CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

E

Viale delle Terme di Caracalla, 00153 Rome, Italy - Tel: (+39) 06 57051 - Fax: (+39) 06 5705 4593 - E-mail: codex@fao.org - www.codexalimentarius.org

Agenda Item 7 **CX/FFP 12/32/7**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON FISH AND FISHERY PRODUCTS

Thirty-Second Session

Bali, Indonesia

1 – 5 October 2012

Proposed Draft Performance Criteria for Reference and Confirmatory Methods for Marine Biotoxins in the *Standard for Raw and Live Bivalve Molluscs*

COMMENTS AT STEP 3

(Kenya, United States of America)

KENYA

We support the inclusion of this document to be included in section 1-8.6. It is a good guide with set limits that helps manufacturers and regulatory authorities on what to be analyzed and ease interpretation of results.

UNITED STATES OF AMERICA

General comment:

The United States would like to thank the members of the Committee for the opportunity to comment on this document and for the work of the Electronic Working Group to prepare this document for the Committee's consideration. While we are in general agreement that the inclusion in the Standard of reference method criteria in lieu of specific reference methods may be desirable, we continue to be concerned that the proposed criteria do not insure that reference methods conform to uniform criteria. In addition, the proposed criteria appear to exclude functionally based methods, including the globally recognized official reference method for saxitoxin, the mouse bioassay.

Bivalve total toxicity, and not toxin composition, is the measure of importance for protecting public health. The ideal reference method would measure total toxicity directly based on biological function. Multi-analog methods do not assure detection of all the toxins contributing to toxicity, and they still rely on a bioassay to convert toxin levels to toxicity. For the saxitoxin group, the mouse bioassay and the receptor binding assay have the characteristics needed of a globally applicable reference method.

The United States uses both multi-analog HPLC and the mouse bioassay for internal regulatory purposes, and has no wish to exclude multi-analog methods. In fact, our suggested changes to the criteria will facilitate the use of multi-analog HPLC methods that may otherwise be excluded in the currently proposed criteria. Multi-analog reference methods provide accurate toxin composition when all potential toxins are measured, and the laboratory has the required budget and expertise. We suggest changes to the proposed criteria to account for the variability of multi-analog method performance with the toxin profile, and to account for the limited availability of certified reference standards for some toxins that can contribute to total toxicity. In addition, we recommend the inclusion of uniform analog toxicity conversion factors to enable the use of multi-analog methods as reference methods. While the United States supports the appropriate use of multi-analog methods, we continue to favor the mouse bioassay as the best current Codex reference method for saxitoxin because it measures total toxicity directly, provides reliable protection under varying conditions, and is practical for all countries.

We suggest modifying the proposed criteria to assure method compliance to uniform quantitative criteria, and to increase method selection flexibility. The suggested changes adhere to *Codex Procedural Manual* guidelines and are inclusive of the mouse bioassay for the saxitoxin group. Appendix 1 contains revised performance criteria incorporating the recommendations in these comments.

Specific comments:

Proposed Draft Heading: Modify as follows:

"Proposed Draft Performance Criteria for Reference ~~and Confirmatory~~ Methods for Marine Biotoxins (for Inclusion in Section I-8.6 in the Standard for Live and Raw Bivalve Molluscs)"

Reason: Methods meeting the criteria are reference methods and not necessarily confirmatory methods (e.g. multi-analog HPLC methods). In addition, confirmatory methods are not necessarily reference methods (e.g., testing the physical/chemical properties).

Add the current section heading from the Bivalve Standard with the following modification:

"I-8.6 Determination of Biotoxins **– Method Performance Criteria**"

Reason: To be consistent with the current Bivalve Standard section heading and numbering system, and to inform that method performance criteria are used.

Background: Move the background information outside of the Bivalve Standard.

Reason: Method criteria background information can be found in the Codex Procedural Manual, Session Reports and Working Group Reports, and is not needed inside the Bivalve Standard.

Background, 1st paragraph: Move.

