# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

| 1. AGENCY USE ONLY *(Leave blank)* | 2. REPORT DATE | 3. REPORT TYPE AND DATES COVERED |
|---|---|---|
| | 3/16/04 | |

4. TITLE AND SUBTITLE
Foodborne Marine Biotoxins

5. FUNDING NUMBERS

6. AUTHOR(S)
Mark A. Poli

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
USAMRIID

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

UNLIMITED DISTRIBU**DISTRIBUTION STATEMENT A**
Approved for Public Release
Distribution Unlimited

20040401 062

13. ABSTRACT *(Maximum 200 words)*

Marine biotoxins, which are globally distributed, are responsible for numberous human intoxication syndromes due to the ingestion of cooked or uncooked seafood. Over 2500 cases of foodborne diseases caused by fsh and shellfsh toxicity were reported to the Centers for Disease Control & Prevention (CDC) between 1993 and 1997 in the United States (1). Marine biotoxins are estimated to cause over 60,000 foodborne intoxications worldwide each year(2). In addition to human intoxications, they cause massive fsh kills, negatively impact coastal tourism and fshery industries, and have been implicated in mass mortalities of birds and marine mammals. The long-term environmental and public health effects of chronic exposure to these toxins are poorly understood; research needs are only beginning to be addressed(2,3). Ingestion of seafood containing marine biotoxins causes six identifable syndromes; paralytic shellfsh poisoning(PS), neurotoxic shellfsh poisoning (NSP), ciguatera fsh poisoning(CFP), diarrhetic shellfsh poisoning(DSP), anmesic shellfsh poisoning(ASP), and azaspiracid poisoning(AZP). With the exception of CFP, which, as the name implies, is caused by eating contaminated fnfsh, all are caused by the ingestion of shellfsh. And, with the exception of ASP, the causative toxins are all isolated from marine dinoflagellates. ASP is notable as a syndrome caused by the only known toxin produced by a diatom. These toxin-producting species are a small minority of the only known toxin produced by a diatom. These toxin-producing species are a small minority of the only known toxin produced by a diatom.These toxin-producing species are a small minority of the thousands of known species of phytoplankton. However, under the correct environmental conditions, they can proliferate to high cell densities known as blooms. During these harmful algal blooms (HABs), they may be ingested in large quantities by zooplankton, flter-feeding shellfsh, or grazing or flter-feeding fshes. Through these intermediates, toxins can then be vectored to higher trophic levels, including humans. In a recent decades, ther has been a perceived increase in both geographic distribution & occurrence of HABs(2,4,5).

| 14. SUBJECT TERMS | 15. NUMBER OF PAGES |
|---|---|
| Marine biotoxins, potential threat, food-borne, description, review | 14 |
| | 16. PRICE CODE |

| 17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
|---|---|---|---|
| UNCLASSIFIED | UNCLASSIFIED | UNCLASSIFIED | UNLIMITED |

# MARCEL DEKKER, INC.

MARCEL DEKKER™

*Register now on* dekker.com *and receive a discount coupon towards your next Dekker purchase. Registered users enjoy these advantages:*

- ✔ Preview content online for free
- ✔ Quickly access your subscriptions and downloads
- ✔ Sign up for email notifications about new products and special offers in your subject of interest

***Send your book order to your regular book supplier or directly to your nearest Marcel Dekker, Inc. office:***

**USA, Canada, South America:**

**5 Ways to Order**

***Website:*** *www.dekker.com*
***Toll-free:*** *1-800-228-1160*
***Fax:*** *1-845-796-1772*
***E-mail:*** *bookorders@dekker.com*
***Mail:*** *Marcel Dekker, Inc., Cimarron Road P.O. Box 5005, Monticello, NY 12701-5185*

**Europe, Africa, Middle East Far East, Australia, India, China:**

**5 Ways to Order**

***Website:*** *www.dekker.com*
***Phone:*** *+41-061-260-6300*
***Fax:*** *+41-061-260-6333*
***E-mail:*** *intlorders@dekker.com*
***Mail:*** *Marcel Dekker, AG Hutgasse 4, Postfach 812 CH-4001 Basel, Switzerland*

***Please include quantity, ISBN, author, title, shipping and billing addresses, telephone number/fax number/e-mail address, method of shipment, and purchase order numbers or credit card information.***

To purchase offprints of chapters that appear in any Marcel Dekker, Inc. book or reprints of articles that appear in any Marcel Dekker, Inc. journal:
**offprints@dekker.com**

To inquire about special sales and bulk purchases of Marcel Dekker, Inc. books or journals:
**bulksale@dekker.com**

**research + dekker.com → results**

DISTRIBUTION STATEMENT A
Approved for Public Release
Distribution Unlimited

# 25

# Foodborne Marine Biotoxins

**Mark A. Poli**

*U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland, U.S.A.*

## I. BACKGROUND

Marine biotoxins, which are globally distributed, are responsible for numerous human intoxication syndromes due to the ingestion of cooked or uncooked seafood. Over 2500 cases of foodborne diseases caused by fish and shellfish toxicity were reported to the Centers for Disease Control and Prevention (CDC) between 1993 and 1997 in the United States (1). Marine biotoxins are estimated to cause over 60,000 foodborne intoxications worldwide each year (2). In addition to human intoxications, they cause massive fish kills, negatively impact coastal tourism and fishery industries, and have been implicated in mass mortalities of birds and marine mammals. The long-term environmental and public health effects of chronic exposure to these toxins are poorly understood; research needs are only beginning to be addressed (2,3).

Ingestion of seafood containing marine biotoxins causes six identifiable syndromes: paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), ciguatera fish poisoning (CFP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), and azaspiracid poisoning (AZP). With the exception of CFP, which, as the name implies, is caused by eating contaminated finfish, all are caused by the ingestion of shellfish. And, with the exception of ASP, the causative toxins are all isolated from marine dinoflagellates. ASP is notable as a syndrome caused by the only known toxin produced by a diatom. These toxin-producing species are a small minority of the thousands of known species of phytoplankton. However, under the correct environmental conditions, they can proliferate to high cell densities known as blooms. During these harmful algal blooms (HABs), they may be ingested in large quantities by zooplankton, filter-feeding shellfish, or grazing or filter-feeding fishes. Through these intermediates, toxins can then be vectored to higher trophic levels, including humans.

In recent decades, there has been a perceived increase in both geographic distribution and occurrence of HABs (2,4,5). While a portion of this increase is undoubtedly a result of increased monitoring and reporting programs as well as improved detection technologies, a global expansion in geographic range of several syndromes has been well documented (2,4,5). It seems likely that anthropogenic effects have contributed to this expansion, but to what extent is currently a subject of debate. What seems certain, however, is that foodborne marine biotoxins will continue to be an important issue to the seafood industry, recreational harvesters, and consumers for the foreseeable future.

