



Interstate Shellfish Sanitation Conference  
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## MEMORANDUM

TO: Biotoxin Committee Members

FROM: Ken B. Moore, Executive Director *Ken B. Moore*

DATE: July 21, 2004

RE: 2004-2005 Committee Charges

This memorandum is to confirm your appointment by the Conference Executive Board Chairman to the Import Assessment Committee. Eric Thompson will serve as Committee Chairman.

The Biotoxin Committee is requested to continue providing guidance and recommendations to the ISSC Executive Board concerning biotoxin issues. Additionally, the committee is assigned the following tasks for 2004-2005:

- Proposal 03-105 Determining closed status of growing area containing toxin-forming organisms
- Proposal 03-106 Paralytic shellfish poison (PSP) standard for Geoduck
- Proposal 03-107 (in conjunction with Laboratory Quality Assurance Committee) Neurotoxic shellfish toxins (mouse bioassay) laboratory evaluation checklist
- Proposal 03-116 Rapid Methods for ASP and PSP detections – The Committee is requested to provide a status report on the use of the Jellett Rapid Test.

If you are unable to participate in the activities of this subcommittee, please contact us at 803-788-7559 or [issc@issc.org](mailto:issc@issc.org). Thank you for your interest and support of the ISSC and we look forward to working with you. Your Committee Chairperson will be contacting you soon.

/nsd

### 2004-2005 Biotxin Committee Roster

|                 |                         |  |
|-----------------|-------------------------|--|
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**PROPOSAL 03-105:**

**Specific Reference:**

Chapter IV. Shellstock Growing Areas @ .04 Marine Biotoxin Control. B. and C. Pages 55-56

**Text of Proposal/  
Requested Action:**

B. Marine Biotoxin Monitoring.

In those areas where and at those times when marine biotoxins are likely to occur in shellfish, representative samples of shellfish and/or water shall be collected during all harvest periods. Samples shall be collected from indicator stations at intervals determined by the Authority, and assayed for the presence of toxins and/or toxin-forming organisms in accordance with §C.

C. Closed Status of Growing Areas

- (1) A growing area, or portions thereof as provided in §A. (4), shall be placed in closed status for the taking of shellstock when the Authority determines that the level of biotoxin present in shellfish meats and/or the level of toxin forming organisms in the growing area is sufficient to cause a health risk. The closed status shall be established based on the following criteria:
  - (a) The concentration of paralytic shellfish poison (PSP) equals or exceeds 80 micrograms per 100 grams of edible portion of raw shellfish; or
  - (b) For neurotoxic shellfish poison (NSP), the harvesting of shellstock shall not be allowed when:
    - (i) ~~Any NSP toxin is found in shellfish meats~~ The concentration of NSP equals or exceeds 20 mouse units per 100 grams of edible portion of raw shellfish; or
    - (ii) The cell counts of ~~Gymnodinium breve~~ Karenia brevis organisms in the water column exceed 5,000 per liter.

**Public Health Significance:**

The Chapter IV. @. 04 B Requirements for Marine Biotoxin Monitoring does not allow for marine biotoxin hazard monitoring through the use of water sampling. However, it does refer to §C and §C contains a water quality criterion for NSP-forming *Gymnodinium breve* (now *Karenia brevis*) as well as a provision (IV. @. 04 C (2)) that "For any marine biotoxin producing organism for which criteria have not been established under this Ordinance, either cell counts in the water column or biotoxin meat concentrations may be used by the Authority as the criteria for not allowing the harvest of shellstock."

A number of states currently utilize monitoring of *K. brevis* cell counts in the water column rather than shellfish tissues and the FDA has accepted practice.

States that utilize water quality monitoring to assess the *K. brevis* hazard also utilize a variety of early warning systems, such as aerial surveillance, satellite imagery, interstate communication, and conditions conducive to proliferation of *K. brevis* to alert them to the impending occurrence of unacceptable concentrations of the organism. In some cases, sampling is conducted only when early warning systems indicate that unacceptable *K. brevis* concentrations are likely to occur rather than during "...all harvest period." The FDA has also accepted that approach.

