STATE OF DELAWARE

Department of Natural Resources
And Environmental Control

DIVISION OF WATER RESOURCES
WATERSHED ASSESSMENT SECTION
SHELLFISH AND RECREATIONAL WATER PROGRAMS

Marine Biotoxin Contingency Plan
2009 Update

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DELAWARE MARINE BIOTOXIN CONTINGENCY PLAN - 2009

I. General Plan Specifications

A. Introduction & Background

Delaware is a member of the Interstate Shellfish Sanitation Conference (ISSC), the administrative body of the National Shellfish Sanitation Program (NSSP), a tripartite organization made of State, Federal, and Industry representatives. NSSP guidance documents provide the public health principles supporting major components of the NSSP and its Model Ordinance (MO) pursuant to regulating the human health-protection elements associated with the growing, harvesting, processing, and interstate transport of molluscan shellfish.

The specifications contained herein provide for the monitoring of marine and estuarine shellfish growing waters for phytoplankton typically associated with the production of toxins. In addition, a total inventory of phytoplankton – including non-toxin-producing species measured as present, common, abundant, actual concentrations, and/or relative abundance – shall be taken both in efforts relating to routine surveillance and in response to blooms. Baseline numeric or cell density data may be used to assess potential toxicological effects. Emergency-closure protocol may be invoked as per Title 7 of the Delaware Annotated Code, as the M.O. is adopted by reference in the "Delaware Shellfish Sanitation Regulations."

Shellfish are filter feeders, and have the ability to concentrate toxigenic dinoflagellates from the water column when present in shellfish growing waters. The toxins produced by these dinoflagellates can cause illness and death in humans. Toxins are accumulated in the viscera and/or other tissues of shellfish, and are transferred to humans when the shellfish are eaten (Gordan et al, 1973). These toxins are not normally destroyed by cooking or processing, and cannot be detected by taste. Most of these toxins are detected through animal testing. However, some involve the use of instrument based or biochemical analyses for detection. Since the dinoflagellates are naturally occurring, their presence in the water column, or traces of their toxin in shellfish meat, does not necessarily constitute a health risk, as toxicity is dependent on various environmental "triggers" for toxin production, and concentration (dose) in the shellfish.

There are three types of shellfish poisonings which are specifically addressed in the NSSP Model Ordinance: paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), and amnesic shellfish

poisoning (ASP), also known as domoic acid poisoning. All three are dangerous toxins. PSP and ASP (or domoic acid) can cause death at sufficiently high concentrations. In addition, ASP can cause lasting neurological damage. PSP is caused by dinoflagellates of the genus *Alexandrium* (formerly *Gonyaulax*). NSP is caused by brevetoxins produced by the dinoflagellates of the genus *Karenia* (formerly *Gymnodinium*). Both of these dinoflagellates can produce "red tides", i.e. discolorations of seawater caused by blooms of the algae. Toxic blooms of these dinoflagellates can occur unexpectedly, or follow predictable patterns. The unpredictably in occurrence of toxic blooms was demonstrated in New England in 1972 when shellfish suddenly became toxic in a previously unaffected portion of the coastline, which resulted in many illnesses (Schwalm.1973). Historically, *Alexandrium* blooms have occurred between April and October along the Pacific coasts, from Alaska to California, and in the Northeast, from the Canadian Provinces to Long Island Sound (U.S. Public Health Service, 1958). The blooms generally last only a few weeks, and most shellfish (with the exception of clams which retain the toxin for longer periods) clear themselves rapidly of the toxin once the bloom dissipates. Global warming may be playing a role with regard to distribution of potentially toxic algae species outside of their traditional range, and with regard to duration of blooms.

