CODEX ALIMENTARIUS COMMISSION

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World Health Organization

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Agenda Item 3 **CX/FFP 14/33/5 Add.1**
ORIGINAL LANGUAGE ONLY

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON FISH AND FISHERY PRODUCTS

Thirty-third Session

Bergen, Norway

17 – 21 February 2014

DRAFT PERFORMANCE CRITERIA FOR REFERENCE AND CONFIRMATORY METHODS FOR MARINE BIOTOXINS (SECTION I-8.6 DETERMINATION OF BIOTOXINS) IN THE STANDARD FOR LIVE AND RAW BIVALVE MOLLUSCS
(At Step 6 of the Procedure)

Comments submitted by African Union, Argentina, Canada, Chile, Morocco, Philippines, United States of America and

AU

The use of animal biossay for biotoxin determination should be discouraged and replaced with alternative methods for ethical and scientific reasons.

ARGENTINA

En relación a los criterios de rendimiento para los métodos de referencia y confirmación para biotoxinas marinas, Argentina considera que sin estar en desacuerdo con adoptar nuevas técnicas diagnósticas surgidas de la investigación, considera que el presente anteproyecto parece excluir el método de carácter biológico conocido como bioensayo en ratón, distinguido mundialmente como el método de referencia oficial para saxitoxinas (AOAC 959.08).

En Argentina, los programas de Control de las Intoxicaciones por Fenómenos Algales, se basan en la determinación de toxina paralizante por medio del bioensayo en ratón (AOAC 959.08) también se desarrollan estudios de las floraciones algales nocivas (FAN) caracterizadas estas por, cambios en la distribución espacial, la estratificación, la concentración de microalgas e incluso la concentración de toxinas presentes y su composición.

Debido a esto, los programas implementados deben contar con metodologías de análisis que permitan cumplir con el objetivo principal de proteger la salud de la población expuesta al riesgo de intoxicación por consumo de mariscos contaminados, sean estos destinados a comercialización o autoconsumo, en este sentido consideramos que la suma de las acciones desarrolladas en los diferentes programas contribuyen fortaleciendo al método de bioensayo como método de referencia.

En lo que respecta al grupo de las saxitoxinas, el bioensayo en ratón (AOAC 959.08) reúne las características requeridas como un método de referencia oficial a nivel mundial, que cumple con las normas del Codex (Principios para el establecimiento de métodos de análisis del CODEX), esto es, método oficial elaborado por una organización internacional cuya seguridad ha sido establecida, de simple practicabilidad para su uso habitual y válido para varios grupos de productos, considerando que permite determinar la toxicidad total directamente.

Conforme a lo anterior, manifestamos claramente que si bien Argentina no desea que se excluyan los métodos de análisis químicos, observamos que estos métodos por su elevado costo y practicabilidad no es posible implementarlos fácilmente en los países en desarrollo. Por ello consideramos que el bioensayo en ratón ha demostrado ser un método altamente efectivo y de fácil aplicación, debiendo mantenerse por lo tanto como el **método principal de referencia para saxitoxinas** y debe ser reconocido como tal, dado que cumple con todos los requisitos anteriormente mencionados.

CANADA

General

- Canada recognizes the significant work done to advance to this point of discussion on draft performance criteria and accepts the most recent discussion at CCMAS which advised that the Codex procedural manual cannot be applied when considering performance criteria for substances with multiple analytes, and thus the performance criteria drafted by CCFFP are not appropriate. The CCMAS electronic working group that has been formed to amend the procedural manual to include an approach for these situations will be important for CCFFP to move forward.

- Canada's preference would be for the standard to include criteria (as opposed to listing methods). However, we acknowledge that the work to be done by CCMAS may take some time. With this in mind, Canada would welcome a discussion by the CCFFP on the following proposed option for moving forward:

Option 1

- A) Postpone any further discussion/advancement by CCFFP on drafting performance criteria for section I-8.6 of the standard and hold this section at step 5/6 until the CCMAS e-WG has completed their task. Until CCFFP understands the outcome of the CCMAS e-WG, further discussion could be contrary or duplicative to that of CCMAS.

- B) Canada understands that when CCMAS elaborates criteria, they provide examples as to how the criteria would suit a particular situation. Thus, Canada suggests that CCFFP request that CCMAS use marine biotoxins, particularly saxitoxin, as their case study/ example when elaborating the new criteria. Consideration may also be given to having CCFFP representation on the CCMAS e-WG. This would facilitate/advance the CCFFP ultimate goal to providing better guidance for the determination of biotoxins.

- C) While limited, the text currently included in Section I-8.5 of the STANDARD FOR LIVE AND RAW BIVALVE MOLLUSCS CODEX (STAN 292-2008) would stand in the interim

Option 2

A) Proceed as per 1 A) and 1B) above, but CCFFP works to develop enhanced, but interim, text for adoption into section I-8.5 in the shorter term (pending conclusion to the ongoing CCMAS/CFFP discussion regarding appropriate performance criteria). Such enhancements could include the following text, which provides some fundamental concepts above what currently exists, and would likely be agreeable to most committee members.