Finally, there are circumstances in which the mouse bioassay for NSP results in the death of one or more mice and the laboratory reporting >20 mouse units per 100 grams of shellfish tissue. In practice, though this could indicate the presence of some NSP, growing areas have not been closed or held in closed status on the basis of mouse bioassay results unless mice die and a determination of  $\geq 20$  mouse units per 100 grams of shellfish tissue is made. That practice has been consistent with FDA advice.

Therefore, the following changes are suggested for the accompanying reasons:

1. Add "...and at those times when..." to IV. @ .04 B. as indicated in the proposed changes to allow for the use of early warning systems to initiate sampling as opposed to sampling during all harvest periods regardless of indications that a marine biotoxin hazard is not likely to occur.
2. Add "...and/or water..." and "and/or toxin forming

organisms..." to IV. @ .04 B. as indicated in the proposed changes to allow fro the use of the water sampling alternative.

3. Add "...and/or the level of toxin forming organisms in the growing area..." to IV. @ .04 C. (1) as indicated in the proposed changes to allow for scenarios in which water sampling rather than meat sampling is used.
4. Replace "Any NSSP toxin is found in shellfish meats" with "The concentration of NSP equals or exceeds 20 mouse units per 100 grams of edible portion of raw shellfish". In IV. @ .04 C (1) (b) (i) as indicated in the proposed changes to allow for circumstances in which there are some indications that some NSP toxin may be present but not enough to precipitate the "20 mouse units" determination.

The NSP method stipulates the reporting language to be used. The method can provide results of "toxin present less than 20 mouse units". The current manual requirement of no toxin present would result in areas remaining closed if toxin is present at less than 20 mouse units. The public health significance of toxin less than 20 mouse units is debatable. However, the low levels could increase closure period without affording any additional public health problems.

**Cost Information  
(if available):**

None submitted.

**ACTION BY 2003 TASK  
FORCE I**

Recommended referral of Proposal 03-105 to appropriate committee as determined by the Conference Chairman and requests that FDA share APHA guidance from 1989 discussions.

**ACTION BY 2003  
GENERAL ASSEMBLY**

Adopted recommendation of 2003 Task Force I.

**ACTION BY USFDA**

Concurred with Conference action.

**PROPOSAL 03-106**

**Specific Reference:**

Chapter IV Shellstock Growing Areas @ .04 Marine Biotoxin Control. C. (1) Page 56

**Text of Proposal/  
Requested Action:**

Amend Chapter IV. @ .04 C. (1) by adding the following new subsection:

**(d) The concentration of paralytic shellfish poison (PSP) equals or exceeds 200 micrograms per 100 grams of geoduck viscera tested.**

**Problem:**

Currently, geoduck fisheries occur in Washington State, British Columbia, and Alaska. Over the past ten years, the average annual harvest of these three areas has been 3,136,628 pounds, 4,283,227 pounds, and 204,60 pounds, respectively. Between 95-99% of this produce was sold live. The commercial fishery in Alaska has been in place since 1986. Most of the production has been processed and frozen with removal of the viscera. This frozen market is no longer economically viable.

The present standard for growing area certification for PSP, the mouse bioassay test, is based on results obtained from composite samples of three visceral balls. This is the only portion of the animal tests. The viscera ball reflects approximately 20% of the entire weight of the "edible portion" of the animal, and is the only section of the animal in which PSP has been found. This testing method (testing only the viscera) is a variation from the Model Ordinance for marine toxins, which requires the sample be composed of the entire mass of the edible portions of the shellfish.

Based on evaluation of consumption patterns, it has been established that the toxin concentration shall not equal or exceed 80 micrograms per 100 grams. We are proposing the geoducks have a species-specific toxin concentration level. For example, in geoducks, where only the visceral ball is sampled, the toxin concentration should be adjusted to reflect an appropriate proportion of the edible portion of the shellfish that the visceral ball represents.

