**Proposal Subject:** Adoption of ELISA as a Type I NSSP Analytical Method to Replace the Mouse Bioassay for Monitoring NSP-Causing Toxins in Molluscan Shellfish

**Specific NSSP Guide Reference:** Section IV Guidance Documents
Chapter II Growing Areas
.10 Approved NSSP Laboratory Tests

**Text of Proposal/ Requested Action** Request adoption of enzyme linked immunosorbent assay (ELISA) as a Type I NSSP analytical method for neurotoxic shellfish poisoning (NSP) toxins in molluscan shellfish, under NSSP Guidance Documents Chapter II.10 Approved National Shellfish Sanitation Program Laboratory Tests: Microbiological and Biotoxin Analytical Methods.

An AOAC collaborative study is planned for the ELISA method. Drs. Jerome Naar and Francie Coblentz at UNCW will be the Principle Investigators. A single lab validation of the method is nearing completion, prior to submission to the AOAC Methods Committee for approval to run the collaborative trial. Results of the AOAC collaborative study will be provided to the ISSC for review by the Laboratory Methods Review Committee.

**Public Health Significance:** Accumulation of the breve toxins, the toxins responsible for Neurotoxic Shellfish Poisoning (NSP) in shellfish can cause illness in human consumers. Monitoring for NSP toxicity is essential to assure the safety of bivalves harvested for food and to protect the industry by sustaining consumer confidence.

The mouse bioassay for NSP has served well since it was developed in the 1970s. The assay is relatively simple, able to detect dangerous levels of toxicity, and appears to be an accurate measure of human oral potency. Nevertheless, there has long been a need for detection methods that are more sensitive and more precise, that do not require live test animals, while still providing an accurate measure of human oral potency. Motivation for finding alternatives includes the ethical concerns and negative public perceptions focused on test methods that use live animals.

The ELISA for NSP provides an excellent alternative to the mouse bioassay, offering far greater sensitivity, greater accuracy, and a reliable measure of toxin contamination in shellfish. In the format developed at the UNCW, it offers very high throughput.

Because of the higher throughput, the use of the ELISA as screening method will allow monitoring programs to increase their capacity to monitor shellfish beds after blooms of breve toxin-producing algae while minimizing the use of live animals. This will allow for shellfish to be tested at shorter time intervals to potentially expedite reopenings.

The ELISA in its current mode is best suited to use in a central lab to which samples are sent. Since this is the way in which most toxin monitoring is now conducted, the ELISA can, with suitable equipment and training, be used where mouse bioassays are currently conducted. In Florida, the state that is the most routinely affected by Karenia brevis red tides, shellfish testing is conducted by the Fish and Wildlife Conservation Commission at the Fish and Wildlife Research Institute (FWRI), which is already equipped and familiar with the use of the ELISA. Researchers from FWRI have been involved in the development of this assay and its current validation.

Implementation:
Progress in implementation of the ELISA has been greatly facilitated by the support from NOAA and the Fish and Wildlife Research Institute, which has funded projects to assist the development and the validation of this assay. Drs. Naar and Coblentz are planning an

AOAC collaborative study of the ELISA with the technical support form various investigators from UNCW, FWRI, FDA and US Army. The AOAC task force on marine biotoxin detection methods, led by Dr. James Hungerford, has identified AOAC validation of the ELISA as a high priority.

Some comparisons of the ELISA with:

Receptor Binding Assay:

A preliminary study performed by several investigators under the lead of Dr Robert Dickey FDA, demonstrated ELISA provides similar results as the receptor binding assay; however, the ELISA does not require the use of any radioactive material.

HPLC/MS:

Side by side analysis of shellfish extracts by ELISA and HPLC-MS was conducted by the FDA and reveal good correlation betweens both methods
However, HPLC/MS require careful filtration of the sample, which is a significant cost, and provide a single path, so throughput per instrument is dependent on run time. Equipment cost and operator skill requirements are also much higher.

**Cost Information (if available):** None

**Action by 2007 Laboratory Methods Review Committee** Recommended referral of Proposal 07-104 to an appropriate committee as determined by the Conference Chairman.

**Action by 2007 Task Force I** Recommended adoption of the Laboratory Methods Review Committee recommendation on Proposal 07-104.

**Action by 2007 General Assembly** Adopted recommendation of 2007 Task Force I.

**Action by USFDA** December 20, 2007
Concurred with Conference action.

**Action by 2009 Laboratory Methods Review Committee** Recommended no action on Proposal 07-104. Rationale: Adequate data has not been submitted.

**Action by 2009 Task Force I** Recommended adoption of the Laboratory Methods Review Committee recommendation on Proposal 07-104.

**Action by 2009 General Assembly** Adopted recommendation of 2009 Task Force I on Proposal 07-104.

**Action by USFDA 02/16/2010** Concurred with Conference action on Proposal 07-104.