The purpose of method criteria is clear in the Codex Procedural Manual, and, as with a list of reference methods, needs no further explanation.

The statement that these methods "can be used by competent authorities to select methods that are adequate for monitoring biotoxins for regulatory purposes" is aside from the main purpose for reference methods in standards (for cases of dispute), and may mislead because any method, including screening methods, that are approved by the competent authority are adequate for regulatory purposes.

Background, 1st paragraph, 2nd line, Footnote #1 – reference method definition: Delete footnote.

Reason: "Reference methods" are adequately defined in the "Principles for the Establishment of Codex Methods of Analysis" (Codex Procedural Manual), which states:

"Reference Methods (Type II). Definition: A Type II method is the one designated Reference Method where Type I methods do not apply. It should be selected from Type III methods (as defined below). It should be recommended for use in cases of dispute and for calibration purposes."

*The Proposed Footnote is taken from the "Guidelines on Good Laboratory Practice in Pesticide Residue Analysis" (CAC/GL 40-1993), which states: "These methods will generally **have** been collaboratively studied and are usually based on molecular spectrometry." The Proposed Footnote was changed to: "These methods generally **haven't** been collaboratively studied and are usually based on molecular spectrometry."*

We believe that biotoxin reference methods should generally be collaboratively studied, and that the pesticide definition is not applicable to algal biotoxins where bioassay reference methods are in general use.

Background, 2nd paragraph: Move.

Reason: Functionally based methods, such as the mouse bioassay and receptor binding assay, do not require information on the toxin profile present. However, this information is critical for the application of multi-analog methods, and should be addressed with definitive criteria (rather than in a background section.)

Background, 3nd paragraph: Move.

Reason: Guidance on using screening methods and evaluating regulatory strategies would better fit in Section 7.2.2.3 "Marine Biotoxin Control" of the Code of Practice for Fish and Fishery Products.

General proposed performance criteria/principles: Delete this section, including provisions (a) - (g), and replace with the following:

"I-8.6.1 General Criteria

(i) Reference methods shall be selected in accordance with Section I-8.6.2 (Method Numerical Criteria) and Section I-8.6.3 (Application of Method Numerical Criteria), and in accordance with the "General Criteria for the Selection of Methods of Analysis" and "General Criteria for the Selection of Single-Laboratory Validated Methods of Analysis" in the *Codex Procedural Manual.*

(ii) The method selected should be chosen on the basis of practicability and preference should be given to methods which have applicability for routine use."

Reason: General qualitative principles are not needed within the Bivalve Standard and may lead to misapplication of the criteria. Method selection under the criteria approach is systematic, and virtually all validated methods that meet the numerical criteria are acceptable.

Adequate general criteria are already contained in the Codex Procedural Manual, and are cited in I-8.6.1(i) above. Most of the proposed criteria are not unique to algal biotoxin methods and need not be repeated in the Standard. However, according to the Procedural Manual the general criterion listed in I-8.6.1 (ii) (above) must be included when using the criteria approach.

Many of the proposed general criteria that use the expression "preference should be given" cover the same parameters as the proposed numerical criteria. These "preferential" criteria may lead to disregard of the numerical criteria, or subjective arguments against using methods that meet the numerical criteria.

Some of the proposed general criteria address attributes that are specific to multi-analog methods, to the apparent exclusion of methods that measure total toxicity directly. Criteria needed specifically for multi-analog methods should be listed separately from general provisions.

We propose adding a new section after the numerical criteria table for the purpose of listing provisions that explain how to apply numerical total toxicity criteria to multi-analog methods (see comments for new section I-8.6.3 "Application of Method Numerical Criteria").

Table

Table Heading – "Method performance parameters for marine biotoxins methods": Revise as follows:

"I-8.6.2 Method Numerical Criteria"

Reason: To be consistent with the term "numerical criteria" used in the Codex Procedural Manual, and to align with the Bivalve Standard section numbering system. (See earlier comment for new section heading "I-8.6 "Determination of Biotoxins – Method Performance Criteria".)