Based on industry processing yields, the proportions are as follows: neck meat (siphon) 22%, body 18%, and visceral ball 10% of the whole animal by weight. These figures should be doubled to reflect their corresponding percentage of the edible portion of the raw shellfish (or percentage without shell). If only 20% of the edible portion of the shellfish is being used to determine the concentration of toxin, the standard of "80 micrograms per 100 gram sample of edible shellfish of raw shellfish" could be raised, for geoducks only, by a factor of five to 400 micrograms per 100-gram sample. Erring on the side of being conservative, a standard of 200 micrograms per 100-gram sample specifically based on the current method of testing geoduck viscera is justifiable.

**Public Health Significance:**

Evaluation of the historical data from Alaska (3210 lot samples from 1982 to 2000) indicates that if the PSP standard were raised to 200 microgram, 91% of the sample results would have certified product for live shipments. It has been demonstrated that when PSP levels in the geoduck viscera are at levels below 1000 micrograms per 100 grams of tissue, there is little or no accumulation in the body meat and siphon.

In Washington State, from the inception of the commercial fishery in 1970 until 1996 no PSP tests, or an insignificant number of PSP tests, were performed on geoducks for certification of growing area. This product was harvested and sold daily on a year-round basis for over 25 years. During that time, over 100 million pounds of geoducks were commercially harvested, sold, and consumed. Tens of millions of these pounds were harvested from all parts of Puget Sound and sold live. There has never been a reported case of illness or death from PSP associated with the consumption of geoducks. These facts are indicative of the reality that the viscera are not consumed. The current practice of testing only the viscera is not in compliance with the Model Ordinance, because it is not representative of the edible portion of this shellfish.

**Cost Information (if available):**

Live geoducks yield approximately \$2.00 - \$10.00 per pond, while eviscerated and frozen geoducks yield approximately 50¢ - \$1.00 per pound. The low price and demand for frozen geoducks make this market no longer economically feasible.

**ACTION BY 2003 TASK FORCE I**

Recommended the proposal be sent to Committee with instructions to provide a report at the 2005 Annual Meeting of the Conference.

**ACTION BY 2003 GENERAL ASSEMBLY**

Adopted recommendation of 2003 Task Force I.

**ACTION BY USFDA**

Concurred with Conference action.

**PROPOSAL 03-107:**

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| <b>Specific Reference:</b>                     | Chapter III Laboratory @ .01 Quality Assurance D. (1) Page 38  |
| <b>Text of Proposal/<br/>Requested Action:</b> | Chapter III@.01.D. (1) add the following to the end of the existing sentence (Laboratory evaluation criteria listed in Section IV Guidance Documents). The suggested NSP checklist is provided in the attached file. |
| <b>Public Health Significance:</b>             | An Nssp standardized NSP laboratory evaluation checklist will allow objective evaluation of laboratory conformance with Nssp requirements.   |
| <b>Cost Information (if available):</b>        | N/A  |
| <b>ACTION BY 2003 TASK FORCE I</b>             | Recommended that Proposal 03-107 be referred the appropriate committee as determined by the Conference Chairman.   |
| <b>ACTION BY 2003 GENERAL ASSEMBLY</b>         | Adopted recommendation of 2003 Task Force I.   |
| <b>ACTION BY USFDA</b>                         | Concurred with Conference action.  |

**PROPOSAL 03-116:**

**Specific  
Reference:**

NSSP Guidance Documents, Chapter II

**CONSTITUTION BY-LAWS and PROCEDURES of the INTERSTATE SHELLFISH SANITATION CONFERENCE**

PROCEDURE XVI. PROCEDURE FOR ACCEPTANCE AND APPROVAL OF ANALYTICAL METHODS FOR THE NSSP

And:

**NATIONAL SHELLFISH SANITATION PROGRAM  
2002 MODEL ORDINANCE WORKING DOCUMENT**

**III. LABORATORY**

**@. 02 Methods.**

C. Biotoxin. Methods for the analyses of shellfish and shellfish harvest waters shall be:

(1) The current AOAC and APHA methods used in bioassay for paralytic shellfish poisoning toxins;

**Text of Proposal/  
Requested Action:**

For many years, there has been an expression of need by regulatory agencies and industry to develop a non-animal PSP test to monitor PSP levels with precision and accuracy.

