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World Health Organization

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Agenda Item 8 **CX/FFP 12/32/8**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON FISH AND FISHERY PRODUCTS

Thirty-second Session

Bali, Indonesia

1 – 5 October 2012

REPORT OF THE ELECTRONIC WORKING GROUP ON THE PROPOSED DRAFT PERFORMANCE CRITERIA FOR SCREENING METHODS FOR MARINE BIOTOXINS IN THE STANDARD FOR RAW AND BIVALVE MOLLUSCS

Prepared by the Electronic Working Group led by Canada

EXECUTIVE SUMMARY

Productive comments and suggestions from many countries were received regarding the tasked objectives for this group. The Electronic Working Group (e-WG) suggests that further discussion at the 32nd Session of the Codex Committee on Fish and Fishery Products (CCFFP) should occur regarding the path forward (including the need) for continuation of this work as the wide range of comments received did not allow for consensus.

The e-WG would like to draw the attention of the CCFFP to the discussion points (see paragraphs 21, 28, 35 and 40) and the recommendations in paragraphs 41 and 42.

BACKGROUND

1. At the 30th session of the CCFFP, the Committee tasked an e-WG, led by Canada, to develop method performance criteria for marine toxins which could be used by governments to select adequate methods for regulatory monitoring.

2. At the 31st Session of the CCFFP (2011), that e-WG tabled a report and proposed method performance criteria/principles for the determination of biotoxins for inclusion in the Standard (Appendix II of CX/FFP 11/31/10). The Committee agreed that as the working document had not been circulated for comments due to lack of time, and as some delegations indicated that they needed to consult with their experts at the national level, the proposed draft performance criteria would be circulated at Step 3 for comments and consideration by the next session.

3. The Committee also agreed with the proposal of the eWG to consider performance criteria for screening methods, for inclusion in the Code of Practice for Fish and Fishery Products.

4. The Committee agreed with the purpose of the new work, as indicated in the "*Proposal for new work for developing performance criteria/parameters for screening methods for determination of biotoxins in the standard for raw and live bivalve molluscs*", and noted that the complete project document would be prepared by Australia and Canada for submission to the 66th Session of the Executive Committee and the 34th Session of the Commission.

5. The Committee agreed that, subject to approval of new work, an electronic working group led by Canada, working in English, would be established with the following mandate:

- Propose criteria/parameters for screening methods for Biotoxins in the Standard for Live and Raw Bivalve Molluscs.
- Consider whether the criteria developed for both Reference and Confirmatory and Screening methods should reside in the Code of Practice and, if so, be applied to other commodities covered by the CCFFP for which biotoxin requirements apply.
- Present a summary report of the work carried out by the e-WG along with recommendations to the 32nd session of the CCFFP.

6. The proposal for new work was approved by the 34th Session of the Commission (July 2011).

7. An invitation to participate in the e-WG was sent to all Codex members and observers in October 2011. In addition to Canada, seventeen (17) countries and the European Union expressed interest in participating. A complete list of the e-WG participants is included (refer to Appendix I – List of Participants).

PURPOSE

8. This report outlines the e-WG's process to address the objectives outlined in the new work as follows:

a) Consider the current Codex definition of Screening Methods as given in the 'Codex Veterinary Drug Residues in Food Glossary', to determine if it is appropriate in this context, and if not, develop a satisfactory definition.

b) Develop Draft Performance Criteria/Principles for Screening Methods for Biotoxins in the Standard for Live and Raw Bivalve Molluscs, taking into account the criteria developed in the "Draft Performance Criteria/Parameters for Reference and Confirmatory Methods for the Determination of Biotoxins in the Standard or Raw and Live Bivalve Molluscs".

c) Determine whether the criteria developed for Reference and Confirmatory methods and Screening methods for raw and live bivalve molluscs can be applied to other commodities covered by the CCFFP.

d) Consider whether the criteria developed for both reference and confirmatory and screening methods should reside in the Code of Practice.

9. It also summarizes the discussions and comments within the e-WG, and identifies recommended next steps for the Committee to consider in further discussing the performance criteria for both screening and reference/confirmatory methods for marine biotoxins in the Standard for Live and Raw Bivalve Molluscs.

