

Proposal Subject: Receptor Binding Assay (rba) for PSP as a Type I NSSP Analytical Method

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

Text of Proposal/ Requested Action Adopt the receptor binding assay (rba) as a Type I NSSP analytical method for PSP testing for use as an alternative to and improvement over the AOAC mouse bioassay under NSSP Guidance Documents Chapter II.10 Approved National Shellfish Sanitation Program Laboratory Tests: Microbiological and Biotxin Analytical Methods.

An AOAC collaborative study is planned for the rba. Dr. Fran Van Dolah at NOAA will be the Principle Investigator. Dr. Van Dolah is nearing completion of a single lab validation, using a HOAc extraction, prior to submission to the Method 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 saxitoxins, the toxins responsible for Paralytic Shellfish Poisoning (PSP) in shellfish can cause illness and death in human consumers. Monitoring for PSP 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 paralytic shellfish poison (PSP) has served well since it was developed in the 1930s. The assay is relatively simple, quickly detects 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, more precise, 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 receptor binding assay (rba) for PSP provides an excellent alternative to the mouse bioassay, offering far greater sensitivity, greater accuracy, and a reliable measure of human oral potency. In the format developed at the NOAA/Charleston laboratory, it offers very high throughput.

The greater sensitivity of the rba will allow monitoring programs to detect the arrival of a PSP event earlier than is possible with the mouse bioassay. By providing more latitude between the detection limit and regulatory limit this will provide a higher level of assurance that growing areas can be closed before violative product is harvested and will also allow growers to get product out of the water while still safe in anticipation of a closure.

The rba 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 rba can, with suitable equipment and training, be used as a direct replacement for the mouse bioassay in many existing biotoxin management programs.

The principal limitation of the rba is that, in its current form, it requires the use of radioactive material. Although the amounts of radioactivity are miniscule and the risk negligible, appropriate procedures for the receipt, use, and disposal of radioactive materials must be implemented to satisfy regulatory requirements. This is a small cost, but must still be recognized. While efforts are underway to develop methods that have the benefits of the receptor assay without requiring radioactive materials, they have not advanced sufficiently to justify delaying implementation of the rba in its current format.

Implementation:

Progress in implementation of the rba has been greatly facilitated by the support of the International Atomic Energy Agency, which has funded several technical cooperation projects to assist developing nations in both setting up the rba and in establishing the necessary infrastructure to ensure that its employment will be useful. As a part of the IAEA program, Dr. Fran Van Dolah is planning an AOAC collaborative study of the rba. The AOAC task force on marine biotoxin detection methods, led by Dr. James Hungerford, has identified AOAC validation of the rba as a high priority.

Molecular basis for validity:

The idea that the rba was a fundamentally valid measure of toxicity of the saxitoxins to mammals arose from a systematic study of structure/activity relationships among carefully purified and characterized saxitoxins aimed at understanding the reasons for differences in observed toxicity to mice and, ultimately, the nature of the highly selective interaction with the binding site. In the course of this work it was found that the mouse intraperitoneal potencies of the various saxitoxins corresponded well with their binding affinities in the rba.

Some comparisons of the rba with:

Mouse bioassay:

The mouse bioassay gives a useful, approximate answer more quickly and will reliably detect a dangerously toxic sample, while the rba produces more results per day, can produce a large number of precise results much more quickly, and is much more sensitive. The limit of sensitivity for the rba is ca 0.5nM STX, vs 0.5 micromolar STX for the mouse bioassay. As usually applied, the rba is 10x to 100x more sensitive than the mouse bioassay.

Immunoassays:

The response spectrum of the rba is better matched to human oral potency than the immunoassays now available so, while the rba can be considered an accurate measure of human oral potency, the accuracy of an immunoassay depends on which toxins are present in the sample and may not accurately reflect toxicity to consumers. On the other hand, some immunoassays can be portable and can be performed by persons with little training, under field conditions.

HPLC, LC/MS:

Both methods are analyses, rather than assays, and thus determine the concentrations of individual toxins. This information can be vital for research and can be useful in regulatory applications. However, HPLC and LC/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, particularly for LC/MS.

**Cost Information
(if available):**

**Action by 2007
Laboratory
Methods Review
Committee**

Recommended referral of Proposal 07-106 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 07-106.
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 07-106. Rationale: Adequate data has not been submitted. If new data becomes available a new proposal will be submitted in the SLV format.
Action by 2009 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 07-106.
Action by 2009 General Assembly	Adopted recommendation of 2009 Task Force I on Proposal 07-106.
Action by USFDA 02/16/2010	Concurred with Conference action on Proposal 07-106.