Revise section I-8.5 to read:

I-8.5 DETERMINATION OF BIOTOXINS

*Provision**	*Methodology*	*Principle*	*Type*
Saxitoxin group	AOAC Official Method 2005.06 (Paralytic Shellfish Poisoning Toxins in Shellfish) four matrices and 12 toxins	LC-FL	II

*** In addition to this method listed, competent authorities may authorise the use of other appropriately validated methods (for this and/or other toxin groups determined to be relevant to the jurisdiction) as appropriate tools for biotoxin management.**

8.5.1 Methods of analysis selected for the determination of biotoxins shall be selected 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*

8.5.2 Whether for screening or confirmatory purposes, the method selected shall be chosen on the basis of practicability, and preference should be given to methods which have applicability for routine use, are appropriately validated, and appropriately address the toxin profile of the region/situation.

8.5.3 Internationally scientifically validated toxin equivalent factors (TEFs) must be used to calculate total toxicity for methods that do not measure total toxicity directly

8.5.4 Methods that do not measure total toxicity directly should be validated and used for the relevant toxin analogues that may contribute to total toxicity.

CHILE (English comments)

Chile would like to thank the Codex Committee members the opportunity to submit comments on this document which shall be submitted for consideration by the Committee.

Regarding the performance criteria for reference and confirmatory methods for marine biotoxins, Chile expresses its concern that the criteria proposed in the table seem to exclude the biological method known as mouse bioassay, distinguished worldwide as the reference official method for saxitoxins (AOAC 959.08).

In Chile, the National Program for the Surveillance and Control of Poisonings caused by Harmful Algal Phenomena is based on the determination of paralytic toxin by mouse bioassay (AOAC 959.08). This program has been successful, as evidenced by the data collected in the 2002-2012 period, which indicate a clear decline in poisonings reported in the geographic areas where large volumes of aquatic resources are produced and which are mostly affected by this type of phenomenon (*Report of Results, National Surveillance and Control of Poisonings caused by Harmful Algal Phenomena in Chile for paralytic shellfish toxin 2002-2012, Institute of Public Health, Ministry of Health, Chile*).

It is known fact that harmful algal blooms (HABs) are dynamic phenomena characterized by frequent changes of the coastline affected area, spatial distribution, stratification, concentration of microalgae and even the cellular concentration of present toxins and its composition. For this reason, the programs implemented by each country should have methodologies of analysis that allow meeting the main objective of protecting the health of the population exposed to the risk of poisoning by consumption of contaminated shellfish, whether these are intended for marketing or consumption, a condition which is clearly met by the bioassay method.

In order to meet the objective of protecting public health, it is necessarily required to know the total toxicity of the affected product. In this regard, the ideal reference method should be able to achieve this aim based on biological functions, a condition that chemical methods- through the identification of multiple analogues- can only achieve by determining the concentration of each toxin, which requires having the respective certified standards. According to this, the chemical methods can quantify the total toxicity of a sample only if three conditions are jointly met:

a) All the toxins present in a sample are detected,

b) Toxicity factors equivalent for each analogue are determined and,

c) There are certified reference standards for all analogues.

Regarding the group of saxitoxins, the mouse bioassay (AOAC 959.08) has the characteristics required as an official standard reference method worldwide, which complies with Codex standards (Principles for the Establishment of Codex Methods of Analysis), ie official method developed by an international organization whose safety has been established, of simple feasibility for normal and valid use for several product groups, considering that it allows determination of the total toxicity directly.

Paralyzing toxins constitute a group of at least 21 structurally related neurotoxins. However, at present, only 13 of these compounds are available as commercially analytical standards (C1 and C2, dcGTX-2 and 3, dcNEO, dcSTX, GTX-1 and 4, GTX-2 and 3, GTX-5, NEO and STX toxins) delivered by a single provider

worldwide. In this regard, it is noted the case of the toxicity profile of the species *Gymnodinium catenatum*, which contains GTX6, C3 and C4 toxins, for which there is still no commercially available standards.

This is why the proposed criteria for selecting methods must consider the large variability of the spatial, temporal, intrapopulation and inter-individual distribution of marine toxins in shellfish, as well as the limited and variable availability of certified reference materials containing them.

According to the above provisions, it is clearly stated that Chile does not want chemical analysis methods for multiple analogues as reference methods to be excluded. However, we note that this is a field of vigorous scientific and technological development which has not yet reached an international consensus stage and, therefore, we believe that the mouse bioassay is the main reference method for saxitoxins and it should be recognized as such, given that it complies with all the above mentioned requirements. Thus, Chile proposes that the performance criteria of methods are carefully examined, as set out in the Table 1, Appendix VII of document REP13/FFP for groups of toxins and, thus, the 959.08 AOAC mouse bioassay is not indirectly excluded.