PROCEDURE

10. The objectives and proposed workplan (process and time frames) were circulated to the e-WG in December 2011.

First Discussion Document:

11. On January 27, 2012 Canada, as lead of the e-WG, shared a discussion document on screening methods with the e-WG for review and comment. This document was prepared in consideration of the key objectives (noted above), as well as relevant country comments specific to screening methods provided by the previous e-WG (on reference methods).

12. The document prompted the e-WG for comments on (i) three proposed definitions for screening methods and (ii) proposed performance criteria (and any appropriate reference documents which could be useful for further drafting of parameters for screening methods). With respect to Objectives III and IV, the document sought general comments and ideas regarding the application

of these performance criteria to other commodities under CCFFP and where these criteria should reside. Comments from 9 countries and the EU were received.

Second draft:

13. Canada, with the substantive assistance of Australia, considered the e-WG comments and prepared a second discussion document. This included revised text for a screening method definition as well as revised text for criteria (Objectives I and II). It also presented options for consideration regarding applicability and preferred location of the criteria (Objectives III and IV). The document was circulated to the e-WG members for comments on April 5, 2012. Comments from 7 countries were received.

The final report:

14. All country comments were considered and key points are reflected in the summary of discussions below. A draft final report was shared with the e-WG on June 12, 2012 and feedback was received..

DISCUSSIONS

General comments:

15. A number of countries provided general comments on the approach taken for criteria for reference methods. These comments highlighted the significant challenge in developing criteria for screening methods while there remains considerable questions and discussion on the reference/confirmatory method criteria. One country suggested that the e-WG recommend continued work on both reference and screening criteria be done together though a physical working group

16. In both rounds of consultation, the subject of the mouse bioassay method was raised, specifically as to whether it meets reference method criteria, its history of use, and consideration of ethical/legislative commitments.

17. In terms of location of screening method criteria, one suggestion among others was that a separate Codex guidance document be developed on overall biotoxin control strategies. This could include a section on screening method criteria, as well as provide educational guidance on other aspects of marine biotoxin control programs (e.g. early warning methods, harvest area opening and closing strategies, and labeling and distribution controls, sampling strategies, sentinel species, plankton monitoring etc.).

Objective I: Definition of Screening Method

18. In considering the three definitions presented in the first discussion document[1], e-WG comments allowed for the drafting of a new definition which borrowed from the preferred elements of each. This definition is as follows:

> *[Biotoxin] screening method: A qualitative or semi-quantitative method of proven reliability characterized by well-established detection of the presence of an analyte or class of analytes at the level of interest. These methods generally (i) have the capability for a high sample throughput, (ii) are relatively rapid, and (iii) are designed to avoid false compliant results.*

19. There was an additional suggestion to use an existing definition for screening methods provided in the *Codex Guidelines on Good Laboratory Practice in Pesticide Residue Analysis (CAC/GL 40-1993)*[2].

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[1] The first definition was from the "*Codex Veterinary Drugs Residues in Foods Glossary*", the second from the EU Comission Decision 2002/657 [1], and the third was a newly drafted alternative combining elements of these and the existing definition for Reference Methods in the "*Guidelines on Good Laboratory Practice in Pesticide Residue Analysis*" (CAC/GL 40-1993).

[2] " A method used to detect the presence of an analyte or class of analytes at or above the minimum concentration of interest. It should be designed to avoid false negative results at a specified probability level (generally β = 5%). Qualitative positive results may be required to be confirmed by confirmatory or reference methods. See Decision Limit and Detection Capability"

20. In conclusion, the definition from the 'Codex Veterinary Drug Residues in Food Glossary' was not considered appropriate, while the definition noted above was generally supported by most commenting countries.

21. Discussion points for the CCFFP:

- Does the CCFFP support the definition identified above for screening methods?
- If not, what additional guidance can the CCFFP provide to advance this aspect of the work?

Objective II: Criteria

22. The first round of consultation resulted in considerable discussion on the draft performance criteria and parameters for screening methods. With respect to the criteria proposed, the sections on 'Selectivity' and 'Quantification' were felt to be appropriate. The section on 'Probability of Detection / Detection Capability' prompted several country recommendations for text regarding false negatives and threshold values.

23. One country suggested that the criteria format (which borrowed from the reference method criteria) may not be suitable for screening method criteria and that such criteria could be better reflected as an element of a separate comprehensive guidance document on overall marine toxin harvest control strategies. Another country supported the development of such a guidance document and further suggested that this document could include both reference and screening criteria.