The method developed by Jellett Rapid Testing Ltd has been presented to the ISSC and other regulatory bodies over the past several years. In cooperation with individuals, governments and those organizations, the analytical method has been refined and improved. The Rapid Test kits have been tested in several states and foreign countries, and independent papers have been published attesting to the validity, reproducibility and reliability of these test kits.

The CONSTITUTION BY-LAWS and PROCEDURES of the INTERSTATE SHELLFISH SANITATION CONFERENCE allows the ISSC, through the Laboratory Methods Review Committee, to accept analytical methods that are sufficiently validated but are not AOAC or APHA methods. This is defined in the Constitution, PROCEDURE XVI. PROCEDURE FOR ACCEPTANCE AND APPROVAL OF ANALYTICAL METHODS FOR THE NSSP. Two possible reasons for considering a method are found in Subdivisions i and ii.

*Subdivision i. Meets immediate or continuing need;*

*Subdivision ii. Improves analytical capability under the NSSP as an alternative to other approved or accepted method(s)*

The need for alternative methods has been expressed by regulatory agencies, governmental organizations and industry for many years. The Jellett Rapid Test for PSP has been validated extensively to demonstrate its simplicity, reliability, precision and accuracy. As a result of ongoing improvements and demonstrations of efficacy, and the need that has been expressed by industry and state agencies, the Jellett Rapid Test for PSP is presented as a screening method for the NSSP as a Type III or Type IV method.

Please see attached list of supporting information.

Suggested wording:

C. Biotoxin. Methods for the analyses of shellfish and shellfish harvest waters shall be:

(1) The current AOAC and APHA methods used in bioassay for paralytic shellfish poisoning toxins;

(2) The Jellett Rapid Test for PSP may be used as a screening method for PSP toxins by regulatory and industry laboratories.

**Public Health  
Significance:**

Public Health Significance: *{Include appropriate documentation of both the problem and public health significance such as literature references or data. Inadequate information may result in a recommendation of "No Action" by the Issue Review Committee or Task Force.}*

Currently, only data from certified laboratories conducting PSP analyses using the Mouse Bioassay (MBA) are considered reliable and acceptable. Because of many significant constraints, in practical terms, this means that only state laboratories (in the US, governmental laboratories in other countries) can provide acceptable data at this time. However, acceptance of the Jellett Rapid Test for PSP would allow harvesters, processors, and regulatory agencies to screen for PSP with an accepted method that provides valid useable data.

The Jellett Rapid Test for PSP was developed over several years in answer to the oft-stated need for a rapid, reliable, non-animal analytical method that could be used to supplement the Mouse Bioassay.

Possible applications for The Jellett Rapid Test for PSP include:

- as a method of screening out negative samples in shellfish regulatory labs;
- as a harvest management tool at aquaculture facilities or in wild shellfish harvest areas (especially near shore areas) to determine if shellfish are free of PSP and safe to harvest;
- as a quality control tool for shellfish processing plants, distributors and wholesalers to ensure incoming shellfish are free of PSP toxins before processing or further distribution (this test could become part of the plant's HACCP program);
- as a tool for water classification for biotoxins;
- to assist in site selection for aquaculture activity;
- as a screening tool for toxic phytoplankton in seawater to provide an early warning for shellfish growers; and
- as a research tool for broad scale ecological monitoring.

