CODEX ALIMENTARIUS COMMISSION E

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World Health Organization

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Agenda Item 10 **CX/FFP 11/31/10**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON FISH AND FISHERY PRODUCTS

Thirty-first Session

Tromsø, Norway

11 – 15 April 2011

PROPOSED DRAFT LIST OF METHODS FOR DETERMINATION OF BIOTOXINS IN THE STANDARD FOR RAW AND LIVE BIVALVE MOLLUSCS

(Prepared by the Electronic Working Group led by Canada)

EXECUTIVE SUMMARY

Good progress has been made by the Electronic Working Group (E-WG) on several areas of the Proposed Draft List of Methods for the Determination of Biotoxins. There remain a number of items where wider discussion at the 31st Session of the Codex Committee on Fish and Fishery Products (CCFFP) needs to take place, specifically on the following:

- The home or location of the performance criteria / principles
- Whether new work is required to develop performance criteria/principles for screening methods
- The information to be included in the Standard versus the Code of Practice i.e should Appendix XI be removed from the Standard and added to the Code.
- Additional work on brevetoxin

BACKGROUND

1. At the 30th Session of the CCFFP (2009), the Committee agreed to establish an electronic Working Group on the Draft List of Methods for the Determination of Biotoxins for Live and Raw Bivalve Molluscs (2009, ALINORM 10/33/18, paras 69 - 80). The Committee also noted that it could not decide at this stage whether specific methods would be included or not in the Standard as this required further consideration, and that the scientific guidance from FAO/WHO would be considered in the process. The Committee recalled that at this stage only the method for saxitoxin was included in the Standard and that the methods in Appendix XI of ALINORM 08/31/18 had been listed for further discussion and would be reviewed by the E-WG to facilitate discussion at the next session. The electronic Working Group, coordinated by Canada, working in English, was given the following mandate:

- Develop proposed performance criteria/principles for analytical methods for Biotoxins, taking into account the criteria described in the Procedural Manual, the updated documentation prepared by the FAO/IOC/WHO Expert Consultation on the section on methods for Biotoxins analysis, existing criteria developed by the Codex Committee on Methods of Analysis and Sampling and other relevant documentation as appropriate.
- Assess the current methods against the proposed performance criteria/principles with a view to revising the Table in Appendix XI.
- Present a summary report of the work carried out by the E-WG along with recommendations to the Codex Committee on Fish and Fishery Products.

2. In May 2010, an invitation to participate to the E-WG was distributed to all Codex members. In addition to Canada, representatives from 21 countries registered to join the group. A complete list of the E-WG participants is included (refer to Annex I – List of Participants).

PURPOSE

3. This report outlines the E-WG's process in developing the revised proposed draft standard, its discussions and proposals that the Committee should consider in further discussing the Proposed Draft List of Methods for the Determination of Biotoxins in the Standard for Live and Raw Bivalve Molluscs.

PROCEDURE

Objectives of the E-WG, workplan and the first draft of the proposed performance criteria/principles

4. The proposed objectives, timeframe and the first draft document (including supporting technical information) were circulated in English to the E-WG on July 23, 2010. At the time of the deadline on September 3, 2010, representatives from eight (8) countries had submitted comments on the first draft.

Second draft document on the proposed performance criteria/principles:

5. The country comments were considered and a second draft document, as well as performance criteria/ principles for screening methods, were circulated on October 26, 2010 to the E-WG members. At the time of the deadline on November 10, 2010, seven (7) countries submitted comments on the second draft.

Third draft document (revised Appendix XI):

6. The country comments received were considered and a revised Appendix XI (ALINORM 08/31/18) was circulated to the E-WG members for comments on December 7, 2010. Seven (7) countries submitted comments on the revised Appendix XI.

The final report and the final revised proposed draft standard

7. The country comments were considered and the revised proposed draft standard was finalized. The final report was sent to the Codex Secretariat on March 10, 2011.

DISCUSSIONS

1st round of consultation

8. Several countries highlighted the fact that the Yessotoxin (YTX), Pectenotoxin (PTX) and cyclic imine (CI) toxins were not mentioned in the Appendices forwarded to the E-WG for comments. These toxins were not considered when developing the Appendices as they are not included in Appendix XI – Draft Standard for Live and Raw Bivalve Molluscs (ALINORM 08/31/18). Also, the Report of the Working Group Meeting to Assess the Advice from the Joint FAO/WHO/IOC ad hoc Expert Consultation on Biotoxins in Bivalve Molluscs (Ottawa, Canada, April 10-12, 2006) recommended that the Codex standard should not identify requirements for YTX, PTX and CI at that time because of the lack of evidence of human illness. As science evolves and data becomes available, this could be reassessed. If there is an interest to have YTX, PTX and CI included in the Codex Standard for Live and Raw Bivalve Molluscs, this should be raised to the Committee at the next session.