24. With respect to the general approach for drafting performance parameters for screening methods, several countries provided suggestions. A number of reference documents were proposed as being potentially relevant in contributing to the parameters, including those related to AOAC INTERNATIONAL's International Stakeholder Panel on Alternative Methods (ISPAM) Working Group on Qualitative Chemistry Guidelines and the *Codex Guidelines on Good Laboratory Practice in Pesticide Residue Analysis (CAC/GL 40-1993).*

25. In the second round of e-WG consultation, a revised version of performance criteria for screening methods was considered (incorporating comments received) and a table elaborating method performance parameters for the toxins listed in the Standard was also included. Additional changes to the revised criteria were proposed by several countries. The comments provided were comprehensive and varied (Refer to Appendix II) ..

26. With respect to the Table 1, consisting of specific method performance parameters for each toxin group, two countries suggested that Table 1 be removed as the information can be readily calculated using the "detection capability" criterion in the proposed performance criteria and the toxin limits in the Standard. It was also suggested that CCMAS be consulted on Table 1 to ensure that the method performance criteria comply with CCMAS guidance and are fit for purpose. It should be noted also that substantial comments to the Table drafted in the previous work on Reference Method Performance Parameters, specifically on recovery values for the various toxins, were provided by one commenting country for further consideration

27. In conclusion, while there seems to be general agreement on the five main headings for criteria, the variety and scope of the comments received pertaining to the criteria demonstrated that there is continued interest among e-WG member countries in having the elements for each of the criteria for screening methods fully elaborated and well defined. More time is required to reconcile the various comments. This reinforces the earlier recommendation from the electronic working group on reference/confirmatory methods regarding the need for screening method criteria.

28. Discussion points for the CCFFP:

- Given the diverse comments received, does the CCFFP have any additional comments to offer on the criteria, and guidance on how best to proceed with this aspect of the work?

- Are specific screening method performance parameters necessary? If so, do the parameters outlined in Table 1 provide a useful basis for discussions, and what guidance can CCFFP provide for advancing the work (e.g. continued discussion, consultation with CCMAS, etc.)?

Objective III: Applying criteria to other commodities under CCFFP

29. When elaborating provisions in Codex standards, the e-WG considered the guidance outlined in the Codex Procedural Manual [3] which states "At Step 4, commodity committees should discuss and report to the Committee on Methods and Analysis and Sampling (CCMAS) on matters connected with, provisions in Codex standards which require analytical or statistical procedure.

30. In this context, any guidance regarding methods of analysis (e.g. criteria) should only be needed in a Codex fish standard when that standard contains a provision (i.e. Maximum Limit) that will require an analysis to facilitate compliance to the standard.

31. There are currently three Codex fish standards (adopted or under development) which have established (specifically or by reference) "Maximum Level" provisions for biotoxins for which analysis would be needed. They are:

- Standard for Live and Raw Bivalve Molluscs (CODEX STAN 292-2008) - Sections I-5.2 and II-5 Contaminants
- Draft Standard for Quick Frozen Scallop Adductor Muscle Meat (at Step 6) (REP11/FFP, Appendix VII) & CL 2011/15-FFP - Section 5.2
- Draft Standard for Fresh/Live and Frozen Abalone (*Haliotis* spp.) (at Step 6) (REP11/FFP, Appendix X). & CL 2011/15-FFP - Sections I-5.2 and II-5 Contaminants"

32. Consideration was given to limiting the scope of future discussions on this objective to the three Codex fish standards listed above. Furthermore, regarding the latter two standards under development, a simple drafting approach for the respective analysis sections could be to refer to the analysis section of the Codex Standard for Live and Raw Bivalve Molluscs.

33. One country commented that the application of the criteria to other commodities should be considered if the Competent Authority has, through risk assessment, deemed it necessary to apply risk management strategies for marine toxins in those species.

34. In conclusion, the outcome of discussion among commenting countries pointed to a general agreement that it is premature to recommend that the criteria be applicable to other commodities beyond those included in the Codex Standard for Live and Raw Bivalve Molluscs. Further discussion on this matter could continue once agreement has been reached on Objectives I and II.

