Proposal Subject: Correction of the wording for the action level for NSP toxins and the incorporation of action levels for AZP and DSP toxins in shellfish in the Guide.

Specific NSSP Guide Reference: Section II. Model Ordinance Chapter IV. Shellstock Growing Areas
@.04 Marine Biotoxin Control C. (1)

Section IV. Guidance Documents Chapter II. Growing Areas
.04 Action Levels, Tolerances and Guidance Levels for Poisonous or Deleterious Substances in Seafood

Text of Proposal/ Requested Action In Section II Model Ordinance, Chapter IV. Shellstock Growing Areas @.04 Marine Biotoxin Control C. (1), correct the wording for NSP toxins and add the action levels for azaspiracids (AZP) and DSP toxins, as follows:

C. Closed Status of Growing Areas.

(1) A growing area, or portion(s) thereof as provided in §A.(4), shall be placed in the closed status for the taking of shellstock when the Authority determines that the number of toxin-forming organisms in the growing waters and/or the level of biotoxin present in shellfish meats is sufficient to cause a health risk. The closed status shall be established based on the following criteria:

PSP – cells/L n/a; 80 µg/100 grams
NSP – 5,000 cells/L or 20 MU/**100 grams** (~~approximate as 80 µg/100 g~~**0.8 mg brevetoxin-2 equivalents/kg)**
AZP – cells/L n/a; 0.16 mg AZA-1 equivalents/kg (0.16 ppm)
DSP – cells/L n/a; 0.16 mg OA equivalents/kg (0.16 ppm)
ASP – cells/L n/a; 2 mg/100 grams (20 ppm)

(a) The concentration of paralytic shellfish poison (PSP) equals or exceeds 80 micrograms per 100 grams of edible portion of raw shellfish; or

(b) For neurotoxic shellfish poisoning (NSP), the harvesting of shellstock shall not be allowed when:
(i) The concentration of NSP equals or exceeds 20 mouse units per 100 grams of edible portion of raw shellfish; or
(ii) The cell counts for *Karenia brevis* organisms in the water column exceed 5,000 per liter; or

I For domoic acid, the toxin concentration shall not be equal to or exceed 20 ppm in the edible portion of raw shellfish.

(d) For azaspiracid shellfish poisoning (AZP), the concentration of azaspiracids shall not be equal to or exceed 0.16 mg/kg (AZA-1 equiv.) in the edible portion of raw shellfish.

(e) For diarrhetic shellfish poisoning (DSP), the concentration of DSP toxins shall not be equal to or exceed 0.16 mg/kg (OA equiv.) in the edible portion of raw shellfish.

And under the Natural Toxins section of Table 1 of the Guidance Documents: Chapter II-Growing Areas; .04 Action Levels, Tolerances and Guidance Levels for Poisonous or Deleterious Substances in Seafood, correct and insert the following:

Substance	Level	Food Commodity[a]	Reference
Neurotoxic Shellfish Poisoning (NSP) toxins	20 MU/100g	Clams, mussels, oysters, fresh frozen or canned	NSSP MO
Azaspiracid Shellfish Poisoning (AZP) toxins	0.16 mg/kg	Clams, mussels, oysters, fresh frozen or canned	NSSP MO
Diarrhetic Shellfish Poisoning (DSP) toxins	0.16 mg/kg	Clams, mussels, oysters, fresh frozen or canned	NSSP MO

Public Health Significance:

NSP Toxins

Neurotoxic shellfish poisoning (NSP) is caused by consumption of shellfish contaminated with brevetoxins. Brevetoxins are a group of lipophilic neurotoxins produced by the marine dinoflagellate *Karenia brevis* and other algal species (e.g., *Chattonella* spp.). Brevetoxins are accumulated and extensively metabolized in filter-feeding molluscan shellfish. Toxicity of shellfish has been historically assessed by mouse bioassay, while efforts are underway to validate alternative methods of analysis (e.g., LC-MS, immunoassay). Shellfish exhibiting any detectable level of toxicity by mouse bioassay are considered potentially unsafe for human consumption. In practice, a value of 20 MU/100 g shellfish tissue has been considered the regulatory limit by the States. Expressed in brevetoxin-2 (PbTx-2) equivalents, this level is 0.8 mg/kg in shellfish tissue. Method alternative to mouse bioassay must provide an equivalent level of public health protection.

The requested action is editorial corrections to the Guide with respect to the current action level.

AZP Toxins

Azaspiracids (AZA) are a group of lipophilic marine algal toxins that accumulate in various shellfish species (Twiner et al., 2008). Consumption of AZA-contaminated shellfish causes the acute illness azaspiracid shellfish poisoning (AZP). AZP is characterized by severe gastrointestinal disturbances; symptoms include nausea, vomiting, diarrhea, abdominal pain and cramps. AZA were first discovered in 1995 following an outbreak linked to consumption of Irish mussels. Since then, several documented outbreaks of AZP have been reported in Europe, and AZA have been isolated from shellfish along the European Atlantic coast from Norway to Portugal, and in Morocco. In 2008, the first recognized cases of AZP in the U.S. were reported, and linked to consumption of imported mussels from Ireland (Klontz et al., 2009). The finding of AZA in the imported product highlights the concern for the consumer safety of molluscan shellfish marketed internationally.