Group	**Toxin**	**Maximum level /kg in shellfish meat**	**Minimum applicable range**	**LOD**	**LOQ**	**Accuracy**	**Recovery percentage**
Saxitoxin group (STX)	Total toxicity	≤ 0.8 milligrams (2HCL) of saxitoxin equivalent	0.4 – 1.2	(*)	(*)	33%	70-120
Okadaic acid group (OA)	Total toxicity	≤ 0.16 milligrams of okadaic acid equivalent	0.05 – 0.27	(*)	(*)	44%	70 - 120
Domoic acid (DA)	Domoic acid	≤ 20 milligrams of domoic acid	13.2 – 26.8	2	4	22%	85-110
Brevetoxin group (BTX)	Total toxicity	≤ 200 mouse units or 0.8 milligrams of BTX2 equivalent	74 – 326 MU (0.26 – 1.34 mg BTX2 eq.)	(*)	(*)	44%	70 - 120
Azaspiracid group (AZP)	Total toxicity	≤ 0.16 milligrams of AZA1 equivalent	0.05 – 0.27	(*)	(*)	44%	70 - 120

(*) Non applicable

In particular, it is proposed to consign, in the specific case of saxitoxins, the minimum applicable range from 0.4 to 1.2 mg of HCl/kg saxitoxin equivalent, considering that the limit of detection (LOD) and limit of quantitation (LOQ) are parameters strictly applicable to individual chemical analogues and not applicable to a group of toxins. The above, in addition to the stated in the Codex Procedural Manual, which states that the criteria for LOD/LOQ can be established as an alternative to the minimum applicable range.

Even though all methods must meet requirements such as LOD and LOQ in general, in the case of those analyses of the bioassay-type, since it is not a chemical method, acceptance criteria based on the Codex guidelines would not be appropriate. Chile proposes that these limits should not be set up until it is properly agreed on how the LOD or LOQ will be addressed in the case of multi-analytes, as well as their relation to the toxicity equivalent factors. This is because the focus of the criteria established in the Codex Procedural Manual applies to analytes, individually.

Undoubtedly, the bioassay method does not have the sensitivity or selectivity of a chromatographic method, however, it has toxicological specificity required for the determination of paralytic toxins allowing safeguarding the health of consumers, with a wide margin of safety, which has been demonstrated for 60 years, period in which this methodology has been applied.

Moreover, it is important to note that the bioassay, as a method for detection of saxitoxin, has represented an adequate control to less developed nations and it has allowed maintaining effective public policies to protect the health of consumers.

We believe it is necessary to enhance that the goal of Codex is to develop standards, guidelines and codes of harmonized international food practices intended to protect the health of consumers and to ensure fair practices in food trade. In this context, the determination of biotoxins must be made by using the official methods globally recognized; however, each competent authority should be able to decide the method to be used, in order to meet the two precepts above mentioned.

For all the above mentioned facts, Chile requests that the mouse bioassay method for determining saxitoxins is not indirectly excluded as a reference method, by establishing criteria such as LOD and LOQ, which are only applicable to methods of instrumental analysis. The bioassay is a monitoring tool widely used by regulatory bodies, because of its effectiveness, speed, low cost and which it has allowed, over the years, providing adequate guarantees for public health safeguarding.

CHILE (Spanish comments)

Chile agradece a los miembros del Comité la oportunidad de presentar observaciones a este documento que será sometido a consideración por parte del Comité.

En relación a los criterios de rendimiento para los métodos de referencia y confirmación para biotoxinas marinas, Chile manifiesta su preocupación respecto a que los criterios propuestos en la tabla, parecen excluir el método de carácter biológico conocido como bioensayo en ratón, distinguido mundialmente como el método de referencia oficial para saxitoxinas (AOAC 959.08).

En Chile, el Programa Nacional de Vigilancia y Control de las Intoxicaciones por Fenómenos Algales Nocivos, se basa en la determinación de toxina paralizante por medio del bioensayo en ratón (AOAC 959.08). Este programa, ha sido exitoso, como lo demuestran los datos recolectados en el período 2002-2012 que indican una clara disminución de las intoxicaciones notificadas en las zonas geográficas donde se producen grandes volúmenes de recursos hidrobiológicos y que son mayormente afectadas por este tipo de fenómeno (*Informe de Resultados, Programa Nacional de Vigilancia y Control de Intoxicaciones por Fenómenos Algales Nocivos en Chile para toxina paralizante de molusco 2002-2012, Instituto de Salud Pública, Ministerio de Salud, Chile*).