35. Discussion points for the CCFFP:

- Does the CCFFP agree this aspect of the work be put on hold pending resolution of the issues identified for work under Objectives 1 and 2?
- If not, what guidance can CCFFP provide for advancing the work (e.g. continued discussion, other steps)?

Objective IV: Location of the criteria

36. In the first round, countries deferred providing any comments on the preferred location of the criteria, advising that it was perhaps premature to make a recommendation on that objective.

37. In the the second round, the e-WG members were asked to provide comment on three options for location of criteria:

[3] Codex Alimentarius Commission - Procedural Manual, 20th Edition, Section II: Elaboration of Codex Texts, Relations Between Commodity Committees and General Subject Committees: Methods of Analysis and Sampling (p. 48)

a. Locate the criteria in the Standard.

b. Locate the criteria in the Code of Practice.

c. Defer discussion on the location of the criteria, pending further progress on the matters of principle

38. There was support for including reference method criteria in the Standard. The feedback regarding screening method criteria was varied. There was support to put the screening method criteria in the Code or in an annex to the Standard, while one country supported housing the screening method criteria within the Standard. Two countries felt it was premature to agree on criteria location until discussion on other matters of principle have progressed.

39. In conclusion, there was general support to house the reference method criteria in the Standard. Further discussion is needed on the location of screening method criteria.

40. Discussion points for the CCFFP:

- Does the CCFFP agree to put the reference method criteria in the Standard?
- Does the CCFFP have a preference regarding the future location of the screening method criteria:
    - in the Code of Practice, or
    - in an Annex to the Standard, or
    - in the Standard, or
    - in a new independent Codex document on marine biotoxin control strategies for regulatory authorities, or
    - to defer the decision on this point pending further discussion.

RECOMMENDATIONS

41. That the Committee consider the e-WG Report as prepared by Canada, and discuss the specific points identified in the body of this report with a view to providing answers to guide further work.

42. If, as a result of the discussions suggested above, the Committee decides to continue the work on criteria for both screening and confirmatory methods, the e-WG recommends that both sets of criteria be discussed in parallel under the same initiative with countries engaging their CCMAS counterparts as appropriate on technical and procedural matters.

Appendix I

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Appendix II

Performance Criteria and Parameters for Screening Methods
The draft text discussed in the e-WG and the country comments submitted during the e-WG discussions

For Information

[Background

Screening methods are being increasingly used for regulatory marine biotoxin management in a number of countries. Due to the ease of implementation, cost-effectiveness and rapid turn-around-times, screening methods provide an attractive option for intensive marine biotoxin monitoring programmes. Given the widespread use of screening tests as front line marine biotoxin management tools, it is imperative that these methods are fit for purpose and meet specified method performance criteria to ensure the suitability of bivalves and other shellfish for human consumption. Competent authorities considering the use of a particular screening method should utilise a confirmatory or reference method as a complement to more accurately determine levels of marine biotoxins in positive samples.

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 for screening methods that can be used by competent authorities to select methods that are adequate for routinely monitoring biotoxins for regulatory purposes. Competent authorities should evaluate potential screening methods against the performance criteria outlined herein.

General Proposed Performance Criteria for Marine Biotoxin Screening Methods

General method principles and performance criteria (General Criteria) are outlined in the Codex Alimentarius Commission PROCEDURAL MANUAL, 20th ed. document (ISBN 978-92-5-106821-2) in the PRINCIPLES FOR THE ESTABLISHMENT OF CODEX METHODS OF ANALYSIS section. The competent authority is advised to refer to this document when considering selecting a marine biotoxin screening method based on this criteria approach. The marine biotoxin method criteria, outlined in Table I: Method performance criteria for marine biotoxin screening methods, are to be considered by the competent authority to be inclusive of methods such as Lateral flow immunochromatography and ELISA.

a) Selectivity

i. Selectivity of a screening method refers to the ability of the test to distinguish the presence of a target compound or class of compounds from other substances in the sample. Screening methods (which are often based on microbiological growth, immunoassays or chromogenic response) may 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.

ii. Test method cross reactivity should be investigated in validation studies prior to implementing the method. 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. Also the response for the different

marine biotoxin group analogues should be well understood and relative to the response of calibrant standards.