---

The views, opinions, and/or findings contained herein are those of the author and should not be construed as an official Department of the Army position, policy, or decision unless so designated by other documentation.

This chapter will briefly describe the six major toxic syndromes mentioned above and the implicated toxins. Space limitations do not allow a discussion of cyanobacterial toxins obtained through contaminated drinking water. Readers interested in this topic should refer to Ref. 6 and references therein.

## II. HUMAN INTOXICATION BY MARINE BIOTOXINS

### A. Paralytic Shellfish Poisoning

PSP results from ingesting shellfish contaminated with a family of heterocyclic guanidines called saxitoxins (Fig. 1). The name saxitoxin (STX) is derived from the giant butter clam *Saxidoma giganteus*, from which the toxin was first isolated (7). While STX was the original toxin isolated, the family of PSP toxins is now known to consist of over 20 derivatives of varying potency. They are associated with species of marine dinoflagellates belonging to the genera *Alexandrium*, *Pyrodinium*, and *Gymnodinium*, as well as several species of freshwater cyanophytes. More recently, the isolation of STX from species of *Moraxella* associated with dinoflagellate cultures suggests a bacterial origin (8). In filter-feeding shellfish such as clams, oysters, mussels, and scallops, toxins accumulate after ingestion of dinoflagellate cells during bloom conditions or resting cysts from the sediment. The toxin profile in the originating dinoflagellates can be modified by metabolic biotransformation reactions in the shellfish (9). Ingestion of toxic shellfish by humans results in the characteristic signs and symptoms of intoxication. These toxic dinoflagellates occur in both tropical and temperate oceans. Approximately 2000 cases of PSP are estimated to occur annually across regions of North America, South America, Europe, Japan, Australia, Southeast Asia, and India (2,5). The overall mortality rate has been estimated at 15% (2). In addition to human intoxications, PSP toxins have also been suspected in the deaths of birds (10) and humpback whales (11).

STX and its derivatives elicit their physiological effects by interacting with the voltage-dependent sodium channels in excitable cells of heart, muscle, and neuronal tissues. High-affinity binding to neurotoxin receptor site 1 on the sodium channel blocks ion conductance across the cellular membranes, thereby inhibiting depolarization. While all voltage-dependent sodium channels are susceptible to saxitoxins, pharmacokinetic considerations make the peripheral nervous system the primary target in seafood intoxications.

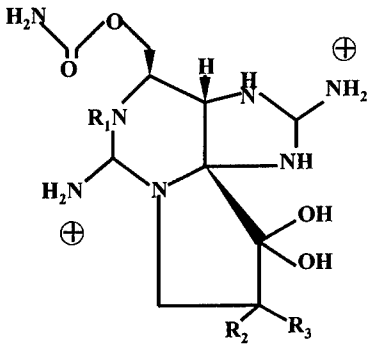



**FIGURE 1** The basic toxin structure of the paralytic shellfish poisons. In the parent carbamate compound (saxitoxin), $R_1$, $R_2$, and $R_3$ = H. A number of sulfated and hydroxylated derivatives at these locations make up the carbamate class of PSPs. In addition, the same sulfated/hydroxylated derivatives occur as carbamoyl or decarbamoyl classes of PSP toxins where the carbamate group is replaced by $OCONHSO_3^-$ or OH, respectively.

Ingestion of PSP toxins results in a rapid-onset (minutes to hours) complex of paresthesias including a prickling, burning, or tingling sensation in the lips and mouth that rapidly progresses to the extremities. At low doses these sensations can disappear in a matter of hours with no sequelae. At higher doses numbness can spread from the extremities to the trunk, followed by weakness, ataxia, hypertension, loss of coordination, and impaired speech. At lethal doses respiratory failure results from paralysis of respiratory musculature (8,12). Children appear to be more susceptible than adults. In an outbreak in Guatemala in 1987 involving 187 cases, the highest attack rate occurred in patients 13–17 years old, and children <6 years of age had a much higher mortality rate (50%) than that of victims 18 years and older (5%) (12,13). The lethal dose for young children may be as low as 25 μg STX equivalents* (13), while that for adults is probably about 1–4 mg STX equivalents (2). In adults, clinical symptoms can occur upon ingestion of approximately 0.7 mg equivalents (14). Shellfish can contain up to 10–20 mg equivalents per 100 g of meat, so ingestion of only a few shellfish can cause illness (2,13,15). Fortunately, clearance from the blood via the urine is rapid. After a series of outbreaks of PSP on Kodiak Island, Alaska, in 1994, serum half-life was estimated to be less than 10 hours. Respiratory failure and hypertension resolved in 4–10 hours in these victims, and toxin was not detectable in urine 20 hours after ingestion (15).

Treatment of PSP centers upon removing unabsorbed material from the gastrointestinal tract and supportive care. In severe cases, mechanical ventilation may be necessary. Neither antidote nor vaccine is currently available.

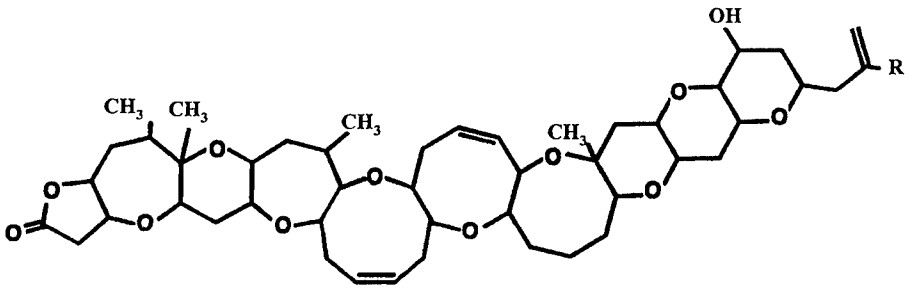
Toxins in clinical samples can be detected by several methods. High-performance liquid chromatography (HPLC) can detect individual toxins, but typically requires derivatization reactions (16,17). While this allows elucidation of toxin profiles that can provide valuable comparative data, it provides no information on toxicity. Receptor-binding assays based upon either rat brain membranes (18,19) or purified STX binding proteins from frogs or snakes (saxiphilins) (20) measure total biological activity without regard to toxin profile. All of these assays have been used to detect PSPs in the urine and serum of intoxicated victims (15). Immunologically based assays can detect major toxins such as STX or neosaxitoxin, but cross reactivity with other PSPs is highly variable. At present, the "gold standard" assay for PSP toxins in shellfish is still the mouse bioassay (21), although a rapid-throughput microtiter plate–based version of the receptor-binding assay (19) shows great promise as a regulatory screening tool.