The minimum concentration of PSP toxin that will cause intoxication in susceptible persons is not known. Epidemiological investigations of PSP in Canada; however, have indicated 200 to 600 micrograms of PSP toxin will produce symptoms in susceptible persons. A death has been attributed to the ingestion of a probable 480 micrograms of PSP toxin. Investigations indicate that lesser amounts of the toxin have no deleterious effects on humans. Shellfish growing areas should be closed at a PSP toxin level, which provides an adequate margin of safety, since in many instances PSP toxicity levels can change rapidly. The NSSP Model Ordinance requires that growing areas be placed in the closed status when the PSP toxin concentration is equal to or exceeds the action level of 80 micrograms per 100 grams of edible portion of raw shellfish (FDA, 1977; FDA, 1985).

In shellfish growing areas where low levels of PSP routinely occur, harvesting for thermal processing purposes may be an alternative to consider. Thermal processing as defined by applicable FDA regulations (21 CFR 113) will reduce but not entirely destroy the PSP content in the shellfish. If thermal processing is practiced, a state's shellfish program authority must develop and implement procedures to control the harvesting and transportation of the affected shellfish to the processing plant.

NSP, which is less common, had been documented to have occurred near Ocracoke Inlet in 1987; however, more commonly further south, particularly in Florida and throughout the Gulf Coast. Delaware experienced a *Karenia spp* bloom (including K. papillonacea and *K. brevis*) in August and September, 2007, with confirmed maximum coastal concentrations of 14,000 cells / liter (Whereat, 2007). The transport mechanism is believed to be an eddy from the Gulf Stream. At those concentrations, no discolored water or other physical signs of the bloom were detected. The bloom was detected via routine surveillance. Bloom-related elevated chlorophyll levels are generally recognized to be visible via satellite at concentrations ranging from 50,000 to 100,000 cells / L. (K.p. is not proven to produce brevetoxin under laboratory conditions. However, New Zealand has a K.p. shellfish bed closure threshold >100K/L.)

In Gulf coast areas, toxicity in shellfish has been associated with red tide outbreaks caused by massive blooms of *K. brevis*. The most common public health problem associated with such blooms is respiratory irritation; however, neurotoxic shellfish poisonings associated with K. *brevis* blooms have been reported in Florida (Center for Disease Control, 1973 [a] and [b]). Uncooked clams from a batch eaten by a patient with neurotoxic symptoms were found to contain 118 mouse units per 100 grams of shellfish meat. However, concentrations of >20 MU/100 grams of shellfish meat were confirmed by Karen Steidinger (Heil, 2007). The NSSP Model Ordinance mandates that growing areas be placed in the closed status when any NSP toxin is found in shellfish meat, or when the cell counts for *K. brevis* in the water column exceed 5,000 cells / liter (cells / L). However, as blooms are often transient in nature, and/or not uniform in concentration and distribution, toxin levels in molluscan shellfish meats, as determined by approved methods, shall take precedence in determining the need for, and/or extent of shellfish growing area closures precipitated by the presence of *K. brevis* in Delaware waters.

ASP is caused by domoic acid, which is produced by diatoms of the genus *Pseudonitzachia*. Blooms of *Pseudonitzachia spp*. are of relatively short duration. However, during the 1991-1992 incident in Washington, high toxin levels persisted for several months (Liston, 1994). The NSSP Model Ordinance requires that growing areas be placed in the closed status when the domoic acid concentration is equal to or exceeds 20 parts per million in the edible portion of raw shellfish.

B. Characterization of Delaware Waters Relative To Algae Blooms

The presence of the above causative organisms has been documented in Delaware waters; however, not typically in sufficient concentrations to cause