Toxin abbreviations: Move abbreviations into the column with the toxin names using parentheses.

Reason: To use the empty column space for toxicity equivalent factors (see next comment).

Add Toxicity Equivalent Factors (TEFs). e.g.:

Toxin	Toxicity Equivalent Factor

Reason: Toxicity equivalent factors should be specified in the criteria to allow multi-analog methods to be used as Codex reference methods. If different TEFs are used by different countries results will not be comparable. (See toxin group-specific comments for recommended TEF values.)

"Units" column and "Maximum Level" column: Merge columns and revise as follows:

		Maximum level /kg of mollusc flesh
Saxitoxin Group	Total Toxicity	**≤ 0.8 milligrams (2HCL) of saxitoxin equivalent**
Okadaic Acid Group	Total Toxicity	**≤ 0.16 milligrams of okadaic equivalent**
Domoic Acid Group	Domoic Acid	**≤ 20 milligrams domoic acid**
Brevetoxin Group	Total Toxicity	**≤ 200 mouse units or (0.8 milligrams BTX2 equivalent)**
Azaspiracids Group	Total Toxicity	**≤ 0.16 milligrams AZA1 equivalent**

Reason: To be consistent with the units and terminology used in Section I-5.2 of the Bivalve Standard (biotoxin provisions).

Limit of Detection/Limit of Quantification: Delete LOD/LOQ columns and criteria.

Reason: Section 1.2 of the "Guidelines for Establishing Numeric Values for Method Criteria and/or Assessing Methods for Compliance Thereof", Codex Procedural Manual (20th edition, page70) clearly indicates that LOD/LOQ criteria are used only as an alternative to minimum applicable range criteria. It states: "As an alternative to establishing minimum applicable range, the criteria could be numeric values for LOD and LOQ."

Minimum Applicable Range" criteria have been established in the proposed criteria. The criteria should not list dual, conflicting provisions for method "applicability".

Minimum applicable range criteria are preferred because LOD/LOQ criteria calculated according the Codex Procedural Manual would unjustifiably restrict the use of widely used instrumental and biological reference methods.

Precision at ML: Revise column heading as follows:

"Precision at ML (**RSD_R**)".

Reason: To show that the precision measure used is the inter-lab relative standard deviation (RSD_R) as specified in the Codex Procedural Manual. (Note that the decimal points in front of the values in the column should be removed.)

Recovery: Revise column heading as follows:

"Recovery **percent**"

Reason: To indicate the values are percentages.

Trueness: Remove column

Reason: The acronym "CRM" is repeated in the column for each toxin. This is more simply handled by a single sentence. (See comment for "All Methods (ii)" in proposed new Section "I-8.6.3 Application of Method Numerical Criteria")

Minimum Range, LOD, LOQ (analog-specific criteria): Remove all numerical criteria associated with individual toxin analogs.

Reason: Analog-specific criteria are inadequate to insure that methods meet required total toxicity criteria. Analog-specific criteria are unnecessary, unrealistic and overly restrictive.

The "Guidelines for Establishing Numeric Values for Method Criteria and/or Assessing Methods for Compliance Thereof" in the Codex Procedural Manual states that "Only the provision for the commodity along with its maximum level is needed when establishing numeric values for method criteria". Individual toxin analogs have no maximum level provisions and therefore cannot have meaningful criteria individually. All criteria need to relate to the total toxicity maximum level provisions in the Bivalve Standard.

The proposed analog-specific criteria do not assure method compliance with essential total toxicity criteria. For example, the sum of the proposed analog-specific minimum ranges is allowed to exceed the total toxicity minimum range.