Como es sabido, las floraciones algales nocivas (FAN) son fenómenos dinámicos caracterizados por, cambios frecuentes del área litoral afectada, la distribución espacial, la estratificación, la concentración de microalgas e incluso la concentración celular de las toxinas presentes y su composición. Debido a esto, los programas implementados por cada país deben contar con metodologías de análisis que permitan cumplir con el objetivo principal de proteger la salud de la población expuesta al riesgo de intoxicación por consumo de mariscos contaminados, sean estos destinados a comercialización o autoconsumo, condición que es claramente cumplida por el método de bioensayo.

Para cumplir el objetivo de proteger la salud pública se requiere necesariamente conocer la toxicidad total del producto afecto. En este sentido, el método de referencia ideal debería poder lograr este objetivo en base a funciones biológicas, condición que los métodos químicos, a través de la identificación de múltiples análogos sólo pueden lograr mediante la determinación de la concentración de cada una de las toxinas presentes, lo que requiere contar con los respectivos estándares certificados. Conforme a esto, los métodos químicos pueden cuantificar la toxicidad total de una muestra solamente si se cumplen conjuntamente tres condiciones:

a) se detectan todas las toxinas presentes en una muestra,

b) se determinan los factores de toxicidad equivalentes para cada análogo y,

c) existen estándares certificados de referencia para todos los análogos.

En lo que respecta al grupo de las saxitoxinas, el bioensayo en ratón (AOAC 959.08) reúne las características requeridas como un método de referencia oficial a nivel mundial, que cumple con las normas del Codex (Principios para el establecimiento de métodos de análisis del CODEX), esto es, método oficial elaborado por una organización internacional cuya seguridad ha sido establecida, de simple practicabilidad para su uso habitual y válido para varios grupos de productos, considerando que permite determinar la toxicidad total directamente.

Las toxinas paralizantes constituyen un grupo de a lo menos 21 neurotoxinas estructuralmente relacionadas. Sin embargo, en la actualidad existen sólo 13 de estos compuestos disponibles como estándares analíticos a nivel comercial (Toxinas C1 y C2, dcGTX-2 y 3, dcNEO, dcSTX, GTX-1 y 4, GTX-2 y 3, GTX-5, NEO y STX) distribuidos por un único proveedor a nivel mundial. En este respecto, cabe destacar el caso del perfil tóxico de la especie *Gymnodinium catenatum*, que contiene toxinas GTX6, C3 y C4, para las cuales aún no hay estándares disponibles a nivel comercial

Es por esto, que los criterios propuestos para seleccionar métodos deben considerar la gran variabilidad de la distribución espacial, temporal, intrapoblacional e interindividual de las toxinas marinas en los moluscos, así como la disponibilidad limitada y variable de materiales de referencia certificados que las contengan.

Conforme a lo anterior, manifestamos claramente que Chile no desea que se excluyan los métodos de análisis químicos para múltiples análogos como métodos de referencia. Sin embargo, observamos que este es un campo de vigoroso desarrollo científico y tecnológico que aún no alcanza una etapa consenso internacional y por ello consideramos que el bioensayo en ratón es el método principal de referencia para saxitoxinas y debe ser reconocido como tal, dado que cumple con todos los requisitos anteriormente mencionados. De esta forma, Chile propone que se examinen cuidadosamente los criterios de rendimiento de los métodos, según lo establecido en la Tabla 1, Apéndice VII del documento REP13/FFP, para los grupos de toxinas y de esta forma no excluir, de manera indirecta, al bioensayo en ratón AOAC 959.08.

Grupo	**Toxina**	**Nivel máximo /kg en la carne de molusco**	**Intervalo mínimo aplicable**	**LOD**	**LOQ**	**Precisión**	**Porcentaje de recuperación**
Grupo de las saxitoxinas (STX)	Toxicidad total	≤ 0,8 miligramos (2HCL) de equivalente de saxitoxina	0,4 – 1.2	(*)	(*)	33%	70-120
Grupo del ácido okadaico (OA)	Toxicidad total	≤ 0,16 miligramos de equivalente de ácido okadaico	0,05 – 0.27	(*)	(*)	44%	70 - 120
Ácido domoico (DA)	Ácido domoico	≤ 20 miligramos de ácido domoico	13,2 – 26,8	2	4	22%	85-110
Grupo de la brevetoxina (BTX)	Toxicidad total	≤ 200 unidades ratón o 0,8 miligramos de equivalente de BTX2	74 – 326 MU (0.26 – 1,34 mg BTX2 eq.)	(*)	(*)	44%	70 - 120
Grupo de los azaspirácidos (AZP)	Toxicidad total	≤ 0,16 miligramos de equivalente de AZA1	0,05 – 0,27	(*)	(*)	44%	70 - 120

(*) No aplica

De manera particular, se propone consignar, en el caso específico de las saxitoxinas, el intervalo mínimo aplicable que va de 0,4 a 1,2 mg de equivalente de saxitoxinas HCl/Kg, en consideración a que los niveles de detección (LOD) y de cuantificación (LOQ) son parámetros aplicables estrictamente a análogos químicos individuales y que no son aplicables a un grupo de toxinas. Lo anterior, sumado a que el Manual de Procedimientos de Codex señala que los criterios de LOD / LOQ pueden establecerse como alternativa al rango mínimo aplicable.