9. Some countries suggested that a separate set of performance criteria/principles should be developed for screening methods and be included in the Standard, in addition to the criteria/principles for Reference methods. Some E-WG members also recommended that specific definitions for screening methods versus reference methods be included in the Standard for clarity purposes. In light of the comments received by E-WG members, Canada drafted separate performance criteria/principles for screening methods based on the "Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programme Associated with the use of Veterinary Drugs in Food Producing Animals CAC/GL 71-2009" as suggested by Australia.

10. Several countries recommended that specific methods should not be referenced in the Standard for Live and Raw Bivalve Molluscs (i.e. removing Appendix XI). If a list of methods is deemed necessary to assist countries in finding the appropriate method to be used, a specific list for reference and screening methods could be included in the Code of Practice as long as the methods have met the criteria/principles identified in the Standard. This approach will ensure that the Standard allows for the use of a variety of

validated methods and does not need to be changed frequently to accommodate newly validated methods as scientific knowledge evolves rapidly in this area.

11. Several countries expressed some concerns with the feasibility of using Certified Reference Materials (CRMs) to assess trueness, particularly in the case of PSP toxins, as CRM's are not available for all analogues.

12. Some countries recommended including a statement that Competent Authorities should give preference to methods that do not utilize animals where validated alternatives exist.

13. Canada prepared a document detailing the method performance parameters which were developed using the guidelines found in the CODEX procedural manual and established by CCMAS. Consideration was given to the various toxin combinations, relative toxicities as well as the ability of various methods to separate the analogues. This document, intended to be an Appendix to the draft performance criteria/ principles to assist countries in the assessment of whether a method met the applicable criteria, was included with documents distributed to the E-WG for consideration.

2nd round of consultation

14. There was general agreement/consensus from the E-WG members that two sets of criteria/principles should be developed, one for screening methods and another set for reference methods. Based on the comments received, Canada developed an initial draft of criteria/principles for screening methods, which can be found in Annex III of this document. E-WG members recommended that the document be formatted such that the headings were consistent with the proposed criteria/principles for reference methods.

3rd round of consultation

15. Several countries noted that clarification as to the process used in assessing whether a method met the performance criteria/ principles would be useful, as there were a number of methods which were suggested for removal from the Table (as proposed in Appendix XI of ALINORM 08/31/18) (see Annex IV) as a result of not meeting the criteria/ principles.

16. Many countries pointed out that the proposed performance criteria/ principles eliminated the use of the mouse bioassay as a reference method despite this method's history of providing an acceptable level of consumer protection. The E-WG lead notes that the mandate of the work was to develop performance criteria/ principles for use by countries, to form a science-based understanding and common ground for discussion of methods should the need arise.

17. There was no clear consensus on the format of presentation of the methods, though in general countries were in agreement that the list of methods would be best placed in the Code of Practice.

18. The E-WG lead would note that due to the limited available information in the first two rounds of consultation, methods for Brevetoxin were not assessed and this is highlighted in the Table in Annex IV).

RECOMMENDATIONS

19. The Committee is invited to consider incorporating the general proposed criteria/ principles found in the document "Draft List of Methods for the Determination of Biotoxin in the Standard for Live and Raw Bivalve Molluscs" and accompanying "Method Performance Parameters for Marine Biotoxin Methods" for inclusion in the Standard (Annex II).

20. The Committee should consider relocating the Table previously proposed for inclusion in section I-8.5 of the Standard (see Appendix XI, ALINORM 08/31/18) to an appropriate location in the Code of Practice on Fish and Fishery Products. The Table could be considered as a list of methods, and could include a footnote advising that the methods not meeting the performance criteria/ principles for reference methods may be used by countries for monitoring and screening purposes (refer to Annex IV). In addition, the Table could be provided to the Codex Committee on Methods of Analysis and Sampling with a request to review any additions with respect to Type of Methods.

21. The Committee may wish to consider the comments by E-WG members regarding the development of similar performance criteria/ principles for screening methods (Annex III) as future work.