Multi-analog method criteria should allow realistic variability in method performance for individual toxin analogs as long as overall total toxicity criteria are met. We recommend using flexible "relational criteria" to assure that multi-analog methods meet the total toxicity criteria. (See proposed new Section "I-8.6.3 Application of Method Numerical Criteria")

Add New Section: as follows:

"I-8.6.3 Application of Method Numerical Criteria:"

Reason: To provide guidance on determining method compliance with total toxicity numerical criteria. To add relational criteria that insure multi-analog methods meet minimum applicable range, precision, and recovery criteria for total toxicity, while allowing flexibility in method performance for individual analogs.

New Section I-8.6.3: Add the following criteria under "All Methods"

"**All Methods**

i. All methods must meet Total Toxicity criteria.

Reason: Clarifies that both biological and instrumental multi-analog methods must meet criteria based on the Bivalve Standard total toxicity maximum levels.

ii. Trueness is evaluated using certified reference material."

Reason: Moved from the Table. No need to repeat "CRM" for each toxin analog in Table.

New Section I-8.6.3: Add the following criteria under "Multi-Analog Methods"

"**Multi-Analog Methods**

i. All methods must be validated for, and used to detect, all the toxin analogs that may possibly contribute to total toxicity in the sample.

Reason: To insure the method is applicable to the all the analogs likely to be encountered. Allows for the addition or exclusion of analogs as appropriate.

ii. Methods must be used with certified reference material for each analyte.

Reason: To assure accuracy in identification and quantification of the analyte.

iii. The toxicity equivalent factors (TEFs) listed in I-8.6.2 must be used to calculate total toxicity. For toxins without listed TEFs, internationally recognized TEFs must be used.

Reason: Uniform TEFs are needed for the use of multi-analog methods as reference methods.

iv. To determine method compliance with "Total Toxicity Minimum Applicable Range" criteria: The sum of the validated limits of quantification (in toxicity equivalents) for each toxin analog that may possibly contribute to total toxicity in the sample must not exceed the lower limit of the "total toxicity minimum applicable range" criterion.

Reason: To determine multi-analog method compliance with total toxicity minimum applicable range criteria. This provision allows varying limits of quantification for each analog, but prevents the use of a method when multiple toxin analogs below the limit of quantification could result in total toxicity over the lower limit of the "Minimum Applicable Range".

v. To determine method compliance with "Total Toxicity Precision" criteria: The method's total toxicity precision (RSD_R), for a sample at the maximum level, is estimated using the validation RSD_R data for the individual analogs at the concentrations found in the sample.

Example (assuming analog variances are independent):

Estimated $RSD_{R\ \text{total toxicity}}$ = sd{a+b+c…}/total mg equivalents/kg × 100

$$\text{sd\{a+b+c+...\}} = \sqrt{\text{sd}\{a\}^2 + \text{sd}\{b\}^2 + \text{sd}\{c\}^2 + \ldots}$$

sd{a} = inter-laboratory standard deviation for toxin {a} (calculated after converting to toxin equivalents) at the concentration in the sample.

Note: sd{a} can be estimated from the validation $RSD_{R\{a\}}$ at the nearest analyte concentration, e.g., sd{a} = $RSD_{R\{a\}}$ × mg equivalents{a}/kg ÷ 100

Reason: To determine multi-analog method compliance with "Total Toxicity Precision" criteria. Precision differs for each toxin analog and level, therefore total toxicity precision should be estimated for the toxin profiles measured.

vi. To determine method compliance with "Total Toxicity Recovery" criteria: Total toxicity recovery is a weighted average of the recoveries of the individual analogs in the sample matrix, weighted by the toxin equivalent levels in the sample."

Reason: To determine multi-analog method compliance with "Total Toxicity Recovery" criteria. Recovery differs for each toxin analog, therefore total toxicity recovery should be calculated for the toxin profile measured.

Saxitoxin Group

General comment:

In accordance with the Joint FAO/IOC/WHO ad hoc "Expert Consultation on Biotoxins in Bivalve Molluscs" (2004), the U.S. supports the mouse bioassay (MBA) as the best choice for the Type II reference method for the saxitoxin group, and agrees that significant limitations in use of multi-analog HPLC methods remain. The Expert Report considered the MBA lower limit of use (0.4 mg STX eq. /kg) as adequate, and states that the MBA is widely used and has successfully protected public health for over 60 years.