b) Precision

i. Preference should be given to methods that have undergone full collaborative inter-laboratory studies. Single lab validation studies must be in accordance with internationally recognized protocols (e.g. those referenced in the harmonized IUPAC Guidelines for Single–Laboratory Validation of Methods of Analysis).

c) Detection capability

i. Screening methods should have false negative rates which are less than 5 % at a level of half the maximum allowable level and there must be no false negatives at the maximum level (Table 1).

ii. The limit of detection (LoD) of the screening methods should be such that they reliably detect (in at least 95% of samples) the biotoxin components of interest at half the maximum level (Table 1).

iii. Where the test is quantitative or semi-quantitative, preference should be given to methods with detection limits less than half the maximum level (Table 1), thereby providing an early warning.

iv. The sensitivity of the method for all relevant analogues (listed in Table 1) in the toxin group being measured must be known.

d) Quantification

i. Screening methods can be either qualitative or semi-quantitative. Regardless, screening methods must distinguish between samples which contain no detectable toxins and positive samples (i.e. those which contain levels above the LoD).

ii. The ability of the test to produce positive results at the maximum allowable level and the LoD values should be confirmed through method validation studies which investigate the probability of detection at different concentrations of toxin(s).

iii. Qualitative positive results should be confirmed by quantitative confirmatory or reference methods.

e) Scope

i. Methods should address all relevant toxin analogues (as listed in Table 1) in the toxin group that is being tested.

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

iii. The relative toxicity of structural analogues should be considered and for screening methods which detect multiple biotoxin analogues, preference should be given to methods that account for the relative method response for the analogues.]

Comments from the USA:

Background: We suggest removing the background because providing only limited information could result in improper adaptation of screening methods. If retained, we suggest the following changes in **bold** and ~~strike-though.~~

Screening methods are ~~being increasingly used~~ **a tool** for regulatory marine biotoxin management ~~in a number of countries. Due to the~~ **, used because of** ease of implementation, cost-effectiveness and rapid turn-around-times**.** ~~, screening methods provide an attractive option for intensive marine biotoxin monitoring programmes. Given the widespread use of screening tests as front line marine biotoxin management tools, it is imperative that~~ **Screening** ~~these~~ methods **should be** ~~are~~ fit for purpose and meet **appropriate** ~~specified method~~ performance criteria **for the biotoxin control strategy used** to ensure the suitability of

bivalves and other ~~shellfish~~ **fishery products** for human consumption. Competent authorities ~~considering the use of a particular~~ **that use a** screening method ~~should~~ **must also** utilise a confirmatory or reference method ~~as a complement~~ to ~~more~~ accurately determine levels of marine biotoxins in positive samples **before releasing the product for human consumption.**

~~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,~~ **D**escribed below are ~~the proposed~~ general performance criteria for screening methods that can be used by competent authorities to select methods that are adequate for routinely monitoring biotoxins for regulatory purposes. ~~Competent authorities should evaluate potential screening methods against the performance criteria outlined herein.~~

General Proposed Performance Criteria for Marine Biotoxin Screening Methods:

We recommend removing the paragraph below. The sections of the Codex Procedural Manual cited apply to quantitative methods, and not to qualitative and semi-quantitative screening methods. The U.S. does not support prescriptive criteria for each toxin, such as listed in proposed Appendix 1. We concur with New Zealand that, if this work is to continue, it would be most useful to develop criteria in general terms only.

> ~~"General method principles and performance criteria (General Criteria) are outlined in the Codex Alimentarius Commission PROCEDURAL MANUAL, 20th ed. document (ISBN 978-92-5-106821-2) in the PRINCIPLES FOR THE ESTABLISHMENT OF CODEX METHODS OF ANALYSIS section. The competent authority is advised to refer to this document when considering selecting a marine biotoxin screening method based on this criteria approach. The marine biotoxin method criteria, outlined in the Table Appendix I: Method performance criteria for marine biotoxin screening methods, are to be considered by the competent authority to be inclusive of methods such as Lateral flow immunochromatography and ELISA."~~

Selectivity, i.: The first sentence is an introductory definition that could be merged with part ii. Note that plankton monitoring is an important biotoxin screening method. The second sentence should be removed because it is not a criteria, and is not specific to screening methods. Neither chromogenic detection of antigen-antibody complexes, nor fluorescent detection of separated compounds, unambiguously identifies a compound. Screening methods need only detect the presence of a toxin, or algal species, of interest.