The rationale for using the Jellett Rapid Test for PSP is that the kits provide a cost-effective screen (especially in low-volume laboratories) for PSP that can substantially reduce the need for live animal testing and the attendant care and disposal considerations. As a harvest management tool, the use of the Jellett Rapid Test for PSP will supplement regulatory agency efforts and help prevent the harvest of contaminated product. Having the ability to conduct tests using an accepted method will allow those processors who choose to use this test to demonstrate that they are truly controlling for PSP hazards in the harvested shellfish.

A simple, rapid, effective, reliable test, available to all harvesters, regulators, and processors, would increase the monitoring and reduce the chance that shellfish containing PSP toxins above the regulatory limit would be harvested or marketed.

**Cost Information  
(if available):**

Each test kit costs \$20. It has been reported that each analysis using the Mouse Bioassay costs approximately the same for a large-volume laboratory, but substantially more for small-volume laboratories. However, the costs cited do not take into account the costs associated with maintaining animal care facilities, proper disposal of the test animals, and the dangers associated with injecting live hand-held animals with toxic materials. In the worst case, it is no more expensive than the Mouse Bioassay.

**ACTION BY 2003 LAB  
METHODS REVIEW &  
LAB QA COMMITTEES**

Recommended adoption as a Type IV method with the following restrictions:

- i. Method can be used to determine when to perform a mouse bioassay in a previously closed area.
- ii. A negative result can be substituted for a mouse bioassay to maintain an area in the open status.

Recommended the Executive Board charge the appropriate committee with reexamination of this method at the 2005 Conference.

Recommended modifying Guidance Document Chapter II Growing Areas Table A. 10. Type III and Type IV Marine Biotxin Methods as follows: Insert Type IV under other for Growing Area Survey & Classification, shellfish with the following footnote: The Jellett Rapid Test for PSP.

**ACTION BY 2003  
TASK FORCE I**

Recommended adoption of Proposal 03-116 as amended by the Laboratory Methods Review Committee and Lab Quality Assurance Committee, with the addition of: iii. A positive result can be used for a precautionary closure.; and Lab Quality Assurance Committee including the checklist

**ACTION BY 2003  
GENERAL ASSEMBLY**

Adopted recommendations of 2003 Task Force I.

**ACTION BY USFDA**

Concurred with Conference action with the following comments:

FDA concurs with adoption of Proposal 03-116 to accept the Jellett rapid PSP test as a tool for states to use in the control of PSP in shellfish. However, several important caveats placed on use of the Jellett rapid PSP test were not clearly stated in the Summary of Actions. They are:

1. Use of the Jellett PSP test must be based on the currently approved procedure for PSP toxin extraction. No other extraction procedures have been validated and approved for use under the NSSP, including those provided by Jellett Rapid Testing Ltd.
2. When employing only the Jellett rapid PSP test to screen for saxitoxins, the Shellfish Authority shall make precautionary closures when positive results occur.

1. Revised submission text
2. FSA report
3. Mackintosh et al. 2002
4. Mackintosh and Smith 2002
5. Jellett et al. 2002a (Toxicon)
6. Jellett et al. 2002b (CWHMA)
7. Vale and Sampayo 2002
8. Silva et al. 2002
9. Rafuse et al. 2002
10. Rafuse et al. in press
11. Laycock et al. 2000
12. Cembella et al. 2002
13. Truman et al. unpublished
14. Instruction sheets from Jellett Rapid Test kits
15. Feedback from Rapid Test users

References for List of Attachments (numbers correspond)

2. FSA Project Code: B04006

FSA Project Title: Assessment and validation of a commercial rapid qualitative assay (MIST Alert™) for detection of amnesic and paralytic shellfish poisons in the UK monitoring programme and as an end-product test

3. Mackintosh, Fiona H., Susan Gallacher, Aileen M. Shanks and Elizabeth A. Smith 2002. Assessment of MIST Alert™, a Commercial Qualitative Assay for Detection of Paralytic Shellfish Poisoning Toxins in Bivalve Molluscs. *Journal of AOAC International* Vol. 85, No. 3: 632-641.