The MBA is the formal reference standard against which all other methods are compared. The MBA was used during epidemiological studies to establish the safe maximum level of 400 Mouse Units per 100 grams meat. To assure public safety, results of other methods must correlate closely with the MBA. The maximum level in the Bivalve Standard (0.8 mg STX equivalent/kg) is an estimate of the Mouse Unit limit made by injecting mice with known quantities of purified saxitoxin. The MBA is also used to determine the relative toxicity of saxitoxin analogs in order to allow the use of multi-analog HPLC methods for regulatory purposes. The MBA is a "Defining Method" (Codex Type I), and is arguably, according to the Codex procedure, the required reference method.

Multi-analog HPLC methods are not confirmatory, therefore the mouse bioassay is often used to confirm HPLC total toxicity results, and mass spectrometry is used to confirm specific compounds detected.

The performance of saxitoxin HPLC methods depends on the toxin profile tested. Performance parameters differ for each analog, and intrinsic measurement errors for each analog accumulate when calculating total toxicity. When many analogs at low levels contribute to high total toxicity, HPLC ability to meet performance criteria may be challenged.

Multi-analog methods are not validated for, and certified reference standards are not available for, some of the toxin analogs that routinely contribute to shellfish toxicity. The European Union commented to the eWG that a multi-analog HPLC method underestimated the total toxicity in samples from Portugal and Spain by half with respect to the mouse bioassay because of the method's inability to detect the presence of GTX6 (B2). Certified reference standards are not available for C3 and C4, which can contribute measurably to molluscan total toxicity, particularly in the Southern Hemisphere.

Developing countries will have difficulty operating multi-analog HPLC laboratories. Equipment maintenance and sample preparation is time consuming and expensive. Highly trained experts and confirmatory mass spectrometry equipment should be available. Long turn-around times result when samples are shipped to specialized labs from distant harvest areas. Attempting to use HPLC methods with limited resources can result in fewer analyses, longer turn-around times, less reliable results, and increased public risk. Many countries have expressed their concerns to the eWG and at plenary sessions about the impracticality of HPLC methods and the need for the mouse bioassay. Without the mouse bioassay as a reference method, these countries may experience an indirect trade barrier.

The U.S. supports the use of instrumental methods where possible, but is opposed to criteria that exclude the mouse bioassay, and that do not take into consideration the limitations of multi-analog HPLC methods.

List of Toxins: Add the following toxins, and the accompanying note:

N-sulfocarbamoyl-gonyautoxin-1 (C3)	
N-sulfocarbamoyl-gonyautoxin-4 (C4)	
Hydroxybenzoates (GC 1-6)	**Contribution of hydroxybenzoates to total toxicity in bivalves needs to be determined.**

Reason: All the analogs that may contribute to total toxicity should be listed. Analogs without validated methods and/or certified reference standards should not be ignored. An analog that cannot possibly contribute to total toxicity need not be quantified. (see comment for proposed section I-8.6.3 (i) above.)

C3 and C4 are known to contribute measurably to bivalve toxicity in the Southern Hemisphere, and they also contribute to a lesser extent in the Northeast Pacific. The lipophilic hydroxybenzoates are a major component in algae and their significance for bivalve species toxicity needs to be determined.

List of Toxins. Add the following note:

"**Multi-analog methods must quantify these analytes directly (or, when standards for the N-sulfocarbamoyl toxins are not available, they must be quantified indirectly via hydrolysis) when these toxins may be present and contribute to total toxicity**."

Reason: Explains the use of the list. Allows hydrolysis for determination of N-sulfocarbamoyl toxins when no certified reference material is available.