> ~~i. Selectivity of a screening method refers to the ability of the test to distinguish the presence of a target~~ **algal species, chemical** ~~compound or class of compounds from other substances in the sample. Screening methods (which are often based on microbiological growth, immunoassays or chromogenic response) may 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.~~

Selectivity, ii.: False positives need to be low and manageable for screening methods to be useful. However, some lack of specificity is a trade-off for the speed, convenience, and cost savings of the screening method relative to a confirmatory method. The last sentence covered under *Detection capability*, part iv. Change as follows:

ii. Test method cross reactivity should be investigated in validation studies prior to implementing the method. Blank matrix fortified with other toxins and structurally related compounds possibly found in samples, should be tested to establish ~~that negative results are~~ **the level of false positive results that may be** obtained when test materials contain these other compounds. ~~Responses should be negative~~ **The level of false positives should be manageably low** when these compounds are present at concentrations that might reasonably be expected to be present in a sample. ~~Also the response for the different marine biotoxin group analogues should be well understood and relative to the response of calibrant standards.~~

Precision, i.: This is an overarching guideline that relates to each of the criteria, not just precision. Therefore, it should be presented at the top. We suggest changing "must" to "should".

i. Preference should be given to methods that have undergone full collaborative inter-laboratory studies. Single lab validation studies ~~must~~ **should** be in accordance with internationally recognized protocols (e.g. those referenced in the harmonized IUPAC Guidelines for Single–Laboratory Validation of Methods of Analysis).

Detection capability, i.: While 100% sensitivity at the ML is ideal, there are other considerations such as, some toxins are lethal and some are not, the maximum level is usually well below the level that causes illness, and replicate samples can be used. While this criteria is appropriate for saxitoxin, it may be too restrictive for some biotoxin scenarios. We agree with Australia that the "must" be changed to a "should".

i. Screening methods should have false negative rates which are less than 5 % at a level of half the maximum allowable level and there **should** ~~must~~ be no false negatives at the maximum level ~~(Appendix 1)~~.

Detection capability, ii.: Remove because this is already contained in part i.

~~ii. The limit of detection (LoD) of the screening methods should be such that they reliably detect (in at least 95% of samples) the biotoxin components of interest at half the maximum level (Appendix 1).~~

Detection capability, iii.: Remove first part because qualitative methods also have limits of detection. The "one half" level is arbitrary and depends on the circumstances. Should also add that preference should be given to methods that can be performed rapidly at the harvest site, thereby providing an early warning.

iii. ~~Where the test is quantitative or semi-quantitative,~~ **P**reference should be given to methods with detection limits ~~less than half~~ **below** the maximum level ~~(Appendix 1)~~, thereby providing an early warning.

Detection capability, iv.: This implies that Appendix 1 defines the relevant analogs, however this would depend on the screening method strategy used (e.g., indicator analogs). While sensitivity is the measure of false negatives, the limit of detection with a selected sensitivity is the value that needs to be known. We agree with France that this should be a "should".

iv. The **limit of detection and** sensitivity of the method for all relevant analogues ~~(listed in Appendix 1)~~ in the toxin group being measured ~~must~~ **should** be known.

Quantification: We recommend moving this information elsewhere because quantification is not an essential element of screening methods. The method need only detect the presence of the analyte(s) at the level of interest.

Quantification i.: We recommend removing this criterion because the criteria for specificity and sensitivity are already covered in more detail under *Selectivity* and *Detection capability* above.

~~i. Screening methods can be either qualitative or semi-quantitative. Regardless, screening methods must distinguish between samples which contain no detectable toxins and positive samples (i.e. those which contain levels above the LoD).~~

Quantification ii.: We recommend deleting this part specific to validating method sensitivity because validation should be presented as an overarching guideline that relates to each of the criteria (see comment for *Precision*, part *i*.).

~~ii. The ability of the test to produce positive results at the maximum allowable level and the LoD values should be confirmed through method validation studies which investigate the probability of detection at different concentrations of toxin(s).~~

Quantification iii.: We recommend removing or elaborating this general guidance on applying screening methods. The follow-up procedure for positive results depends on the management strategy and the parameters of the test. Certainly a confirmatory method is used before releasing positive product for human consumption, even if the screening method is quantitative. However, it is not necessary to run a confirmatory method when screening tests are positive, if a decision is made not to allow harvest.