Annex I

List of Electronic Working Group Participants

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***** - "Active" E-WG countries which have replied at least once by providing comments or by indicating to the E-WG that they had no comments.

Annex II

E-WG –Draft Performance Criteria / Parameters for Methods for the Determination of Biotoxins in the Standard for Raw and Live Bivalve Molluscs

Background

As scientific knowledge evolves rapidly in the area of biotoxin methods, it is understood that a list of very specific methods may become out of date. In view of the difficulties this would present, described below are the proposed general performance criteria and principles for reference methods[1] that can be used by competent authorities to select methods that are adequate for monitoring biotoxins for regulatory purposes. Preference should be given to methods that have applicability for routine use.

Prior to selection of a potential method of analysis for biotoxins, each competent authority must have knowledge concerning the relative hazard presented by the toxins present in their territorial waters. This includes a 'farm to fork' understanding which includes the algae that contribute to the toxin formation, toxin analogues generally or usually present in the shellfish (at a minimum) and in the source organism (where possible) from their territorial waters, the bivalve species impacted and the mechanisms by which the toxins impact human health.

Competent authorities considering the use of a particular method may utilize a screening method as a complement to reference methods to gain efficiencies for routine biotoxin monitoring. Competent authorities should evaluate their entire biotoxin testing strategy against the performance criteria outlined herein.

General proposed performance criteria/principles:

General method principles and performance criteria (General Criteria) are outlined in the *CODEX Alimentarius Commission PROCEDURAL MANUAL, 19th ed.* document (ISBN 978-92-5-106493-1) in the PRINCIPLES FOR THE ESTABLISHMENT OF CODEX METHODS OF ANALYSIS section. Analytical terms are further defined in the CODEX document Guidelines on Analytical Terminology (CAC/GL 72-2009). The competent authority is advised to refer to these documents when considering the following Marine Biotoxin method principles and criteria.

The following Marine Biotoxin principles and method criteria are a specific application of the General Criteria. The Marine Biotoxin principles and method criteria, outlined in the Table *Appendix I: Method Performance Parameters for Marine Biotoxin Methods*, are to be considered by the competent authority to be inclusive of analytic approach.

(a) Selectivity

(i) Group specific i.e., the method used should be applicable to the appropriate toxin group it is testing.

(ii) Preference should be given to methods that can be used to test multiple toxin analogues and, when applicable, multiple toxin groups.

(b) Trueness and Recovery

(i) Group trueness i.e., differences in recovery may exist but is acceptable if the overall trueness (to estimate toxicity) is correct.

(ii) Preference should be given to methods that minimize bias and have minimized recovery corrections.

(c) Precision

[1] Reference method: Quantitative analytical method of proven reliability characterised by well-established trueness, specificity, precision and detection power. These methods generally haven't been collaboratively studied and are usually based on molecular spectrometry. The reference method status is only valid if the method is implemented under an appropriate QA regime. (Guidelines on Good Laboratory Practice in Residue Analysis CAC/GL 40-1993, Rev.1-2003).

(i) Methods that have undergone inter-laboratory or collaborative studies based on internationally recognized protocols (such as AOAC International or Codex GL 64) are preferred.

(ii) Consideration should be given to intra-laboratory or single lab validation studies, using internationally accepted validation protocols or guidelines, which may have been published in peer reviewed journals.

(d) Detection Capability

(i) Methods should be sufficiently capable to detect the named biotoxin components at the performance limits outlined in Appendix I.

(ii) Preference should be given to methods with detection limits less than in (i) thereby providing an early warning.

(e) Quantification

(i) Methods that detect groups of analogues should be capable of estimating total toxicity.

(ii) Preference should be given to methods that can provide biotoxin profile information and should be given to methods that can provide quantitative information.

(f) Scope

(i) The relative toxicity of structural analogues should be considered when determining method performance requirements. Preference should be given to methods that express the values in terms of relative toxicity.

(ii) Preference should be given to methods that detect a greater number of biotoxin analogues within a particular group.

(g) Measurement Uncertainty

(i) The measurement uncertainty associated with all analytical results should be estimated.