Add the following Toxicity Equivalent Factors:

Toxin	Toxicity Equivalent Factors
Saxitoxin (STX)	**1.000**
Neosaxitoxin (NEO)	**0.924**
Decarbamoyl-saxitoxin (dcSTX)	**0.513**
Decarbamoyl-neosaxitoxin (dcNEO)	
Gonyautoxin-1 (GTX1)	**0.994**
Gonyautoxin-2 (GTX2)	**0.359**
Gonyautoxin-3 (GTX3)	**0.638**
Gonyautoxin-4 (GTX4)	**0.726**
Gonyautoxin-5 (B1)	**0.064**
Gonyautoxin-6 (B2)	
Decarbamoyl-gonyautoxin-2 (dcGTX2)	**0.154**
Decarbamoyl-gonyautoxin-3 (dcGTX3)	**0.377**
N-sulfocarbamoyl-gonyautoxin-1 (C3)	**0.013**
N-sulfocarbamoyl-gonyautoxin-2 (C1)	**0.006**
N-sulfocarbamoyl-gonyautoxin-3 (C2)	**0.096**
N-sulfocarbamoyl-gonyautoxin-4 (C4)	**0.058**
Hydroxybenzoates (GC1 - 6)	

Reason: TEFs are needed (See earlier comment.) The U.S. recommends using the internationally recognized TEFs published by Oshima (1995). Oral TEFs have not yet been established, while Oshima's mouse intraperitoneal TEFs have been used successfully to protect public health for over 15 years.

Total Toxicity, Minimum Range:

Change the range from "0.26 - 1.34" to "**0.40 - 1.20".**

Reason:

To correct the "Minimum Applicable Range" value, in compliance with Section 1.1 of the "Guidelines for Establishing Numeric Values for Method Criteria and/or Assessing Methods for Compliance Thereof" in the Codex Procedural Manual. The appropriate concentration ratio for calculating the "Minimum Applicable Range" is 10^{-6} (0.000,001), rather than 10^{-7} (0.000,000,1), because this value is closest to the analyte concentration ratio at the maximum level. The exact concentration ratio is 0.8 mg/1,000,000 mg, or 0.000,000,8, which yields a "Minimum Applicable Range" of 0.403 to 1.197.

This change is in agreement with the comments from the Netherlands and Norway during the first round of the biotoxin working group, and matches the result from the "Nordic Committee on Food Analysis, Codex Calculation Spreadsheet" cited by Norway.

Note that the Horwitz/Thompson equation, on which this calculation is based, is a continuous empirically derived curve that plots predicted inter-lab RSD versus analyte concentration ratio; therefore it is appropriate to use the closest, or exact, concentration ratio.

Note that this correction allows the use of AOAC 959.08 (mouse bioassay), which functions satisfactorily within this range.

Total toxicity, Precision at ML: Change the RSD_R criteria from ".44%" to "**33%**"

Reason: When the exact concentration ratio is used (0.000,000,8), the calculation for precision (Codex Procedural Manual, p. 53) yields a maximum RSD_R = 33.1% (See comment on concentration ratio for "minimum range" above.)

Domoic Acid Group

Epi-Domoic Acid: Delete this analog from the toxin list.

Reason: Only Domoic Acid has a maximum level in the Bivalve Standard. Epi-domoic acid cannot be included in criteria without changing the ML provision in the Standard.

In nature epi-domoic exists at very low levels relative to domoic acid; Domoic acid exceeds the maximum level before epi-domoic can be quantified. No validated method or toxicity equivalence factor has been established for epi-domoic acid.

Okadaic Acid Group

Add Toxicity Equivalence Factors as follows:

Toxin	Toxicity equivalence factors
OA	**1**
DTX1	**1**
DTX2	**0.6**
Esters of OA, DTX1 and DTX2 (FA-ESTERS)	**Parent TEF**

Reason: TEFs are needed. TEFs are from: "Opinion of the Scientific Panel on Contaminants in the Food chain on a request from the European Commission on marine biotoxins in shellfish – okadaic acid and analogues", The EFSA Journal (2008) Journal number, 589, 1-62.