toxicological effects. Typically, on the East Coast of North America, the presence of the above organisms in concentrations sufficient to cause toxicological effects in humans occur only in waters to the north of, or to the south of Delaware. This is due to an accident of geography, latitude, and Ocean currents. The Gulf Stream laps at the shore of the Outer Banks, coming within twenty to thirty miles of Cape Hatteras. Despite periodic eddies, frequent, routine surveillance for algae – both potentially toxic and non-toxin-producing species – reveals that the Gulf Stream is not generally a conduit for southern species of potentially toxin-producing phytoplankton entering into Delaware's near-shore environment, despite its movement at approximately three feet per second. However, there are exceptions, such as the 2007 Karenia spp. bloom. The late-season propensity for northeast winds increases the probability for development of an Gulf Stream eddy developing – the probable transport mechanism for the Kareina bloom. (However, Karenia spp prefer low-nutrient water – not a characteristic of the Delaware near-shore environment. As such, such blooms are potentially relegated to a short duration once in near-shore Delaware waters.) The general / net movement of Ocean water to Delaware is south due to the Labrador current. However, water temperatures become too warmed at these latitudes to support northern species of potentially toxic phytoplankton at bloom-like concentrations.

Alexandrium sp. is not known to exist in bloom-like concentrations south of Cape Cod. In the U.S. it is primarily a problem on the West Coast, and in George's Bank, on the East Coast. *Pseudonitzchia spp*. are primarily, also, a problem in cooler waters. *Karenia brevis* is primarily a warm-water species / phenomenon.

Of particular concern in Delaware are *Dinophysis spp*., and *Prorocentrum spp*. during the cooler months, with a caution about *Pseudonitzchia spp*. primarily, also, during the cooler months. Both routine and emergency-response sampling have revealed *Dinophysis spp*. (including *D. norvegica*) and *Prorocentrum spp*. at significant levels; however, not at bloom-like concentrations. *Pseudonitzchia pungens* and *seriata*, as well as *Chatonella spp*., and *K. mikimotoi*, believed to produce brevetoxin, have been found in Delaware waters. In addition, *Pfiesteria spp*. are also common to abundant.

Other potentially toxic species commonly found in Delaware waters include; but are not limited to: *Chaetocerosis spp*., *Karlodinium veneficum*, *Ditylum spp*., *Rhizosolenia spp*., *Anabaena spp*., *Amphidinium spp*., *Ceratium spp*., *Heterosigma akashiwo*, *Chatonella spp*.

Non-toxin-producing species most commonly found in Delaware waters include; but are not limited to: *Heterocapsa rotundata*, *Kryptoperidinium*

foliaceum, *Scrippsiella spp*., As well as *Chaetoceros spp*. and *Skeletonema costatum* (diatoms). Other species are also present at background or periodic bloom-like concentrations, with new discoveries occurring at a high frequency.

C. Biotoxin Control Plan Specifications

These procedures are instituted to provide constructive notice to the public regarding toxic or potentially-toxic algae blooms (early warning system), and to define the severity and extent of the occurrence pursuant to minimizing illness. Protocol for algae identification, administrative procedures, laboratory support, sample collection procedures, and patrol procedures, are contained herein, including adequate surveillance, public notification, including both a characterization of the severity of occurrences, and contingencies to minimize illness, including shellfish bed closures, and returning shellfish beds to the Approved / open status. Parameter organisms shall include; but are not limited to all those listed herein.

1. Sample Observation, Analyses, and Speciation

Observations of toxic and non-toxic phytoplankton (genus profiles, and species identification when such expertise allows it) shall be made to augment existing data regarding the presence of potentially toxic phytoplankton, and shall constitute baseline information as to typical and normal concentrations of said organisms.

Field observation (sample collection and analyses / speciation) and satellite and/or aerial surveillance shall take place when ever practicable, and at a minimum of two times per month during warmer months, and whenever practicable during cooler months. Observations shall be made to augment existing data regarding the presence of potentially toxic phytoplankton, and shall constitute baseline information as to typical and normal concentrations of said organisms. Additionally, excursions in response to possible blooms shall be conducted for the purpose of making observations as to the concentration of these organisms relative to baseline concentrations, and any possible effects that may have occurred or may be occurring at the time of the excursion. Effects may include: dead or dying fish; behavioral anomalies in fish; dead, dying, or behavioral anomalies in fish predators; discolored water (may be mahogany or milky colored); an "off-odor" to the water (may be a "sweet" smell); or neurological effects in humans which may include disorientation, a tingling sensation, asthma-like symptoms, or neuromuscular effects. These effects may be induced by direct water

contact, consumption of contaminated shellfish, or due to the aerosolizing of the toxins.