Method performance parameters for marine biotoxin methods

Group	Toxin		Units	Maximum Level	Minimum Range	Limit of Detection	Limit of Quantification	Precision at ML	Recovery[a,b]	Trueness
Saxitoxin Group	Total Toxicity		mg STXdiHCl eq/kg[c]	0.8	0.26 - 1.34	0.08	0.16	#44%	70-120	CRM
	Saxitoxin	STX			0.04- 1.34	0.04	0.08			
	Neosaxitoxin	NEO			0.04- 1.34	0.04	0.08			
	Decarbamoyl-saxitoxin	dcSTX			0.04- 1.34	0.04	0.08			
	Decarbamoyl-neosaxitoxin	dcNEO			0.04- 1.34	0.04	0.08			
	Gonyautoxin-1	GTX1			0.04- 1.34	0.04	0.08			
	Gonyautoxin-4	GTX4								
	Gonyautoxin-3	GTX3			0.04- 1.34	0.04	0.08			
	Gonyautoxin-2	GTX2								
	Gonyautoxin-5	GTX5			0.04- 1.34	0.04	0.08			
	Gonyautoxin-6	GTX6			0.04- 1.34	0.04	0.08			
	Decarbamoyl-gonyautoxin-2	dcGTX2			0.04- 1.34	0.04	0.08			
	Decarbamoyl-gonyautoxin-3	dcGTX3								
	N-sulfocarbamoyl-gonyautoxin-2	C1			0.04- 1.34	0.04	0.08			
	N-sulfocarbamoyl-gonyautoxin-3	C2								
Domoic Acid Group	Domoic Acid	DA	mg DA/kg	20	13.2 - 26.8	2	4	#22%	85-110 %	CRM
	epi-Domoic Acid	epiDA		Method should detect this analyte						
Okadaic Acid Group	Total Toxicity		mg OA eq/kg	0.16	0.05 -0.27	0.016	0.032	# 44%	70-120	CRM
	Okadaic Acid	OA			0.01-0.27	0.01	0.03			CRM
	Dinophysistoxin-1	DTX1			0.01-0.27	0.01	0.03			CRM
	Dinophysistoxin-2	DTX2			0.01-0.27	0.01	0.03			
	Esters of OA, DTX1 and DTX2	FA-ESTERS		Method should detect this analyte directly or after hydrolysis						
Azaspiracids Group	Total Toxicity		mg AZA1 eq/kg	0.16	0.05 -0.27	0.016	0.032	#44%	70-120	CRM
	Azaspiracid-1	AZA1			0.01 -0.27	0.01	0.03			
	Azaspiracid-2	AZA2			0.01 -0.27	0.01	0.03			
	Azaspiracid-3	AZA3			0.01 -0.27	0.01	0.03			
Brevetoxin Group	Total Toxicity		mg/kg PbTx-2 eq	0.8	0.26 - 1.34	0.08	0.16	#44%	70-120	CRM
	Brevetoxin-1	BTX1								
	Brevetoxin-2	BTX2								
	Brevetoxin-1 derivatives[d]	devBTX1			0.01 -0.27	0.01	0.03			
	Brevetoxin-2 derivatives[d]	devBTX2			0.01 -0.27	0.01	0.03			

Annex III

E-WG – Draft list of Proposed Performance Criteria/Principles for Screening Method for the Determination of Biotoxins in the Standard for Raw and Live Bivalve Molluscs

- Screening methods can be either qualitative or semi-quantitative. The focus of screening methods is to distinguish between samples with no detectable toxins above a threshold value (i.e., a negative sample) and those with a toxicity above the level (i.e., positives).
- Attention to the establishment of threshold values based on statistical treatment of false positive and false negatives is necessary for screening methods.
- Sensitivity for a screening method is defined as the lowest concentration at which a target analyte may be detected reliably based on established statistical limits.
- Sensitivity of the method for all analogues in the specific toxin group being measured must be known.
- Selectivity of a screening method refers to the ability of the test: i) to determine that samples producing a negative response are truly negative; and ii) to distinguish the presence of a target compound or class of compounds from other substances in the sample. Screening method, which are often based on microbiological growth, immunoassays or chromogenic response, do not unambiguously identify a compound and thus, the selectivity may be increased when it is used in combination with a separation technique prior to detection. The selectivity rate should be shown at a confidence rate of a minimum of 90%. A false negative rate less than 5 % at a level of ½ MRL is recommended and there should be no false negatives at the MRL.
- Upon establishing confidence in the method in terms of selectivity, cross reactivity testing must be determined. Blank matrix fortified with other toxins and structurally related compounds possibly found in samples, should be tested to establish that negative results are obtained when test materials contain these other compounds. Responses should be negative when these compounds are present at concentrations that might reasonably be expected to be present in a sample.
- The “cut-off” or threshold value for the test for a particular compound or class of compounds is established by conducting concentration-response experiments.