~~iii.Qualitative positive results should be confirmed by quantitative confirmatory or reference methods.~~

Scope i.: We recommend removing this part because it is similar to *Detection capability*, part *iv*. (see our comment above). Screening methods may not always address/detect all relevant toxin analogs if appropriate indicators are used.

~~i. Methods should address all relevant toxin analogues (as listed in Appendix 1) in the toxin group that is being tested.~~

Scope ii.: Not sure why one should prefer multi-analog or multi-group methods over using several different methods. It seems that total cost, speed, sensitivity and specificity would be the determining factors.

ii. ~~Preference should be given to~~ **M**ethods that can be used to ~~test~~ **detect** multiple toxin analogues and, when applicable, multiple toxin groups **should be considered**.

Scope iii.: We agree with the first part of the sentence, the second part is unclear. For screening method purposes, methods where analog levels are converted to toxin equivalents are not necessarily preferred over rapid immunoassays that cross-react to a subset of analogs. We agree with Australia that the toxicity equivalence factors proposed by the U.S. for reference methods (Table I-8.6.2, U.S. comments) should be used.

iii.The relative toxicity of structural analogues **detected** should be considered ~~and for screening methods which detect multiple biotoxin analogues, preference should be given to methods that account for the relative method response for the analogues.~~

Appendix 1: Appendix 1 should be removed. The table implies prescribed criteria, where they are only suggested ("should") values. The numbers are redundant and easily calculated from "Detection capability", criterion i., and the Molluscan Standard. There are issues with the analog listings (e.g., the okadaic acid language needs to be more specific; monitoring only brevatoxins 1, 2, 3 & 7 may not be protective).

Comments from France:

Background:

Screening Methods **can be** ~~are being increasingly~~ used for regulatory marine biotoxin management ~~in a number of countries. **D**~~ d**ue** to the ease of implementation, cost-effectiveness and rapid turn-

around times **S**creening methods ~~provide an attractive option for intensive marine biotoxin monitoring programmes. Given the widespread use of screening tests as front line marine biotoxin management tools, it is imperative that these methods are~~ **have to be** fit for purpose and meet specified method performance criteria to ensure the suitability of bivalves and other shellfish for human consumption. Competent authorities considering the use of a particular screening method should utilise a confirmatory or reference method as a complement to more accurately determine levels of marine biotoxins in positive samples.

Screening Methods can be either qualitative or semi-quantitative. Regardless, screening methods must distinguish between samples which contain no detectable toxins and positive samples. The ability of the test to produce positive results at the maximum allowable level and the LoD values should be confirmed through method validation studies which investigate the probability of detection at different concentrations of toxin(s). Qualitative positive results should be confirmed by quantitative confirmatory or reference methods.

~~f) Selectivity~~

~~Selectivity of a screening method refers to the ability of the test to distinguish the presence of a target compound or class of compounds from other substances in the sample. Screening methods (which are often based on microbiological growth, immunoassays or chromogenic response) may 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.~~

ii. Test method cross reactivity should be investigated in validation studies prior to implementing the method. 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. Also the response for the different marine biotoxin group analogues should be well understood and relative to the response of calibrant standards.~~

*the important point is to be aware of the limitations of the test (% of false positive…)

g) Detection capability

i. Screening methods should have false negative rates which are less than 5 % at a level of half the maximum allowable level and there must be no false negatives at the maximum level ~~(Appendix 1)~~

~~ii. The limit of detection (LoD) of the screening methods should be such that they reliably detect (in at least 95% of samples) the biotoxin components of interest at half the maximum level (Appendix 1).~~

iii. ~~Where the test is quantitative or semi-quantitative p~~ **P**reference should be given to methods with detection limits ~~less than half~~ **below** the maximum level ~~(Appendix 1),~~ thereby providing an early warning.

iv. The sensitivity of the method for all relevant analogues (listed in Appendix 1) in the toxin group being measured ~~must~~ **should** be known.

v. The limit of detection of the method for all relevant analogues should be defined whether it is possible.

d) Quantification

Fully agree with US: screening method is not always quantitative and its objective is to detect a group of toxins at a defined level of interest (for instance to target the analysis with a quantitative confirmatory method)

~~i. Screening Methods can be either qualitative or semi-quantitative. Regardless, screening methods must distinguish between samples which contain no detectable toxins and positive samples (i.e. those which contain levels above the LoD).~~

~~ii. The ability of the test to produce positive results at the maximum allowable level and the LoD values should be confirmed through method validation studies which investigate the probability of detection at different concentrations of toxin(s).~~

~~Qualitative positive results should be confirmed by quantitative confirmatory or reference methods.~~

e) Scope

i. Methods should address all relevant toxin analogues ~~(as listed in Appendix 1)~~ in the toxin group that is being tested.