The first risk assessment for AZA was conducted by the Food Safety Authority of Ireland (FSAI) in 2001. In 2002, the European Commission set the regulatory limit for AZA (AZA-1, -2, and -3) at 0.16 mg/kg, based on the FSAI data and the limit believed to be detectable by mouse bioassay (EC, 2002). This regulatory limit was strengthened by a second risk assessment conducted by the FSAI (FSAI, 2006). The latter incorporated new data with respect to tissue distribution of AZA in mussels, ratios of different analogues, and the effects of cooking. The calculated median acute reference dose (ArfD, 0.63 µg/kg b.w.) was comparable to the intake value for a 60 kg individual consuming 250 g mussels contaminated with AZA at the 0.16 mg/kg regulatory limit.

EC regulation allows for the use of alternative methods (e.g., LC-MS, immunoassay) to the reference test (mouse bioassay) for AZA in shellfish (EC,2005). These methods must

be capable of detecting the AZA analogues AZA-1, -2, and -3. And they must provide an equivalent level of public health protection to the biological method. The EU-harmonized mouse bioassay and LC-MS methods were recently demonstrated equivalent in their effectiveness in implementation of this regulatory limit (Hess et al., 2009).

The FSAI risk assessment did recognize the uncertainties inherent in its outcome, particularly relating to limitations in the available epidemiological data. Moreover, the toxicity of AZA analogues, and their distribution and metabolism in various shellfish species, have not been well characterized. Chronic and low dose effects of AZA are unknown. Refinement of the risk assessment and revision of regulatory limit may be necessary when additional toxicological and epidemiological data become available.

The requested action is adoption of a regulatory limit for azaspiracids (AZA) of 0.16 mg/kg in molluscan shellfish, in accordance with that set by the European Commission (EC, 2002). By using LC-MS, this limit is based on the sum of the individual azaspiracid toxin analogues AZA-1, -2, and -3, expressed in AZA-1 equivalents. AZA-1 is the only certified analytical standard presently available. AZA-1 equivalents of AZA-2 and -3 are calculated by weighting their relative response factor (RRF)-corrected concentrations with their toxic equivalence factors (TEFs). TEF multipliers derived from initial studies on mice are 1, 1.8, and 1.4 for AZA-1, -2, and -3, respectively (Ofuji et al., 1999).

DSP Toxins

Diarrhetic shellfish poisoning (DSP) is caused by consumption of molluscan shellfish contaminated with toxins of the okadaic acid (OA) group, the origin of which is principally marine dinoflagellates (e.g., *Dinophysis, Prorocentrum* spp.) DSP is characterized by acute gastrointestinal disturbance (e.g., diarrhea, nausea, vomiting, abdominal pain). Toxins responsible are primarily okadaic acid (OA) and the related dinophysistoxins (DTXs) and their acyl esters. Pectenotoxins (PTX) and yessotoxins (YTX) may co-occur, the former of similar toxic potency.

DSP outbreaks were first reported in 1976 in Japan, and in the 1980s in Europe. The first documented outbreak in N. America occurred in 1990, in eastern Canada (Qulliam et al., 1993). There have been no reported cases of DSP to date in the U.S. However, in 2008, toxin-producing *Dinophysis*, and DSP toxins in shellfish above the proposed action levels, were recorded for the first time in the Gulf of Mexico (Deeds, pers. comm.). *Dinophysis* has been found along the east and west coast of the U.S. Since DSP toxin-producing organisms occur throughout the world, DSP toxins in molluscan shellfish are a significant public health concern.

DSP toxins in shellfish have been assessed traditionally by mouse bioassay, and more recently by instrumental methods (LC-FTD, LC-MS), immunoassay, and pharmacology-based assays (protein phosphatase assay). Current EU regulatory limit is 0.16 mg OA equivalents/kg shellfish meat (EC, 2002, 2005). This level represents the sum of that of OA, DTXs, and PTXs. Methods alternative to mouse bioassay incorporate a base hydrolysis step for conversion of DTX acyl esters to free acid forms.

The requested action is adoption of a regulatory limit for DSP toxins of 0.16 mg/kg (OA equivalents) in molluscan shellfish. This limit is based on the sum of OA, DTXs (including acyl esters), and PTXs. Revision of regulatory limit may be necessary when additional toxicological and epidemiological data become available.

References

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Cost Information (if available):

Action by 2009 Task Force I

Recommended referral of Proposal 09-101 to an appropriate committee as determined by the Conference Chairman. The Committee should be directed to gather more information on the standards, methods and costs.

Action by 2009 General Assembly

Adopted recommendation of 2009 Task Force I on Proposal 09-101.

Action by USFDA 02/16/2010

Concurred with Conference action on Proposal 09-101.