Many countries, including the United States, have established regulatory limits for PSP and closely monitor the commercial shellfish banks. In the United States the guidance level is 80 μg/100g of shellfish meat (22), and individual states monitor the toxin levels by mouse bioassay. Thus, commercially purchased shellfish is typically of low risk to the consumer. Recreationally harvested shellfish, however, are not monitored, and private individuals may not be cognizant of commercial regulatory restrictions. The greatest risk to the consumer is from shellfish harvested outside the regulatory sphere. For the recreational fisherman, the greatest safety will come from being aware of any harvesting prohibitions in the area and restricting recreational harvesting accordingly. While visible signs of toxicity, such as discoloration of the water or dead or dying fish, may be present, they are not reliable indicators of the shellfish safety.

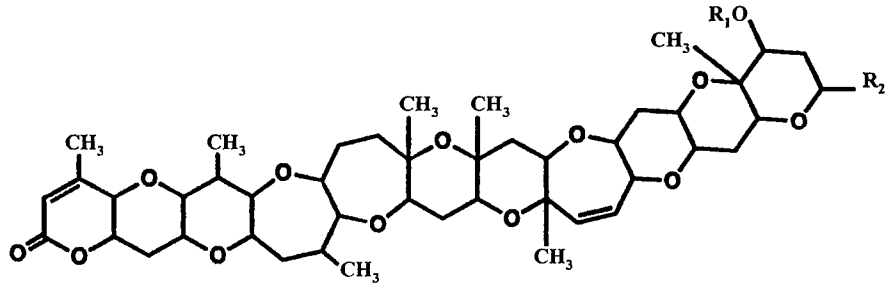
## B. Neurotoxic Shellfish Poisoning

NSP results from the consumption of shellfish contaminated with brevetoxins (Fig. 2), a group of cyclic polyether neurotoxins produced by the marine dinoflagellate *Karenia brevis* (formerly

* Toxicity in shellfish is routinely measured using a mouse bioassay. This assay measures the composite toxicity of the solution, which consists of a mixture of PSP components of varying toxicity. The assay results are compared to a standard curve derived from reference standard saxitoxin, and thus the total activity of the extract is expressed as "saxitoxin equivalents." For pure saxitoxin, 1 mouse unit (MU) = approximately 0.2 μg.




A




B

**FIGURE 2** Polyether backbone structure of the PbTx-2–type (A) and PbTx-1–type (B) brevetoxins. Derivatization at R, $R_1$, and $R_2$ account for the nine known members of this class of toxins, which are the causative agents in neurotoxic shellfish poisoning.

*Ptychodiscus brevis*). Like PSPs, brevetoxins accumulate in filter-feeding molluscs that are then consumed by humans (23). Unlike PSP, however, it appears that the causative agents in NSP are actually molluscan metabolites of the parent brevetoxins (24). This syndrome has historically been limited to the American states bordering the Gulf of Mexico, although in 1993 an outbreak of shellfish poisoning in New Zealand was identified as NSP. The causative organism was a novel *Gymnodinium* species (*Gymnodinium* cf. *breve*), which produced brevetoxin-like neurotoxins (2). In 1987, an outbreak of NSP was reported in North Carolina. In this case, a mass of Florida Gulf Stream water containing *K. brevis* was trapped in a warm-core eddy and was carried into coastal waters, where it remained intact for several weeks (25). During this time, 48 cases of NSP were reported. However, this rare concurrence of a sea surface temperature anomaly and favorable local conditions has not been repeated. Recently, a bloom of *Chattonella verruculosa* appeared in Rehoboth Bay, Delaware, coincident with mass mortalities of menhaden. Cell samples were found to contain significant levels of brevetoxins (Dr. Carmelo Tomas, University of North Carolina at Wilmington, personal communication). While no cases of human illness were reported, this event opens the possibility of a range extension of NSP into the Delaware and Chesapeake Bays.

Because *K. brevis* is an unarmored dinoflagellate and therefore relatively fragile, it is easily lysed by wind or wave action. Consequently, blooms are frequently associated with massive fish kills when lysed cells release toxins into the water column. These lipophilic compounds easily diffuse across gill membranes in fish, where they rapidly exert their toxicity. In an analogous manner,

brevetoxins can be aerosolized by wind, wave, and surf action and cause irritation, coughing, and burning of the throat and upper respiratory tract in beachgoers during coastal red tide blooms. In 1996, a mortality event occurred when at least 149 manatees were unable to escape a Florida red tide (26).

Brevetoxins and their metabolites bind to neurotoxin receptor site 5 on voltage-sensitive sodium channels where they alter the voltage-dependence of activation and inhibit channel inactivation (27,28). This results in inappropriate and prolonged channel opening.

Symptoms of NSP can manifest within an hour of consumption of contaminated shellfish. These typically include nausea, oral paresthesias, ataxia, myalgia, and fatigue. In severe cases, tachycardia, seizures, and loss of consciousness can occur, but a fatal case of NSP has never been reported. Treatment consists of removing unabsorbed material from the gastrointestinal tract and supportive care. Patients typically improve dramatically in 24–48 hours.

Brevetoxins are eliminated primarily in the bile, although urinary excretion plays a significant role. Animal models suggest that most of the toxin is eliminated within 48–72 hours, although some residue remains in lipophilic tissues for extended periods (29,30). Human pharmacokinetic data are very limited. However, a severe NSP outbreak occurred in Florida in 1996 when a family ingested whelks collected in Sarasota Bay. Two children were hospitalized with severe symptoms, including seizures. Brevetoxin metabolites were detected in urine samples collected 3 hours postingestion but were undetectable 4 days later (24). With supportive medical care, symptoms resolved in 48–72 hours.

The toxic dose of brevetoxins in humans has not been established, although it is clear that eating only a few shellfish can result in severe intoxication. Toxins in clinical samples can be detected either by HPLC coupled to mass spectrometry, receptor-binding assays, or immunoassays (24). Because metabolic conversion of the parent toxins occurs in shellfish, and metabolites are less pharmacologically active than the parent toxins, it appears at this time that immunological assays are preferable as screening tools. However, the question of secondary metabolism in humans may impact this issue, and it awaits further study.

The FDA guidance level for brevetoxins in shellfish is 80 μg/100 g of shellfish tissue (22), and Gulf Coast shellfish are closely monitored. State laboratories monitor both toxin activity in the shellfish and *K. brevis* cell counts in the water column. Fishing grounds are closed when cell counts are significant and reopened when toxin activity in shellfish reach safe levels. For this reason, commercially harvested shellfish are very safe. Once again, the greatest risk occurs from recreational harvesters. As with PSP, avoiding intoxication is best accomplished by limiting consumption to commercially caught shellfish and avoiding privately caught shellfish of unknown origin. Recreational harvesters should keep abreast of closures and harvesting limitations set forth by state agencies.

