicate the mussels had been exposed to either organophosphate or carbamate pesticides. Cholinesterase activity in living mussels from the kill site and 3 miles below the kill site was depressed by an average of 65 to 73% compared to mussels collected at the reference site.

Organophosphate and carbamate pesticides are quick acting, with animals dying within minutes to hours after exposure. This is consistent with necropsy findings that the mussels died acutely. Chemical analysis of mussel tissues showed no organophosphate or carbamate pesticides. This is to be expected as these chemicals are rapidly broken down once absorbed by animals. Rarely do we find these chemicals in animal tissues following poisoning. Based on available data, it appears that mussels were exposed to organophosphate or carbamate pesticides which probably caused the die-off."