Si bien, en general todos los métodos deben cumplir con requerimientos tales como LOD y LOQ, en el caso de aquellos análisis del tipo del bioensayo, al no tratarse de un método químico, el criterio de aceptabilidad en base a las directrices del Codex no sería adecuado. Chile propone que no se establezcan estos límites hasta acordar debidamente como se abordarán los LOD o LOQ cuando se trate de multianalitos, como así también su relación respecto de los factores equivalentes de toxicidad. Lo anterior, debido a que el enfoque

de los criterios establecidos en el Manual de Procedimientos del Codex aplica a los analitos en forma individual.

Sin duda, el método de bioensayo, no cuenta con la sensibilidad ni selectividad de un método cromatográfico, sin embargo, cuenta con la especificidad toxicológica requerida para la determinación de toxinas paralizantes permitiendo resguardar la salud de los consumidores, con un amplio margen de seguridad, lo cual ha sido demostrado durante 60 años, período en que esta metodología ha sido aplicada.

Por otra parte, es importante señalar que el bioensayo como método de detección de saxitoxinas, ha representado un adecuado control para naciones menos desarrolladas y ha permitido mantener políticas públicas efectivas, para la protección de la salud de los consumidores.

Consideramos que es necesario realzar que el objetivo de Codex es elaborar normas, directrices y códigos de prácticas alimentarias internacionales armonizadas destinadas a proteger la salud de los consumidores y asegurar prácticas equitativas en el comercio de los alimentos. En este contexto, la determinación de biotoxinas debe realizarse aplicando los métodos oficiales mundialmente reconocidos, sin embargo, cada autoridad competente debe tener la posibilidad de decidir el método a utilizar, con el fin de cumplir los dos preceptos antes mencionados.

Por todo lo señalado anteriormente, Chile solicita que el método de bioensayo en ratón para la determinación de saxitoxinas, no sea excluido de manera indirecta como método de referencia, estableciendo criterios tales como el LOD y LOQ, que son aplicables sólo a metodologías de análisis instrumental. El bioensayo constituye una herramienta de control utilizada ampliamente por los Organismos Reguladores, debido a su efectividad, rapidez, bajo costo y a que ha permitido a lo largo de los años, otorgar garantías adecuadas para el resguardo de la salud pública.

MOROCCO

Méthodes adoptees par le Maroc pour la surveillance et le contrôle des biotoxines marines

Toxines analysées	Méthodes de références utilisées
Toxines paralysantes (PSP)	Méthode du test biologique sur souris (AOAC 959.98, 2000)
Toxines lipophiles LSP: regroupant le groupe de l'acide okadaïque et dinophisistoxines (OADTXs) ainsi que le groupe des azaspiracides (AZP)	Méthode du test biologique sur souris (EURLMB, version 5 juin 2009)
Toxines amnésiantes (ASP): groupe de l'acide domoïque (AD)	HPLC/UV (EURLMB, version 1, juin 2008)

PHILIPPINES

General Comments:

The Philippines supports the inclusion of the proposed criteria in Sec. I-8.6 and recommends the inclusion of the mouse bioassay along with the numerical criteria values for biotoxin in Table 1 following the agreement reached during the 32nd CCFFP Session to include the said test. Moreover, the Philippines also concurs with the 34th CCMAS Session to include the toxicity factors in Table 2.

Specific Comments:

Inclusion of Numerical Criteria Values for Biotoxins in Bivalve Molluscs, to include specific LOD and LOQ for the mouse bioassay (MBA) in Table 1 under I-8.6.1 as follows:

Table 1.

Group	Toxin	Methods	Maximum Level/kg of Mollusk flesh	Minimum Applicable Range	LOD	LOQ	Precision (RSDR)	Recovery Percent
Saxitoxin (STX) group	Total Toxicity	**Mouse Bioassay**	**< 0.8 milligrams (2HCl) of saxitoxin equivalent**	**0.4-1.2**	**0.35**	**0.70**	**33%**	**70-120**
		Other Methods	≤ 0.8 milligrams (2HCl) of saxitoxin equivalent	0.4-1.2	0.08	0.16	33%	70-120
Okadaic Acid (OA) group	Total Toxicity		≤ 0.16 milligrams of okadaic acid	0.05-0.27	0.016	0.032	44%	70-120
Domoic acid (DA) group	Domoic Acid		≤ 20 milligrams domoic acid	13.2-26.8	2	4	22%	85-110
Brevetoxin (BTX)	Total Toxicity		≤ 200 Mouse Units or (0.8 milligrams BTX2 equivalent)	74-326 MU (0.26-1.34 mg BTX2 eq.)	20 (0.08)	40 (0.16)	44%	70-120
Azaspiracid (AZA group)	Total Toxicity		≤ 0.16 milligrams AZA 1 equivalent	0.05-0.27	0.016	0.032	44%	20-120

Justification:

The CCFFP 32nd Session had endorsed the adoption of MBA for screening tests. Likewise, the MBA conforms well with the first and second criteria on the selection of the type of method to be used and their practicability and applicability for routine use.