## C. Ciguatera Fish Poisoning

CFP is a syndrome caused by exposure to ciguatoxins through the consumption of fresh fish. Like brevetoxins and PSP toxins, ciguatoxins originate with dinoflagellates, in this case the benthic species *Gambierdiscus toxicus*. This organism is an epiphyte, growing in association with filamentous algae on coral reefs and reef lagoons. Specific strains of *G. toxicus* produce precursors of ciguatoxins, which are ingested by grazing herbivorous fish and invertebrates (31). As these precursors move up the food chain to higher trophic levels through predation, they are metabolically modified to form a family of very potent neurotoxins (Fig. 3) (32,33). At present, over 20 members of this family have been identified. The Pacific form of the toxin varies slightly from the Caribbean form, although both are long cyclic polyether compounds reminiscent of the brevetoxins. Large, predatory reef-dwelling carnivores such as grouper, snapper, barracuda, and jacks are especially recognized as frequent carriers of ciguatoxins. However, small reef-dwelling herbivores can also cause ciguatera, especially when consumed whole. This is especially true in the tropical Pacific, where these small herbivores are more widely eaten.

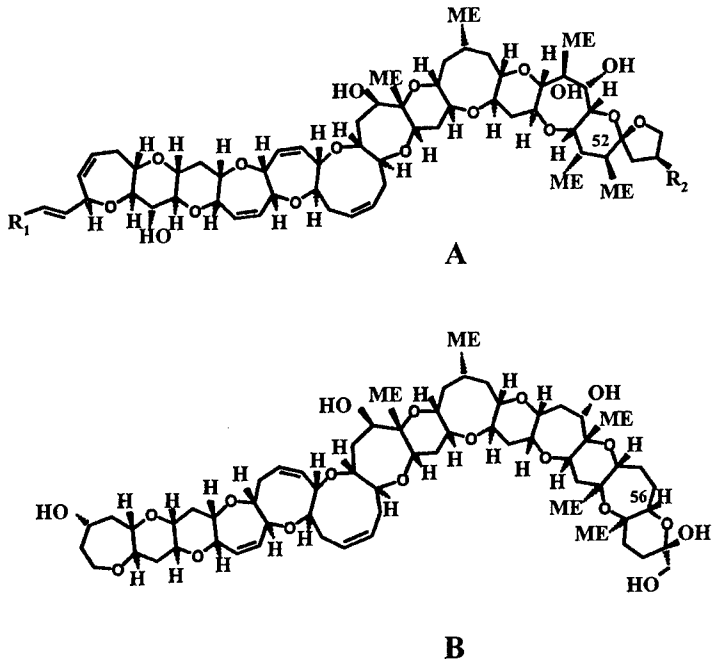



**FIGURE 3** Polyether backbone structure of the Pacific (A) and Caribbean (B) forms of ciguatoxin, the causative agent in ciguatera fish poisoning. Derivatization at $R_1$ and $R_2$ and less energetically favorable epimers make up the more than 20 members of this class.

Although CFP occurs globally in tropical and subtropical latitudes approximately paralleling the distribution of reef-building corals, it occurs most frequently in the Pacific Ocean, western Indian Ocean, and the Caribbean. However, modern advances in the shipping of fresh fish has expanded the range of CFP to virtually anywhere in the world. Even within endemic regions, however, it is highly variable and spotty in distribution. In most areas only a small percentage of the large fish are toxic. Difficulties in predicting toxic areas and detecting toxicity in fish has always been a major impediment to the implementation of control measures.

CFP is estimated to affect more than 25,000 people annually (33), although substantial underreporting undoubtedly occurs. The symptomatology is complex. In severe cases, symptoms can develop in as little as 30 minutes; in milder cases onset can be delayed 24 hours or more. The early symptoms are typically gastrointestinal, including nausea, vomiting, diarrhea, and abdominal pain. These generally last only 24–48 hours and may co-occur with neurological symptoms such as tingling of the lips and extremities, reversal or abnormalities in hot/cold temperature sensations, and severe localized itching. These neurological symptoms occur in nearly all cases and are often accompanied by a wide range of other signs and symptoms (34). Fatigue, muscle and joint pain, and mood disorders such as anxiety or depression occur in 50% or more of cases (33). Severe cases may also manifest cardiac symptoms such as bradycardia and hypotension. There are also regional differences in symptomatology, probably resulting from regional differences in toxins (33). Although the gastrointestinal symptoms resolve early, neurological symptoms often persist for weeks or even months. Late in the course of recovery, symptoms may become episodic, recurring during periods of stress or after consumption of certain foods or alcohol.

Like brevetoxins, ciguatoxins bind to neurotoxin receptor site 5 on the voltage-dependent sodium channel and cause a hyperpolarizing shift in the voltage dependence of channel activation (33). For mammals they are the most potent sodium channel toxins known.

Treatment of CFP consists primarily of symptomatic care and preservation of electrolyte and fluid balances. While not beneficial in all cases, patients diagnosed early may respond to intravenous mannitol treatment (35,36). During the recovery period, avoiding the consumption of fish and alcohol are recommended.

Ciguatoxins in fish are best detected with analytical methods such as liquid chromatography coupled to mass spectrometry. However, such analytical techniques are not useful for analyzing large numbers of samples. Because levels in fish tissues are typically in the parts-per-billion range or below, most techniques lack the required sensitivity. Development of immunological assays has been hindered by the lack of purified toxins with which to vaccinate animals for specific antibody production. Synthesis of the backbone structures is extremely difficult, but progress is being made for some of the Pacific forms (37,38). At present, the most useful in vitro assay available is still the competitive receptor-binding assay with rat brain membranes (18,31). This assay takes advantage of the fact that ciguatoxins and brevetoxins bind to a common receptor site and therefore radiolabeled brevetoxin can be used as the probe. It is sufficiently sensitive to detect ciguatoxins at levels that are believed to cause human intoxication but not to provide the desired safety margin for regulatory testing. Improved assays for these toxins are desperately needed in many parts of the world.

At present, there is no regulatory guidance level for ciguatoxins and the commercial fish harvests are not monitored. Some local jurisdictions have implemented bans on the sale of certain toxic species. The primary control measure for CFP is avoidance of toxic fish. For consumers this means being aware of the local situation with regard to prevalence and implicated species. It is prudent to avoid eating locally implicated species in particular and large coral reef-dwelling predators in general. If in doubt, eating small portions reduces the risk. Although screening individual high-risk fish is not yet fully feasible for either the seafood industry or the individual consumer, research continues for new assay technologies to achieve this goal.

## D. Diarrhetic Shellfish Poisoning

Diarrhetic shellfish poisoning occurs after consumption of shellfish containing okadaic acid or its derivatives (Fig. 4). Okadaic acid was named for the black sponge *Halichondria okadai*, from which it was first isolated. However, it was later determined that the origin of the toxin was actually dinoflagellates of the genera *Prorocentrum* and *Dinophysis*. These organisms also produce at least seven okadaic acid derivatives denoted dinophysistoxins (DTXs). Other toxins, such as the pectenotoxins and yessotoxins, often co-occur with the DTXs in shellfish tissues. However, because these toxins differ from the DTXs in their mechanism of action and are much less potent orally, there is some question as to whether they should be considered as part of the DSP complex (8).