List of toxins: OA, DTX1, DTX2: Add the following note:

"**Multi-analog methods must quantify these analytes**"

Reason: To explain the use of the list and to be consistent with the note for the FA-ESTERS".

FA-ESTERS, "Methods should detect this analyte directly or after hydrolysis": Revise as follows:

"**Multi-analog m**ethods **must quantify** ~~detect~~ this analyte directly or after hydrolysis"

Reason: If these analogs can contribute to total toxicity they must be quantified. Biomolecular activity based methods do not need to quantify specific toxins.

Azaspiracids Group

Add the following toxicity equivalent factors:

Toxin	Toxicity equivalence factors
Azaspiracid-1 (AZA1)	**1**
Azaspiracid-2 (AZA2)	**1.8**
Azaspiracid-3 (AZA3)	**1.4**

Reason: TEFs are needed. TEFs are from: "Opinion of the Scientific Panel on Contaminants in the Food chain on a request from the European Commission on marine biotoxins in shellfish – azaspiracids", The EFSA Journal (2008), 723, 1-52.

List of Toxins. Add the following provision:

"**Multi-analog methods must quantify these analytes**"

Reason: To explain the use of the list.

Brevetoxin Group

Toxin list: Add a note to the toxin list as follows:

"**The derivatives that may contribute to brevetoxin group total toxicity in shellfish still need to be determined.**"

Reason: Brevetoxin 1 and 2 are derivatized in bivalve molluscs and may be insignificant. The significant brevetoxin derivatives depend on the bivalve species, and need to be determined.

Maximum Level: List the maximum level in mouse units (MU) with the BTX-2 equivalents in parentheses as follows:

"**74 – 326 MU (0.26 – 1.34 mg BTX2 eq.)**"

Reason: The brevetoxin maximum level in the Bivalve Standard is in terms of mouse units and must be listed as such in the criteria. The American Public Health Association Mouse Bioassay used predominantly and is recommended in the FAO/IOC/WHO Expert Report. For consistency in terminology, "BTX2" is used instead of "PbTx-2".

Appendix 1: Revised Draft Performance Criteria

I-8.6 Determination of Biotoxins

I-8.6.1 General Criteria

(i) Reference methods shall be selected in accordance with Section I-8.6.2 (Method Numerical Criteria and Section I-8.6.3 (Application of Method Numerical Criteria), and in accordance with the "General Criteria for the Selection of Methods of Analysis" and "General Criteria for the Selection of Single-Laboratory Validated Methods of Analysis" in the *Codex Procedural Manual.*

(ii) The method selected should be chosen on the basis of practicability and preference should be given to methods which have applicability for routine use.

I-8.6.2 Method Numerical Criteria

Group	Toxin	Toxicity Equivalent Factors	Maximum level /kg of mollusc flesh	Minimum applicable range	Precision (RSD_R)	Recovery percent
Saxitoxin (STX) group	Total Toxicity		≤ 0.8 milligrams (2HCL) of saxitoxin equivalent	0.4 – 1.2	33%	70 – 120
	Saxitoxin (STX)	1.000	Multi-analog methods must quantify these analytes directly (or, when standards for the N-sulfocarbamoyl toxins are not available, they must be quantified indirectly via hydrolysis) when these toxins may be present and contribute to total toxicity.			
	Neosaxitoxin (NEO)	0.924				
	Decarbamoyl-saxitoxin (dcSTX)	0.513				
	Decarbamoyl-neosaxitoxin (dcNEO)					
	Gonyautoxin-1 (GTX1)	0.994				
	Gonyautoxin-2 (GTX2)	0.359				
	Gonyautoxin-3 (GTX3)	0.638				
	Gonyautoxin-4 (GTX4)	0.726				
	Gonyautoxin-5 (B1)	0.064				
	Gonyautoxin-6 (B2)					
	Decarbamoyl-gonyautoxin-2 (dcGTX2)	0.154				
	Decarbamoyl-gonyautoxin-3 (dcGTX3)	0.377				
	N-sulfocarbamoyl-gonyautoxin-1 (C3)	0.013				
	N-sulfocarbamoyl-gonyautoxin-2 (C1)	0.006				
	N-sulfocarbamoyl-gonyautoxin-3 (C2)	0.096				
	N-sulfocarbamoyl-gonyautoxin-4 (C4)	0.058				
	Hydroxybenzoates (GC1 - 6)		Contribution of hydroxybenzoates to total toxicity in bivalves needs to be determined.			
Okadaic acid (OA) group	Total Toxicity		≤ 0.16 milligrams of okadaic equivalent	0.05 – 0.27	44%	70 - 120
	Okadaic acid (OA)	1.0	Multi-analog methods must quantify these analytes.			
	Dinophysistoxin-1 (DTX1)	1.0				
	Dinophysistoxin-2 (DTX2)	0.6				