2. Emergency Observation, Analyses, and Speciation

In addition to routine monitoring, waters shall be monitored for toxic phytoplanktonic forms in response to observations which may indicate their presence, such as those listed above.

See Appendix 2 analyses parameters

II. Emergency Shellfish Sampling, Assay, Public Notification and Re-Opening of Closed Shellfish Beds

The following is specifically written to address *Karenia brevis* blooms; however, shall constitute a template to address any and all toxic or potentially toxic algae blooms that occur in Delaware waters.

A. Background / Contingency Sampling & Testing

The phenomenon known as "red tide" occurs when K. brevis concentrations increase above normal background levels of 1,000 cells/liter. Concentrations equal to or greater than 250,000 cells/liter can cause fish kills; however, much lower concentrations (greater than or equal to 5,000 cells/liter) may cause shellfish to become toxic if exposure is sustained. Shellfish become toxic by filter-feeding on the dinoflagellates, and absorbing the toxin into their digestive tissues. Toxic shellfish meats, when ingested, may cause illness to humans and animals. The period of exposure required to elevate NSP levels in shellfish to levels toxic to humans depends on environmental factors. Since this is a warm-water phenomenon, shellfish are assumed to be pumping during blooms. However, hydrographic conditions may limit exposure. In the field, shellfish may retain toxicity for 2 to 4 weeks.

The NSSP Model Ordinance mandates that growing areas be placed in the closed status when any NSP toxin is found in shellfish meat, or when the cell counts for *K. brevis* in the water column exceed 5,000 cells / L. However, as blooms are often transient in nature, and/or not uniform in concentration and distribution, toxin levels in shellfish meats, as determined by approved methods, shall take precedence in determining the need for, and/or extent of shellfish growing area closures precipitated by the presence of K. brevis in Delaware waters. Shellfish meats shall be tested for toxicity by an approved procedure during and after a *K. brevis* bloom. If a shellfish growing area is closed (for either exceeding 5,000 *K. brevis* cells /

L, or due to toxicity in shellfish meats), the area shall only be re-opened / returned to the Approved status pending testing / negative (<MDL) results for brevetoxin from shellfish meats from shellfish harvested therein.

B. Early Warning System

Delaware's early warning system shall include; but is not limited to routine surveillance, as described earlier in this Plan, observations by DNREC staff, satellite and aerial surveillance.

1. Defining Severity and Extent of Occurrences

When elevated concentrations (>1,000 cells/liter) of *K. brevis* are found, the initial sampling program shall continue, and be expanded. Sampling shall be conducted at predetermined stations, which shall include both Citizen Monitor sites and sites in proximity to observed concentrations >1,000 cells / L, and or where effects are observed, including; but not limited to dead or dying fish; behavioral anomalies in fish; dead, dying, or behavioral anomalies in fish predators; discolored water (may be mahogany or milky colored); an "off-odor" to the water (may be a "sweet" smell); or neurological effects in humans which may include disorientation, a tingling sensation, asthma-like symptoms, or neuromuscular effects.

Closure of an area will occur as follows:

Due to the possible dynamic nature of phytoplankton blooms, including non-uniformity in concentration and distribution, and not necessarily occurring in association with discolored water, odor, or obvious effects - shellfish growing area closure boundaries shall be large enough to accommodate a significant margin of safety. A reasonable and significant margin of safety shall be determined on a case-by-case basis; however, may include all of Rehoboth Bay (growing area 2) and/or Indian River Bay (growing area 3). The Delaware Bay may be divided into zones of closure on a case-by-case basis. The area(s) of closure shall be established using well defined and clearly visible points of land, navigation markers, and/or other easily-recognizable landmarks.