Toxic species of *Dinophysis* and *Prorocentrum* are distributed worldwide. Consequently, DSP is also widespread. It occurs seasonally and is a major problem to the shellfish industry in Europe and Japan, but has also been documented in South America, South Africa, New Zealand, Australia, Thailand, Mexico, Scandinavia, and Canada (2,39). The primary vector to humans is cultured or

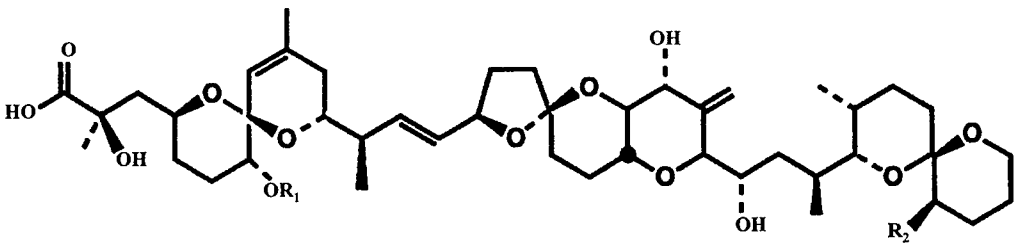



**FIGURE 4** Diarrhetic shellfish poisons. In okadaic acid, $R_1$ and $R_2$ = H. Methylated or acylated derivatives make up dinophysistoxins 1–3.

wild mussels, which accumulate toxins in the digestive glands after filter-feeding. Although the toxin-producing species *Prorocentrum lima* has been found associated with cultured mussels in Maine and low levels of okadaic acid have been measured in shellfish from the Gulf of Mexico (40), no outbreaks of DSP have yet been reported in the United States.

Symptoms of DSP intoxication in humans can occur within 30 minutes of ingestion and consist entirely of gastroenteritis, including nausea, vomiting, diarrhea, and abdominal pain. Symptoms typically resolve in 2–4 days. Treatment is symptomatic, including maintenance of fluid and electrolyte balances, and no deaths have been reported.

DSPs are potent inhibitors of serine/threonine protein phosphatases. Activity is highest for the PP2A class, lower for the PP1 class, and minimal or absent for the PP2B and PP2C classes of phosphatases (2,8,12). Diarrhea is thought to be a result of hyperphosphorylation of proteins controlling sodium secretion by intestinal cells (39), causing impaired water balance and fluid loss. In addition, through their effects on diverse protein phosphorylation and dephosphorylation reactions, DTXs can impact such diverse cellular processes as signal transduction, memory, cell division, and apoptosis (8). Whether these effects are important in human intoxications is not fully understood.

Both okadaic acid and at least one of the DTX derivatives are potent tumor promoters (41,42). The mechanism of this activity is thought to be increased phosphorylation of critical cellular proteins and/or intermediate filaments and changes in DNA gene expression resulting from phosphorylation of suppressor elements (12). Again, it is not known whether this activity poses a significant public health threat to the seafood consumer. However, the wide distribution of toxic *Prorocentrum* and *Dinophysis* species and detection of low levels of DTXs in shellfish in diverse regions raises the question of whether chronic ingestion of subsymptomatic doses of these compounds could pose a health risk. One study, although only tentative in its conclusions, has suggested an association between long-term regular shellfish consumption and digestive cancers in France (43). More study of this issue is urgently needed.

Currently, the U.S. regulatory action level for DSP toxins is 20 μg/100 g (22). The minimum dose of the major DSP toxins necessary to produce symptoms in humans is estimated to be 35–40 μg (12). Internationally, guidance levels vary. Canada has informally adopted the Japanese limit of 5 MU/100 g of meat (about 20 μg/100 g) (44); the European community has no current standard but is in the process of adopting a limit of 16 μg/100 g of shellfish (Douglas McLeod, President, European Mollusc Producers Association, personal communication; Dr. Kevin James, Cork Institute of Technology, personal communication).

Detection of DSP toxins at the regulatory level is still dependent primarily upon the mouse bioassay (45). Other techniques, such as an in vitro phosphatase inhibition assay, immunoassays, and a variety of in vitro bioassays have been proposed and are currently in various stages of validation (17). In most areas of the world where DSP is a problem, shellfish stocks are closely monitored for the presence of toxins and the waters monitored for toxic dinoflagellates. Thus, as with other shellfish intoxication syndromes, the best recourse for avoiding DSP is to restrict consumption to commercially regulated shellfish products. Recreational harvesters, as always, should closely monitor the local restrictions on harvesting and remember that there will probably be no visible indicators of shellfish toxicity.

## E. Amnesic Shellfish Poisoning

ASP first came to the attention of public health authorities during an outbreak in Prince Edward Island, Canada, during the winter of 1987. In this event, over 100 people became ill after eating contaminated mussels, and 3 people died. The causative agent was soon identified as domoic acid (Fig. 5) (46). Domoic acid was not an unknown compound; it had been isolated from red macroalgae in 1958 (47) and was the active ingredient in an algal extract used as an antihelmitic in fishing villages in rural Japan. It had been evaluated and subsequently rejected as a potential insecticide. Consequently, it was quite surprising to discover link between domoic acid and an outbreak of human seafood intoxication. Even more surprising was the identification of the diatom *Nitzschia*

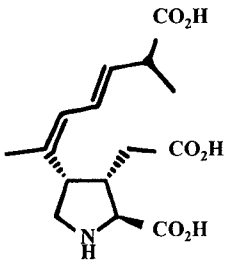



**FIGURE 5** Domoic acid, the causative agent in amnesic shellfish poisoning.

*pungens* f. *multiseries* (now known as *Pseudo-nitzschia multiseries*) as the causative organism. This remains the first and only known seafood toxin produced by a diatom. Since that time, several other species of *Pseudo-nitzschia* around the world have been demonstrated to produce domoic acid (48).

Since the first outbreak in Canada in 1987, the wide distribution of domoic acid–producing species of *Pseudo-nitzschia* has become clear. In 1991 a die-off of numerous cormorants and pelicans occurred in Monterey Bay, California. These birds had been feeding on anchovies containing high levels of domoic acid in their guts after filter-feeding during a bloom of *P. australis*. This bloom later moved up the coast and caused the toxification of razor clams and Dungeness crabs in Washington and Oregon. Several cases of human intoxication are thought to have resulted from the ingestion of these razor clams, although a definite connection was not made (49). In 1998, over 400 California sea lions died and numerous others displayed signs of neurological impairment in the Monterey Bay area during another bloom of *P. australis*. Again, high levels of domoic acid were detected in anchovies and in the feces of the sea lions (50). Domoic acid has since been found to be seasonally widespread along the Pacific coast of the United States (51) as well as the Gulf of Mexico. Around the world, domoic acid has been reported in such diverse locales as New Zealand, Mexico, Denmark, Spain, Portugal, Scotland, Japan, and Korea. Occasionally, levels in shellfish become sufficient to stimulate bans on harvesting. Fortunately, since the initial 1987 Canadian outbreak and the suspected cases in Washington in 1991, no further human cases have been reported. This is no doubt attributable to effective survey and monitoring programs.