	Esters of OA, DTX1 and DTX2 (FA-ESTERS)	Parent TEF	Multi-analog methods must quantify these analytes directly or after hydrolysis.			
Domoic acid (DA) group	Domoic Acid (DA)		≤ 20 milligrams domoic acid	13.2 – 26.8	22%	85 - 110
Brevetoxin (BTX) group	Total Toxicity		≤ 200 Mouse Units or (0.8 milligrams BTX2 equivalent)	74 – 326 MU (0.26 – 1.34 mg BTX2 eq.)	44%	70 - 120
	Brevetoxin-1 (BTX1)		The derivatives that may contribute to brevetoxin group total toxicity in shellfish need to be determined.			
	Brevetoxin-2 (BTX2)					
	Brevetoxin-1 derivatives (devBTX1)					
	Brevetoxin-2 derivatives (devBTX2)					
Azaspiracid (AZP) group	Total Toxicity		≤ 0.16 milligrams AZA1 equivalent	0.05 – 0.27	44%	70 - 120
	Azaspiracid-1 (AZA1)	1.0	Multi-analog methods must quantify these analytes.			
	Azaspiracid-2 (AZA2)	1.8				
	Azaspiracid-3 (AZA3)	1.4				

I-8.6.3 Application of Method Numerical Criteria:

All Methods:

i. All methods must meet "Total Toxicity" numerical criteria

ii. "Trueness" is evaluated using certified reference material.

Multi-Analog Methods:

i. Methods must be validated for, and used to detect, all the toxin analogs that may possibly contribute to total toxicity in the sample.

ii. Methods must be used with certified reference material for each analyte.

iii. The toxicity equivalent factors (TEFs) listed in I-8.6.2 must be used to calculate total toxicity. For toxins without listed TEFs, internationally recognized TEFs must be used.

iv. To determine method compliance with "Total Toxicity Applicable Minimum Range" criteria: The sum of the validated limits of quantification (in toxicity equivalents) for each toxin analog that may possibly contribute to total toxicity in the sample must not exceed the lower limit of the "total toxicity minimum applicable range" criterion.

v. To determine method compliance with "Total Toxicity Precision" criteria: The method's total toxicity precision (RSD_R), for a sample at the maximum level, is estimated using the validation RSD_R data for the individual analogs at the concentrations found in the sample.

Example (assuming analog variances are independent):

Estimated RSD_R total toxicity = sd{a+b+c…}/total mg equivalents/kg × 100

$$sd\{a+b+c+...\} = \sqrt{sd\{a\}^2 + sd\{b\}^2 + sd\{c\}^2 + \dots}$$

sd{a} = inter-laboratory standard deviation for toxin {a} (calculated after converting to toxin equivalents) at the concentration in the sample.

Note: sd{a} can be estimated from $RSD_{R\{a\}}$ at the nearest concentration, e.g.,

sd{a} = $RSD_{R\{a\}}$ × mg equivalents{a}/kg ÷ 100

vi. To determine method compliance with "Total Toxicity Recovery" criteria: Total toxicity recovery is a weighted average of the recoveries of the individual analogs in the sample matrix, weighted by the toxin equivalent levels in the sample.