4. Mackintosh F.H. and E. A. Smith 2002. Evaluation of Mist Alert™ Rapid Test Kits For The Detection of Paralytic and Amnesic Shellfish poisoning Toxins in Shellfish. *Journal of Shellfish Research*, Vol. 21, No. 2: 455-460.

5. Jellett, Joanne F., Raymond L. Roberts, Maurice V. Laycock, Michael A. Quilliam, Richard E. Barrett. 2002. Detection of paralytic shellfish poisoning (PSP) toxins in shellfish tissue using MIST Alert™, a new rapid test, in parallel with the regulatory AOAC® mouse bioassay. *Toxicon* 40: 1407-1425

6. Jellett, J.F., Laycock, M.V., Belland, E.R., Bishop, P.C., Thériault, B.L., Roberts, R.L., Quilliam, M.A. and Cembella, A.D. 2001. Rapid toxin tests: MIST Alert™ for PSP and ASP. In: *Proceedings of the 7<sup>th</sup> Canadian Workshop on Harmful Marine Algae*. Edited by J.N.C. Whyte. *Can. Tech. Rep. Fish. Aquat. Sci.* 2386: 23-25.

7. Vale, Paulo, Maria Antónia de M. Sampayo. 2002. Evaluation of extraction methods for analysis of domoic acid in naturally contaminated shellfish from Portugal. *Harmful Algae* 1: 127-135.

8. Silva, M.A., Jellett, J.F., Laycock, M.V., Quilliam, M.A. and Cembella, A.D. 2001. Phytoplankton monitoring using a rapid field test: MIST Alert™ for Paralytic Shellfish Poisons. In: *Proceedings of the 7<sup>th</sup> Canadian Workshop on Harmful Marine Algae*. Edited by J.N.C. Whyte. *Can. Tech. Rep. Fish. Aquat. Sci.* 2386: 28-34.

9. Rafuse, Cheryl, Allan Cembella, Maurice Laycock, Joanne Jellett. 2001. Rapid Monitoring of Toxic Phytoplankton and Zooplankton with a Lateral-Flow Immunochromatographic Assay for ASP and PSP Toxins. In: Steidinger, K. (Ed) *Proceedings of the 10<sup>th</sup> International Conference on Harmful Algal Blooms* St. Petersburg, Florida. Oct 21-25, 2002. *Intergov. Oceanogr. Comm.*, Paris, in press.

10. Rafuse C., M.A. Silva, J.F. Jellett, and A.D. Cembella. 2001. Rapid Monitoring of Toxic Phytoplankton Using the MIST Alert™ for ASP and PSP Toxins at Aquaculture Sites in Atlantic Canada. *Aquanet Forum*. Dalhousie University Aug. 22, 2001.

11. Laycock, M.V., Jellett, J.F., Belland, Elizabeth R., Bishop, Pamela C., Thériault, Brigitte L., Russell-Tattie, Audra L. Quilliam, Michael A. Cembella, Allen D. Richards, Robert C. 2001. MIST Alert™: A Rapid Assay for Paralytic Shellfish Poisoning Toxins. *Proceedings of the 9<sup>th</sup> International Conference on Harmful Algal Blooms*, Hobart, Australia, 7-11 February 2000, Hallegraeff, G.M., Blackburn, S.I., Bolch, L.J., Lewis, R.J. (Eds.) *IOC of UNESCO*, 2001.

12. Cembella, A. D., M. A. Quilliam, N. I. Lewis, A. G. Bauder, C. Dell'Aversano, K. Thomas, J. Jellett and R. R. Cusack. 2002. The toxigenic marine dinoflagellate *Alexandrium tamarense* as the probable cause of mortality of caged salmon in Nova Scotia. *Harmful Algae*, Volume 1, Issue 3: 313-325