Domoic acid is a neuroexcitatory amino acid, structurally related to kainic acid. It binds with high affinity to the kainate and α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) subtypes of the glutamate receptor throughout the central nervous system and elicits nonsensitizing or very slowly sensitizing currents. The nonsensitizing nature of these currents causes a protracted influx of cations into the neurons through the receptor channels, stimulating a variety of intracellular biochemical events that lead to cell death in susceptible cells (52). Kainate and AMPA glutamate receptor subtypes are present in high concentrations in the hippocampus, a portion of the brain associated with learning and memory processing.

Symptoms of intoxication occur within hours of ingestion and include vomiting, diarrhea, or abdominal cramps within 24 hours and potentially confusion, disorientation, memory loss, and, in serious cases, seizures, coma, and death. The memory loss involves primarily short-term memory, and in the Canadian outbreak was more prevalent in elderly patients (53). Diverse neurological deficits may occur and can persist for months. Potential treatment regimens are discussed in Ref. 53.

Based upon levels measured in Canadian shellfish, it was estimated that mild symptoms can occur after ingesting about 1 mg/kg domoic acid; severe symptoms occur when 2–4 mg/kg are ingested. The current regulatory limit for domoic acid in shellfish in Canada, the European Community, and the United States is 20 μg/g (22). Assuming a serving size of 250 g, this approximates an oral dose of 0.1 mg/kg or less, 10-fold less than the lowest reported toxic dose (3). Even so, the European Community is in the process of reducing their regulatory limit to 4.6 μg/g in harvested

shellfish. The 20 μg/g limit will still apply to sales (Kevin James, Cork Institute of Technology, personal communication).

The official regulatory testing method for domoic acid in the United States and the European Community utilizes analytical HPLC. However, both immunological methods and a very simple and inexpensive thin-layer chromatographic method have been reported to work very well (17).

As with other recognized shellfish intoxications, avoiding toxic shellfish is paramount. Effective monitoring programs are in place and commercial products are typically safe.

## F. Azaspiracid Poisoning

In November 1995, at least eight people in the Netherlands became ill after consuming cultured mussels harvested in Killary Harbor, Ireland. The symptomatology included nausea, vomiting, diarrhea, and stomach cramps, and thus was reminiscent of DSP. However, analysis of the offending shellfish demonstrated negligible levels of either DSP or PSP toxins. After further investigation, a new class of cyclic polyether shellfish toxins, known as the azaspiracids (Fig. 6), was isolated from these toxic mussels (54). After the initial outbreak in the Netherlands, further outbreaks in 1997 and 1998 occurred in Ireland, France, and Italy, all of which traced back to mussels harvested in Ireland. During 1998–2000, monitoring efforts in Ireland showed that most of the major shellfish-producing areas experienced periods of contamination by azaspiracids (55). The causative organism in AZP is not yet known. However, the cyclic polyether nature of the molecules and their seasonal occurrence suggests a dinoflagellate origin (54).

Although the human symptoms of AZP are quite similar to those of DSP, animal studies have demonstrated major differences. As with okadaic acid, azaspiracid, when administered orally to mice at 500–700 μg/kg, caused necrosis, erosion of epithelial cells, and fluid accumulation in the small intestine. Unlike okadaic acid, however, azaspiracid also caused dilation of the stomach, hepatitis and fatty accumulation in the liver, and decreased lymphocyte counts in the thymus and spleen (56). While the effects of okadaic acid were transient, the deleterious effects of azaspiracids lasted for many days. These results were later confirmed in chronic exposure studies, which revealed that the stomach and intestinal damage took several months to heal (57). Even more importantly, this latter study revealed a tumorigenic property of the azaspiracids. At much lower doses (20–50 μg/kg) than that required for GI damage, azaspiracids caused lung tumors and hyperplasia in the stomach. And unlike the DSP toxins, these tumors occurred in the absence of added initiators.

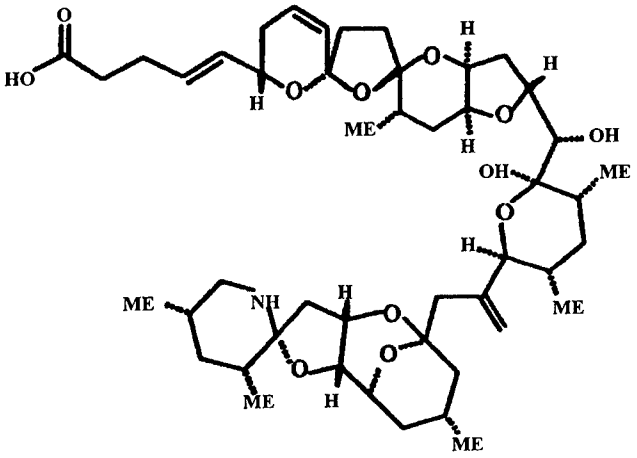



**FIGURE 6** Azaspiracid. Methylated and acylated derivatives have also been isolated and associated with azaspiracid poisoning.

To date, only azaspiracids from mussels cultured in Ireland have caused human intoxication. ASP is a serious threat to the local industry, not only because of the seriousness of the intoxication, but because it occurs in the winter when shellfish are free of contamination by DSP toxins. In addition, toxicity can extend for many months. However, azaspiracids are not restricted to Irish waters. Levels below the current regulatory limits have recently been demonstrated in mussels from northeastern England and southwestern Norway (58). Once the causative organism is identified, a wider distribution of these toxins in shellfish will likely come to light. The European community is expected to soon endorse a regulatory limit of 16 μg/100 g shellfish tissue for all member nations (draft Commission Decision SANCO/2227/2001 Rev-4). An immediate need is increased worldwide surveillance.

Azaspiracids are inefficiently extracted by the procedures used to extract DSP or PSP toxins. However, efficient extraction techniques and a sensitive HPLC-MS assay procedure have been developed (59). Many regions may detect low levels of azaspiracids in shellfish, well below those required for acute intoxication. However, the tumorigenic properties identified by (56) will no doubt make this a sensitive issue. Much more research is urgently needed to better delineate appropriate safety levels for these compounds.

## III. SUMMARY

Marine foodborne biotoxins are a potential threat to human health through the consumption of various seafood products. Because these toxins occur naturally in fresh and otherwise wholesome foods, possess no visual or olfactory clues to their presence, and are impervious to typical cooking temperatures, they can be a difficult problem for both the industry and the consumer. To combat this problem most nations have employed vigorous monitoring programs to ensure the quality and wholesomeness of their seafood. For the most part, these programs are extremely effective. However, occasional outbreaks of poisoning occur, primarily from recreationally harvested seafood. The exception to this is CFP, where present technology is insufficient to adequately test the product. Luckily, CFP is relatively rare; only 205 cases were reported to the CDC from 1993 to 1997 (1). Because this number undoubtedly reflects significant underreporting, and because many areas of the world have a much higher incidence of CFP than the United States, there is an urgent need for the development of new testing technologies for these toxins.