Suggest:

The Philippines recommends the inclusion of a column on toxicity factor.

Table 2. Toxin Analogues to consider

Group	Toxin	Toxicity Factor*
Saxitoxin (STX) group	Saxitoxin (STX)	**1.00**
	Neosaxitoxin (NEO)	**0.92**
	Decarbamoyl-saxitoxin (dcSTX)	**0.51**
	Decarbamoyl-neosaxitoxin (dcNEO)	
	Gonyautoxin-1 (GTX1)	**0.99**
	Gonyautoxin-2 (GTX2)	**0.36**
	Gonyautoxin-3 (GTX3)	**0.64**
	Gonyautoxin-4 (GTX4)	**0.73**
	Gonyautoxin-5 (B1)	**0.06**
	Gonyautoxin-6 (B2)	
	Decarbamoyl-gonyautoxin -2(dcDTX2)	**0.65**
	Decarbamoyl-gonyautoxin -3(dcDTX3)	**0.75**
	N-sulfocarbamoyl-gonyautoxin-1(C3)	**0.01**
	N-sulfocarbamoyl-gonyautoxin-2(C1)	**<0.01**
	N-sulfocarbamoyl-gonyautoxin-3(C2)	**0.01**
	N-sulfocarbamoyl-gonyautoxin-4(C4)	**0.06**

*Source: Oshima 1995

Reference:

Ohima, Y. 1995. Post-Column derivatization HPLC methods for paralytic shellfish poisons. In Manual on Harmful Marine Microalgae, G.M. Hallegraeff, D.M. Anderson, A.D. Cembella and H.O. Enevolsen (eds), IOC, Manuals and Guides No. 33, UNESCO, Paris, pp. 81-94

Justification

This is in conformance with the suggestion of the CCMAS and as guidance.

USA

General Comments

The U.S. believes that the criteria require additional modification in order to ensure that they reflect current scientific understanding, are practical for implementation by Codex members, and effectively protect public health. In particular, we support the principle contained in paragraph 2 of Section 1-8.6, i.e., that methods "should be chosen on the basis of practicability and that preference should be given to methods which have applicability for routine use." If the criteria do not ensure that methods meeting them are practical for routine use by Codex members, then public health protection cannot be ensured. In our view, the paralytic shellfish poison (PSP) AOAC 959.08 mouse bioassay is the gold standard, as it is the only method that can determine saxitoxin-equivalents and is practical for routine use by all member countries.

We would like to make a few points that we believe are important to consider in review of the draft criteria:

- Criteria must be based on the total toxicity equivalent limit in the Bivalve Standard because that is the applicable measure and protects public health. Criteria for individual toxin congeners are not valid and may not protect the public.
- Criteria must include the paralytic shellfish poison (PSP) AOAC 959.08 mouse bioassay because, as stated above, it is technically the only method that can determine saxitoxin-equivalents. It is also the only method that meets the need for a practical method for routine use by all member countries. (PSP HPLC laboratories are expensive and impractical for many countries.)
- Multi-congener methods must include toxicity equivalent factors (TEFs) to be applicable to the total toxicity equivalence limits in the Standard, and these TEFs must be uniform across all methods qualifying as reference methods under the criteria. Because TEFs are essential to the applicability of the method, they need to be included in the criteria.
- TEFs are determined by intraperitoneal (IP) mouse bioassay because the 0.8 mg/kg saxitoxin-equivalent limit is based on IP mouse bioassay of shellfish causing human illness. We question the applicability of oral TEFs based on mice because mouse oral toxicity may not correspond to human oral toxicity. In addition, the toxin profiles used to establish the maximum limit would have included the congeners that are less toxic orally than by IP injection. Since the maximum limit is based on the consumption of congener mixtures, the limit may not provide a sufficient safety margin if only pure saxitoxin were ingested, without other congeners that are less toxic orally than by IP injection. Because of this, mouse oral TEFs should not be standardized to the IP toxicity of pure saxitoxin, as this would lower the TEFs overall and effectively lower the established safety limit when using multi-congener methods. Orally-based TEFs will be difficult to establish without human challenge studies, and a re-evaluation of the current limit.
- Codex method conversion into criteria only applies to single-analyte methods. Validation data for multi-congener methods (that determine a single total toxicity value) cannot be converted into general criteria because the data are inherently for single congeners and some congener mix subsets. These data are not directly applicable to performance analyzing the variety of congener mixtures possible in nature. Because of limited validation data, and toxin profiles that are difficult to analyze, multi-congener methods cannot be determined to meet reference method criteria before the analysis is performed and the toxin profile identified.