C. Embargo of Potentially Toxic Shellfish

Potentially toxic shellfish constitute a deviation from a critical limit. This requires that a certified dealer take corrective action either by following a corrective action that is appropriate for the particular deviation, or by segregating and holding the affected product until a review can determine

the acceptability of the affected product for distribution. Corrective actions include, when necessary, reconditioning, seizure, or destruction of affected product to ensure that no product enters commerce that is either injurious to health or is other wise adulterated as a result of the deviation.

Stop Sale Action shall be immediate, and destruction of shellstock determined on a case-by-case basis.

D. Patrol Program

The Delaware Department of Natural Resources and Environmental Control (DNREC) Division of Fish and Wildlife Enforcement Section, along with assistance from State Parks personnel in shellfish growing waters adjacent to state parks, under the DNREC Division of Parks and Recreation, shall enforce emergency harvest restrictions, as specified under Title 7 of the Delaware Annotated Code.

E. Procedures to Disseminate Information

Procedures for disseminating information regarding emergency closure of Delaware shellfish growing areas due to a toxic phytoplankton bloom shall include; but not be limited to direct notification of harvesters and processors, direct notification to the Delaware Department of Health and Social Services / Division of Public Health, direct notification to other states, ISSC, and U.S. FDA, a press release, emergency signage, and enhanced patrols, as referenced above.

F. Procedures to Re-Open Closed Shellfish Harvest Areas:

Following area or zone closure, water samples are collected at key and/or representative sampling stations for *K. brevis* identification and cell counts. Shellfish shall be collected concurrently for toxicity analyses using approved methods. Negative (<MDL) results shall result in immediate re-opening of the potentially affected / affected area(s).

III. References

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APPENDIX 1

PROJECT ORGANIZATION AND RESPONSIBILITY AND CONTACT INFORMATION

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APPENDIX 2

LABORATORY ANALYTICAL PROCEDURES

(Detection Limits and Quality Assurance Objectives)

Note: These are laboratory procedures, and do not necessarily constitute field observation procedures.

Phytoplankton grab sample - l liter cubitainer approximately three quarters filled from which l milliliter is pipetted after thorough mixing. Sample is placed into a Sedgewick-Rafter plankton counting cell which has been calibrated. A one-strip count is made which represents 135 one-fourth square millimeter fields. To determine the number of phytoplankton cells per milliliter, the total strip count is then multiplied by a derived enumeration factor (54.2) representing the portion of the S-R cell counted. After the initial strip count is made, the entire perimeter of the cell is scanned along with two diagonal cell scans representing an additional 521 one-fourth square milliliter fields. This procedure examines for any additional plankters not encountered in the initial strip count. These are documented if present (less than 54.2 cells per ml).

General Analyses Parameters:

. Relative Abundance (cell densities);
. Dominant Organisms;
. Percent Composition (diatoms, green algae, pigmented flagellates, bluegreen algae);
. Sketches and Measurement of Unidentified Orgamisms;
. Notation and Count of Associated Organisms (ciliates, rotifers, non-pigmented flagellates, etc.)

The number of plankton in the S-R cell are derived from the following:

$$\text{No./ml} = \frac{C \times 1000\ mm3}{L \times D \times W \times S}$$

C = number of organisms counted
L = length of each strip (S-R cell length), mm,
D = depth of a strip (S-R cell depth), mm,
W = width of a strip (Whipple grid image width), mm, and
S = number of strips counted

If cell densities are high, less fields may be counted and appropriate multiplication correction factors applied.

Detection Limit - 1 organism

Precision - Utilizing Standard Methods for the Examination of Water and Wastewater (latest edition) assuming the distribution of organisms in the counting cell is random, the counting error may be estimated. The approximate 95% confidence limits, as a percentage of the number of units counted (N) equals:

2 - (100%) Generally 100 or more units are counted
N - whereby the 95% confidence limits would approximate
+ 20% of the mean.

Analytical References

Field and Laboratory Methods (EPA-670/4-73-100, July 1973) Standard Methods For The Examination of Water and Wastewater, 17th Edition APHA-AWWA-WPCF, 1989.