For the recreational shellfish harvester, the best defense against biotoxins is to keep abreast of information from the state monitoring labs and apply the same standards as the industry. Consumers are best served by consuming only commercially harvested shellfish or that harvested personally or by a known source.

In the event of outbreak of seafood poisoning from any source, victims should seek medical care immediately. Urine and serum samples should be collected and frozen, and any remaining implicated seafood frozen and retained for investigators. Call the U.S. Food and Drug Administration's Center for Food Safety and Applied Nutrition for further instructions. These steps can aid in timely diagnosis and treatment of victims as well as supply important information on human pharmacokinetics and elimination of toxins. And, as was the case in Canada in 1987 and Ireland in 1995, new types of marine biotoxins are often discovered in this manner.

## REFERENCES

1. SJ Olsen, LC MacKinon, JS Goulding, NH Bean, L Slutsker. Surveillance for foodborne disease outbreaks—United States, 1993–1997. MMWR 49(SS01):1–51, 2000.
2. FM Van Dolah. Marine algal toxins: origins, health effects, and their increased occurrence. Environ Health Perspect 108(suppl 1):133–141, 2000.
3. FM Van Dolah, D Roelke, RM Greene. Health and ecological impacts of harmful algal blooms: risk assessment needs. Hum Ecol Risk Assess 7:1329–1345, 2001.

4. DM Anderson. Toxic algal blooms and red tides: a global perspective. In: Red Tides: Biology, Environmental Science and Toxicology. New York: Elsevier, 1989, pp. 11–16.
5. GM Hallegraff. A review of harmful algal blooms and their apparent global increase. Phycologia 32:79–99, 1993.
6. WW Carmichael. Health effects of toxin producing cyanobacteria; "the cyanoHABs." Human Ecol Risk Assess 7:1393–1407, 2001.
7. EJ Schantz, JD Mold, DW Stanger, J Shavel, FJ Riel, JP Bowden, JM Lynch, RS Wyler, B Riegel, H Summer. Paralytic shellfish poison. IV. A procedure for isolation and purification of the poison from toxic clams and mussels. J Am Chem Soc 79:5230–5235, 1957.
8. AH Duranas, M Norte, JJ Fernandez. Toxic marine microalgae. Toxicon 39:1101–1132, 2001.
9. VM Bricelj, SE Shumway. Paralytic shellfish toxins in bivalve molluscs: occurrence, transfer kinetics, and biotransformation. Rev Fish Sci 6:315–383, 1998.
10. IC Nesbit. Paralytic shellfish poisoning: effects on breeding terns. Condor 85:338–345, 1983.
11. JR Geraci, DM Anderson, RJ Timperi, DJ St. Aubin, GA Early, JH Prescott, CA Mayo. Humpback whales fatally poisoned by dinoflagellate toxin. Can J Fish Res Aquat Sci 46:1895–1898, 1989.
12. T Aune. Health effects associated with algal toxins from seafood. Arch Toxicol Suppl 19:389–397, 1997.
13. DC Rodrigue, RA Etzel, S Hall, E de Porras, OH Valesquez, RV Tauxe, EM Kilbourne, P Blake. Lethal paralytic shellfish poisoning in Guatemala. Am J Trop Med Hyg 42:267–271, 1990.
14. RE Levin. Paralytic shellfish toxins: their origin, characteristics, and methods of detection: a review. J Food Biochem 15:405–417, 1991.
15. BD Gessner, P Bell, GJ Doucette, E Moczydlowski, MA Poli, F Van Dolah, S Hall. Hypertension and identification of toxin in human urine and serum following a cluster of mussel-associated paralytic shellfish poisoning outbreaks. Toxicon 35:711–722, 1997.
16. JF Lawrence, C Menard. Liquid chromatographic determination of paralytic shellfish poisons in shellfish after prechromatographic oxidation. J Assoc Off Anal Chem 74:1006–1012, 1991.
17. MA Quilliam. Phycotoxins. J AOAC Int 82(3):773–781, 1999.
18. MA Poli. Three-dimensional binding assays for the detection of marine toxins. In: Proceedings of the Workshop Conference on Seafood Intoxications: Pan American Implications of Natural Toxins in Seafood. Miami: University of Miami, 1996.
19. GJ Doucette, MM Logan, JS Ramsdell, FM Van Dolah. Development and preliminary validation of a microtiter plate-based receptor binding assay for paralytic shellfish poisoning toxins. Toxicon 35: 625–636, 1997.
20. LE Llewellyn, E Moczydlowski. Characterization of saxitoxin binding to saxiphilin, a relative of the transferrin family that displays pH-dependent ligand binding. Biochem 33:12312–12322, 1994.
21. WN Adams, JJ Miescier. Commentary on AOAC method for paralytic shellfish poisoning. J Assoc Off Anal Chem 63:1336–1343, 1980.
22. U.S. Food and Drug Administration. Fish and Fisheries Products Hazards and Controls Guidance. 3rd ed. Washington, DC: Department of Health and Human Services, 2001, p. 74.
23. DG Baden. Brevetoxins: unique polyether dinoflagellate toxins. FASEB J 3:1807–1817, 1989.
24. MA Poli, SM Musser, RW Dickey, PP Eilers, S Hall. Neurotoxin shellfish poisoning and brevetoxin metabolites: a case study from Florida. Toxicon 38:381–389, 2000.
25. PA Tester, RP Stumpf, FM Vukovich, PK Fowler, JT Turner. An expatriate red tide bloom: transport, distribution, and persistence. Limnol Oceanog 36:1053–1061, 1991.
26. GD Bossart, DG Baden, RY Ewing, B Roberts, SD Wright. Brevetoxicosis in manatees (*Trichechus manatus latirostrus*) from the 1996 epizootic: gross, histologic, and immunochemical features. Toxicol Pathol 26:276–282, 1998.
27. JMC Huang, CH Wu, DG Baden. Depolarizing action of a red tide dinoflagellate brevetoxin on axonal membranes. J Pharmacol Exp Ther 229:615–621, 1984.
28. MA Poli, TJ Mende, DG Baden. Brevetoxins, unique activators of voltage-sensitive sodium channels, bind to specific sites in rat brain synaptosomes. Mol Pharmacol 30:129–135, 1986.
29. MA Poli, CB Templeton, WL Thompson, JF Hewetson. Distribution and elimination of brevetoxin PbTx-3 in rats. Toxicon 28:903–910, 1990.
30. M Cattet, JR Geraci. Distribution and elimination of ingested brevetoxin (PbTx-3) in rats. Toxicon 31:1483–1486, 1993.
31. MJ Holmes, RJ Lewis, MA Poli, NC Gillespie. Strain-dependent production of ciguatoxin precursors (gambiertoxins) by *Gambierdiscus toxicus* (Dinophyceae) in culture. Toxicon 29(6):761–776, 1991.