The Draft Criteria recognize this limitation, and require that multi-congener methods perform for the toxin profile encountered. Under the Draft Criteria, a method is determined to meet the criteria after the analysis of all the congeners that can contribute to toxicity. In most circumstances, the HPLC method will easily comply with the criteria because only a few toxin congeners predominate; however, for certain toxin

profiles/matrices, performance calculations will show that the method is non-compliant. Countries can practically determine when the method easily applies, and when to calculate estimated performance and/or perform additional validation studies. This approach assures that multi-congener methods are used appropriately as reference methods without risking public safety.

In addition, we would observe that AOAC method 2005.06 for PSP, currently listed in the Standard, is not valid as a reference method because it does not determine saxitoxin equivalents, and therefore is not applicable to the limit in the Standard. If the criteria approach does not replace this method, it should be removed. As recommended by the FAO/IOC/WHO Expert Report[1], the AOAC mouse bioassay is the best single PSP reference method to list in the Bivalve Standard.

Finally, we note that there has been some confusion about Codex Method Type (I/II) terminology. The intent of Type I classification is to indicate that only one method can be used to establish the accepted value of the provision in the standard. Since technically the PSP mouse bioassay is the only method that can determine saxitoxin equivalents, it could be listed as the reference method in the Standard, eliminating continued debate over TEFs and which methods meet criteria.

In addition to requesting comments on the Draft Performance Criteria, CL 2013/16/FFP also requested information on the TEFs for all biotoxins listed in the Standard, as requested by the Codex Committee on Methods of Analysis and Sampling (CCMAS). The U.S. would favor revision of the criteria to include the TEFs proposed in Norway's comments, along with the calculations used to determine that a multi-congener method meets the criteria for the toxin profile encountered.

Specific Comments

General: We suggest numbering each paragraph in the Draft Criteria for easy reference.

Title: Edit as follows:

> Proposed Draft Performance Criteria for **Assays and Multi-Analog** Reference ~~and Confirmatory~~ Methods for Marine Biotoxins in the Standard for Live and Raw Bivalve Molluscs

Reason:

- To assure understanding that the criteria are intended to include assays, such as the mouse bioassay.
- Only reference methods are needed for the Bivalve Standard. Confirmatory methods, such as mass spectrometry, peak shifting/conversion, spiking, etc., used to confirm a questionable HPLC result, are beyond the scope of these criteria.

I-8.6 Determination of Biotoxins (1st paragraph) - edit as follows:

> ~~Type II and Type III~~ **Reference** methods should be selected 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.*

Reason: Type should not be listed because, by default, methods meeting criteria are Type II/III methods. If there is a Type I method, then criteria cannot exist. Codex Method Type is based on review by CCMAS.

I-8.6 Determination of Biotoxins (3rd paragraph) – edit as follows:

All Method**s** ~~shall~~ **should** meet the numerical criteria listed in Table 1 ~~and may either need the minimum applicable range, or LOD and LOQ criteria listed~~.

Reason:

- "All" is used because some of the proposed criteria are only applicable to multi-congener methods.
- See comment below, proposing removal of LOD/LOQ criteria from Table 1.
- Table 1 should follow this provision that refers to Table 1.

I-8.6 Determination of Biotoxins (4th paragraph) – Move this provision directly after Table 2 (toxin list), and edit as follows:

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[1] Report of the Joint FAO/IOC/WHO ad hoc Expert Consultation on Biotoxins in Bivalve Molluscs, Oslo, Norway, Sept. 26-30, 2004: http://www.fao.org/docrep/007/y5486e/y5486e00.htm#Contents

Multi-analogue methods **are determined to meet** total toxicity criteria ~~are established~~ for **the** toxin profile~~s~~ encountered **based on** ~~using~~ **appropriate** validation study data.

The appropriate calculations are shown below:

Reason:

- Validation studies are used to determine compliance with criteria.
- A section needs to be reinstated that shows how to determine if a multi-congener method meets the criteria to ensure better understanding.
- This provision and new section is best positioned after Table 2.

I-8.6.1 Numerical Criteria Values for Biotoxins in Bivalve Molluscs – Remove section number, and use the text as the heading for Table 1, as follows:

Table 1, Numerical Criteria Values for Biotoxins in Bivalve Molluscs

Reason: Editorial. There is no second section.

Table 1, LOD/LOQ: Remove the LOD and LOQ columns.

Reason:

- The Codex Procedural Manual clearly indicates that criteria may use LOD/LOQ as an alternative to Minimum Applicable Range.
- Listing both causes confusion and is unnecessary because they both measure essentially the same thing.