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

iii. The relative toxicity of structural analogues **detected** should be considered (agree with US) ~~and for screening methods whch detect multiple biotoxin analogues, preference should be given to methods that account for the relative method response for the analogues.~~

Comments from Chile:

Objective II, General proposed Performance Criteria

First Paragraph indicates, as an example, a couple of screening methods. Chile considers that no screening methods should be quoted as examples, because this could lead to confusion if every existing screening method is not considered.

Objective II, a) Selectivity, i. Chile suggests the elimination of the phrase "(which are often based on microbiological growth, immunoassays or chromogenic response)", because it doesn't contribute to a better understanding of the paragraph.

Objective II a) Selectivity ii. "Test method cross reactivity should be investigated in validation studies prior to implementing the methods." Chile agrees with the above stated. However, considering that validation responsibility is a matter still under discussion in CCMAS, we suggest that the responsible entity for validation (private or public) is not indicated in this document.

Comments from Norway:

We would like to suggest including a reference to the work of CCMAS regarding proprietary methods (ref. REP 12/MAS Appendix V para. E) and we might include a new paragraph on proprietary methods under b) Precision in the Objective chapter.

A proprietary method should be either fully collaboratively validated or validated and reviewed by an independent third party according to internationally recognized protocols. The results of such studies should be made available for CCMAS. If a proprietary method has not been validated by a full collaborative trial, it may be eligible for adoption into the Codex system as a Codex Type IV method, but not as a Type I, II or III method.

Comments from the Netherlands:

d) Quantification

(i) Screening methods can be either qualitative or semi-quantitative. Regardless, screening methods must distinguish between samples which contain no detectable toxins and positive samples (i.e. those which contain levels above the LoD).

- Please refer to either appendix 1 or include "(i.e. which contain levels above the (**maximal**) LoD)

In the various sections reference is made to negative results and positive results where the definition is about noncompliant results. Maybe in order to create consistency replace negative and positive results by respectively compliant and noncompliant results (i.e. a-ii, d-i and d-iii)

b) Precision

We assume that precision is only for semi-quantitative screening methods just as in 2002/657/EC. This is not clear from the current text.

Table 1: Proposed Method Performance Parameters for Marine Biotoxin Screening Methods

Group	***Analogues***	***Units***	***Maximum Allowable Level (ML)***	***Maximum Limit of Detection (LoD)***	***False Negative Rate at ½ ML***	***False Negative Rate ML***
Saxitoxin Group	• *Saxitoxin* • *Neosaxitoxin* • *Decarbamoyl-saxitoxin* • *Decarbamoyl-neosaxitoxin* • *Gonyautoxins (1-6)* • *Decarbamoyl-gonyautoxins (1-4)* • *N-sulfocarbamoyl-gonyautoxins (1-4)*	*mg STXdiHCl eq/kg*	*0.8*	*0.4*	*<5%*	*0%*
Domoic Acid Group	• *Domoic acid* • *Iso domoic acid C*	*mg DA/kg*	*20*	*10*	*<5%*	*0%*
Okadaic Acid Group	• *Okadaic acid* • *Dinophysistoxin-1* • *Dinophysistoxin-2* • *Fatty acid esters of okadaic acid (DTX3)*	*mg OA eq/kg*	*0.16*	*0.08*	*<5%*	*0%*
Azaspiracids	• *Azaspiracid-1* • *Azaspiracid-2* • *Azaspiracid-3*	*mg AZA1 eq/kg*	*0.16*	*0.08*	*<5%*	*0%*
Brevetoxin Group	• *Brevetoxin-1* • *Brevetoxin-2* • *Brevetoxin-3* • *Brevetoxin-7*	*mg PbTx-2 eq/kg*				