32. RJ Lewis, MJ Holmes. Origin and transfer of toxins involved in ciguatera. Comp Biochem Physiol 106C:615–628, 1993.
33. RJ Lewis. The changing face of ciguatera. Toxicon 39:97–106, 2001.
34. R Bagnis, T Kuberski, S Laugier. Clinical observations on 3009 cases of ciguatera (fish poisoning) in the South Pacific. Am J Trop Med Hyg 28:1067–1073, 1979.
35. NA Palafox, LG Jain, AZ Pinano, TM Gulick, RK Williams, IJ Schatz. Successful treatment of ciguatera fish poisoning with intravenous mannitol. JAMA 259:2740–2742, 1988.
36. JH Pearn, RJ Lewis, T Ruff, T Tait, J Quinn, W Murtha, G King, A Mallet, NC Gillespie. Ciguatera and mannitol: experience with a new treatment regimen. Med J Aust 151:77–80, 1989.
37. L Eriksson, S Guy, P Perlmutter, R Lewis. A short synthesis of the A/B ring systems of the Pacific ciguatoxins P-CTX-3C and dihydroxy-P-CTX-3C. J Org Chem 64:8396–8398, 1999.
38. S Pauillac, M Sasaki, M Inoue, J Naar, P Branaa, M Chinain, K Tachibana, A-M Legrand. Characterization of mice antisera elicited with a tetracyclic synthetic ring fragment (JKLM) conjugated to carrier proteins. Toxicon 38:669–685, 2000.
39. P Cohen, CFB Holmes, Y Tsukitani. Okadaic acid: a new probe for the study of cellular regulation. Trends Biochem Sci 15:98–102, 1990.
40. RW Dickey, GA Fryxell, HR Granade, D Roelke. Detection of the marine toxins okadaic acid and domoic acid in shellfish and phytoplankton in the Gulf of Mexico. Toxicon 30:355–359, 1992.
41. M Suganuma, H Fujiki, H Suguri, S Yoshizawa, M Hirota, M Nakayasu, M Ojika, K Wakamatsu, K Yamada, T Sugamura. Okadaic acid: an additional non-phorbol-12-tetradecanoate-13-acetate-type tumor promotor. Proc Natl Acad Sci USA 85:1768–1771, 1988.
42. H Fujiki, M Suganuma, H Suguri, S Yoshizawa, K Tagaki, N Uda, K Wakamatsu, K Yamada, M Murata, T Yasumoto, T Sugimura. Diarrhetic shellfish toxin, dinophysis-1, is a potent tumor promotor on mouse skin. Jpn J Cancer Res 79:1089–1093, 1988.
43. S Cordier, C Monfort, L Miossec, S Richardson, C Belin. Ecological analysis of digestive cancer mortality related to contamination by diarrhetic shellfish poisoning toxins along the coasts of France. Env Res (Section A) 84:145–150, 2000.
44. V Burgess, G Shaw. Pectenotoxins—an issue for public health. A review of their comparative toxicology and metabolism. Environ Intl 27:275–283, 2001.
45. OB Stabell, I Steffenak, T Aune. An evaluation of the mouse bioassay applied to extracts of 'diarrhoetic' shellfish toxins. Food Chem Toxicol 30:139–144, 1992.
46. JLC Wright, RK Boyd, ASW de Freitas, M Falk, RA Foxall, WD Jamieson, MV Laycock, AW McCulloch, AG McInnes, P Odense, VP Pathak, MA Quilliam, MA Ragan, PG Sim, P Thibault, JA Walter. Identification of domoic acid, a neuroexcitatory amino acid, in toxic mussels from eastern Prince Edward Island. Can J Chem 67:481–490, 1989.
47. T Takemoto, K Daigo. Constituents of *Chondria arata*. Chem Pharm Bull 6:578, 1958.
48. SS Bates. Ecophysiology and metabolism of ASP toxin production. In: DM Anderson, AD Cembella, GM Hallegraff, eds. Physiological Ecology of Harmful Algal Blooms. Berlin: Springer-Verlag, 1998, pp. 405–426.
49. JLC Wright. Domoic acid—ten years after. Nat Toxins 6:91–92, 1998.
50. KA Lefebvre, CL Powell, M Busman, GJ Doucette, PDR Moeller, JB Silver, PE Miller, MP Hughes, S Singaram, MW Silver, RS Tjeerdema. Detection of domoic acid in northern anchovies and California sea lions associated with an unusual mortality event. Nat Toxins 7:85–92, 1999.
51. VL Trainer, NG Adams, JC Wekell. Domoic acid-producing *Pseudo-nitzschia* species off the U.S. west coast associated with toxification events. Proceedings of the Ninth International Conference on Harmful Algal Blooms, Hobart, 2000.
52. DR Hampson, LJ Manolo. The activation of glutamate receptors by kainic acid and domoic acid. Nat Toxins 6:153–158, 1998.
53. ECD Todd. Domoic acid and amnesic shellfish poisoning—a review. J Food Prot 56:69–83, 1993.
54. K Ofuji, M Satake, T McMahon, J Silke, KJ James, H Naoki, Y Oshima, T Yasumoto. Two analogs of azaspiracid isolated from mussels, *Mytilus edulis*, involved in human intoxication in Ireland. Nat Toxins 7:99–102, 1999.
55. KJ James, A Furey, M Satake, T Yasumoto. Azaspiracid poisoning: a new shellfish toxic syndrome in Europe. Proceedings of the Ninth International Conference on Harmful Algal Blooms, Hobart, Australia, 2000.
56. E Ito, M Satake, K Ofuji, N Kurita, T McMahon, K James, T Yasumoto. Multiple organ damage

caused by a new toxin azaspiracid, isolated from mussels produced in Ireland. Toxicon 38:917–930, 2000.
57. E Ito, M Satake, K Ofuji, M Higashi, K Harigaya, T McMahon, T Yasumoto. Chronic effects in mice caused by oral administration of sublethal doses of azaspiracid, a new marine toxin isolated from mussels. Toxicon 40:193–203, 2002.
58. KJ James, A Furey, M Lehane, H Ramstadt, T Aune, P Hovgaard, S Morris, W Higman, M Satake, T Yasumoto. First evidence of an extensive northern European distribution of azaspiracid poisoning (AZP) toxins in shellfish. Toxicon 40:909–916, 2002.
59. K Ofuji, M Satake, Y Oshima, T McMahon, KJ James, T Yasumoto. A sensitive and specific determination method for azaspiracids by liquid chromatography mass spectrometry. Nat Toxins 7:247–250, 1999.