Proposal Subject: Adoption of LC-MS as a Type I NSSP Analytical Method to Replace the Mouse Bioassay for Monitoring NSP

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 liquid chromatrography-mass spectrometry (LC-MS) 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 LC-MS method. Dr. Steven M. Plakas at FDA will be the Principle Investigator. 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: Neurotoxic shellfish poisoning (NSP) is caused by consumption of shellfish contaminated with algal brevetoxins. 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 toxic shellfish has served well since it was developed in the 1960s. The assay is relatively simple and detects dangerous levels of toxins. However, there has long been a need for detection methods that are more sensitive and precise, that do not require live test animals, while still providing a reliable measure of human oral potency. Motivation for finding alternatives includes ethical concerns and negative public perceptions focused on test methods that use live animals.

The LC-MS method provides an excellent alternative to the mouse bioassay, offering far greater sensitivity and specificity. Greater sensitivity provides a higher level of assurance that growing areas can be closed before violative product is harvested, and enable growers to harvest product while still safe in anticipation of a closure. Greater specificity enables unambiguous identification of toxins present as indicators of human oral potency.

The LC-MS 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, LC-MS can, with suitable equipment and training, be used as a direct replacement for the mouse bioassay in many existing □upernat management programs. The principal limitation of LC-MS is the high initial cost of capital equipment.

Implementation:
A single lab validation of the LC-MS method is now in progress. An AOAC collaborative study of the method is planned. The AOAC task force on marine biotoxin detection methods, led by Dr. James Hungerford, has identified validation of the LC-MS method as a high priority.

Validity:
The idea that the LC-MS provides a valid measure of toxicity of brevetoxin-contaminated shellfish arose from a systematic study of the fate of these toxins in the Eastern oyster, along with comparison of alternative methods to that of mouse bioassay of field samples. LC-MS and ELISA data correlated well with other, and with those of mouse bioassay. LC-MS provides unambiguous identification of brevetoxins, while other in vitro methods and

mouse bioassay cannot.

Some comparisons of the LC-MS method with:

Mouse bioassay:
The mouse bioassay gives a useful, approximate answer quickly and will reliably detect a dangerously toxic sample. However, LC-MS offers high specificity and is much more sensitive (by several orders of magnitude). Field studies in Eastern oyster have provided a useful approximation of the levels of toxin by LC-MS equivalent to the toxicity guidance level by mouse bioassay.

Immunoassays:
In field studies of Eastern oyster, LC-MS data were highly correlated with those of enzyme-linked immunoassay (ELISA). ELISA, as performed, measures a composite of brevetoxins present in the sample that share common structural features, while LC-MS offers a higher level of specificity. However, ELISA can be portable and performed by persons with little training, under field conditions.

Receptor binding assay (RBA):
RBAs are generally believed to reflect toxin potencies better than the structurally-based methods (LC-MS and ELISA). However, in field studies with Eastern oyster, mouse bioassay data were more highly correlated with LC-MS, compared with RBA. RBA also has the disadvantage of requiring the use of radioactive materials, which adds considerable costs. Appropriate procedures for the receipt, use and disposal of radioactive materials must be implemented to satisfy regulatory requirements.

Cost Information (if available): None

Action by 2007 Laboratory Methods Review Committee Recommended referral of Proposal 07-105 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 07-105.

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 07-105. Rationale: Additional information requested has not been submitted.

Action by 2009 Task Force I Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 07-105.

Action by 2009 General Assembly Adopted recommendation of 2009 Task Force I on Proposal 07-105.