Annex IV

LIST OF METHODS FOR DETERMINATION OF BIOTOXINS (DRAFT STANDARD FOR LIVE AND RAW BIVALVE MOLLUSC SECTION I-8.5 DETERMINATION OF BIOTOXINS)

Provision	***Methodology***	***Principle***	***Type***
Saxitoxin Group	*AOAC International Mouse Bioassay*	~~*AOAC Mouse Bioassay*~~[1, a]	~~*III*~~
	*	*Radiolabelled Receptor Binding Assay(Immunochemical)*[2]	*III*
	*	~~*Immunochemical*~~[f]	*III*
	*	*LC-MS*[3, d]	*III*
	*	~~*AOAC LC precolumn oxidation-fluorescence*~~[4, 5, b]	
	*	*LC-post column oxidation-fluorescence*[6,7, b]	
	*	~~*Abraxis PSP ELISA (Immunochemical)*~~[8, b]	
	*	~~*JRT lateral flow immunoassay (Immunochemical)*~~[9, b]	
	*	~~*Surface plasmon resonance (Immuochemical)*~~[10 , b]	
Okadaic Acid Group	*	*LC-MS*[11, 24, d]	*II*
	*	~~*Mouse Bioassay*~~[12, a, c, d]	~~*III*~~
	*	~~*Rat Bioassay*~~[12, b]	
	*	~~*PP2A*~~[d, e]	~~*III*~~
	*	*Fluorescence PP2A phosphatase inhibition assay*[13, b]	
	*	*Recombinant PP2A phosphatase inhibition assay*[14, b]	
		~~*LC FL*~~[15, a]	*III*
	*	~~*ABRAXIS DSP ELISA*~~[16, a, d]	~~*III*~~
	*	~~*JRT Lateral flow immunoassay*~~[17, b]	
Domoic Acid Group	*Quilliam LC-UVD method*	*LC-UV (acid extraction)*[18]	*II*
	*	*LC-UV (aqueous methanol extraction)*[19]	
	*	*Biosense ELISA*[20, 21]	*III*

[a] *Method that was listed in Appendix XI but did not meet the proposed criteria/principles for reference methods.*
[b] *Method that was not previously listed in Appendix XI but was recommended for consideration by E-WG members.*
[c] *When using the MBA for detecting lipophilic marine biotoxins, false positives may occur due to the presence of other substances such as YTX, PTX and CI, which are not known to cause human illness. When false positives are suspected, confirmatory testing, using an internationally validated method, can be carried out in order to identify the type(s) of marine biotoxins present.*
[d] *Further method development (e.g. interlaboratory validation, CRM availability) needed prior to submission for endorsement by CCMAS*
[e] *PP2A removed from list of methods and replaced by Fluorescence phosphatase inhibition assay (PP2A) and Recombinant phosphatase inhibition assay (PP2A)*
[f] *Immunochemical removed from the list of methods and replaced by Radiolabelled Receptor Binding Assay (Immunochemical), Abraxis PSP ELISA (Immunochemical), JRT lateral flow immunoassay (Immunochemical) and Surface plasmon resonance (Immuochemical)*
** Official/recognized method title to be identified*

~~*Strikeout*~~*: Is used to identify existing Appendix XI methods or methods proposed by the E-WG which were determined not to meet the proposed performance criteria/principles for reference methods.*

Note: Additional methods meeting the performance criteria/ parameters likely exist but were not proposed, considered or evaluated as part of this exercise. Other methods exist which may be suitable for screening or monitoring purposes.

Provision	***Methodology***	***Principle***	***Type***
	*	*LC-MS* [11]	*III*
	*	~~*LFIC (JRT Lateral flow immunoassay)*~~ [~~9, a, d~~]	~~*III*~~
	*	*Surface plasmon resonance*[22]	
Brevetoxin Group	*	*LC-MS*[d]	*II*
	*	*ELISA*[d]	*III*
	APHA mouse bioassay	*bioassay*[c]	*III*
Azaspiracid Group	*	*LC-MS* [11, 24, d]	*II*
	*	~~*Mouse bioassay*~~[~~23, a, c~~]	~~*III*~~
		~~*Rat bioassay*~~[~~23, b~~]	

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