I-8.6 Determination of Biotoxins (5th paragraph) – Remove as follows:

~~International scientifically validated toxicity equivalent factors (TEFs) must be used to calculate total toxicity for methods that do not measure total toxicity directly.~~

Reason:

- We have added TEFs back into the draft in our comments, therefore this provision is no longer needed. Mouse IP TEFs are used for limits based on mouse IP studies
- TEFs must be included with the criteria in order for multi-congener method criteria to be applicable to the provisions in the Standard.
- TEFs are needed with the criteria in order to determine multi-congener method compliance with criteria for different toxin profiles.

I-8.6 Determination of Biotoxins (6th paragraph) – Edit as follows.

Methods that do not measure total toxicity directly be should be validated **for,** and used **to detect, all** ~~for~~ the ~~relevant~~ toxin analogues that may **possibly** contribute to total toxicity. Currently known **applicable** toxin analogues ~~to consider~~ are listed in Table 2.

Reason:

- Multi-congener methods must be able to detect applicable toxin congeners in order to be a reference method, and in order to determine if the method meets the criteria for a reference method for the toxin profile encountered.

Table 2, Toxin analogues to consider – Edit Table heading as follows:

Table 2, **Applicable** Toxin **A**nalogues ~~to consider,~~ **and Toxicity Equivalence Factors for Multi-Analogue Reference Methods**

Table 2, Toxin analogues to consider - Add toxicity equivalence factors (TEFs) as follows:

Group	Toxin	Toxicity Equivalence Factors
Saxitoxin (STX) group	Total Toxicity	
	Saxitoxin (STX)	**1**
	Neosaxitoxin (NEO)	**1**
	Decarbamoyl-saxitoxin (dcSTX)	**0.6**
	Decarbamoyl-neosaxitoxin (dcNEO)	**0.4**
	Gonyautoxin-1 (GTX1)	**1**
	Gonyautoxin-2 (GTX2)	**0.4**
	Gonyautoxin-3 (GTX3)	**0.6**
	Gonyautoxin-4 (GTX4)	**0.7**
	Gonyautoxin-5 (B1)	**0.1**
	Gonyautoxin-6 (B2)	**0.1**
	Decarbamoyl-gonyautoxin-2 (dcGTX2)	**0.2**
	Decarbamoyl-gonyautoxin-3 (dcGTX3)	**0.4**
	N-sulfocarbamoyl-gonyautoxin-1 (C3)	**0.01**
	N-sulfocarbamoyl-gonyautoxin-2 (C1)	**0.006**
	N-sulfocarbamoyl-gonyautoxin-3 (C2)	**0.1**
	N-sulfocarbamoyl-gonyautoxin-4 (C4)	**0.1**
	11-hydroxy-STX	**0.3**
Okadaic acid (OA) group	Total Toxicity	
	Okadaic acid (OA)	**1.0**
	Dinophysistoxin-1 (DTX1)	**1.0**
	Dinophysistoxin-2 (DTX2)	**0.5**
	Esters of OA, DTX1 and DTX2 (FA-ESTERS)	**Parent TEF**
Domoic acid (DA) group	Domoic Acid (DA)	
Brevetoxin (BTX) group	Total Toxicity	
	Brevetoxin-1 (BTX1)	
	Brevetoxin-2 (BTX2)	
	Brevetoxin-1 derivatives (devBTX1)	
	Brevetoxin-2 derivatives (devBTX2)	
Azaspiracid (AZP) group	Total Toxicity	
	Azaspiracid-1 (AZA1)	**1.0**
	Azaspiracid-2 (AZA2)	**1.8**
	Azaspiracid-3 (AZA3)	**1.4**

Reason: See comments above. To include the TEFs included in Norway's comments, which are averages, properly rounded to one significant figure.

New Section – Add a new section after the revised 4th paragraph that was moved after Table 2, as follows (also showing moved 4th paragraph):

Multi-analogue methods are determined to meet total toxicity criteria for the toxin profile encountered based on appropriate validation study data.

The appropriate calculations are shown below:

i. **To determine method compliance with "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.**

ii. To determine method compliance with "Precision (RSD_R)" criteria: The method's total toxicity precision (RSD_R), for a sample near 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+...} = $\sqrt{sd\{a\}^2 + sd\{b\}^2 + sd\{c\}^2 + \ldots}$

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 $RSD_{R\{a\}}$ at the nearest concentration, e.g.,

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

iii. To determine method compliance with "Recovery percent" 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:

- Needed for better understanding of application of the criteria, and to show how multi-congener methods can be determined to meet total toxicity.
- These calculations were introduced with the Draft Criteria used as a basis for discussion during the CCFFP 32nd Session, in Bali. Several members of the in-session working group, who had reviewed the proposal beforehand, noted agreement with the calculations, however, the calculations were removed from the Draft Criteria because some countries did not have time to review them.
- While some HPLC methods may not meet the criteria for some theoretical toxin profiles (e.g., low levels of every applicable toxin, or high levels of toxins with high RSD for the matrix), countries can use real regulatory results to determine that HPLC methods do meet the criteria for toxin profiles generally encountered.