PARALYTIC SHELLFISH POISONING

BRUCE W. HALSTEAD
Director
International Biotoxicological Centre,
World Life Research Institute,
Colton, CA, USA

in collaboration with

E. J. SCHANTZ
Food Research Institute,
University of Wisconsin,
Madison, WI, USA

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1. INTRODUCTION

The shellfish industry of the world is large, about 71.28 million tonnes for 1979, and according to all projections, the consumption of molluscs in the future should increase considerably. With a rapidly increasing world population and a growing demand for protein food resources, the demand for food molluscs is also increasing. As well as being a valuable food resource, however, molluscs are also a source of potential health problems; there is extensive documentation on the spread of diseases following their consumption. One of the more important and deadly of these shellfish-borne diseases is paralytic shellfish poisoning (PSP).

This guide has been produced in response to the recommendations of a WHO meeting on paralytic shellfish poisoning, which was held in Berlin (West), 5-8 December 1978.\(^a\) The meeting expressed the opinion that recent national and international health problems associated with shellfish consumption justified intensifying the surveillance, prevention, and control of paralytic shellfish poisoning. The aim of the guide is to help prevent outbreaks of PSP, which appear to be on the increase in both incidence and geographical spread. This is in line with a resolution adopted by the Thirty-first World Health Assembly (WHA31.48) on the prevention and control of zoonoses and foodborne diseases due to animal products, which inter alia requested the Director-General to further the development of national, regional, and global strategies and methods of surveillance for the prevention and control of foodborne diseases due to animal products.

\(^a\) The meeting was supported by a financial contribution from the Federal Republic of Germany, which also gave a grant towards the cost of publication of the guide.
2. DEFINITION OF PARALYTIC SHELLFISH POISONING

Paralytic shellfish poisoning is a type of biological intoxication resulting from the ingestion of marine molluscs containing potent neurotoxins derived from planktonic unicellular organisms known as dinoflagellates. Since dinoflagellates possess the basic morphological and nutritional characteristics of both plants and animals, they are sometimes known as "plant animals", and their toxins are referred to as algal toxins or phytotoxins. Paralytic shellfish poisoning is caused by a well-defined group of such toxins, the best known being saxitoxin. This disease is sometimes called mussel poisoning, clam poisoning, or dinoflagellate poisoning, but paralytic shellfish poisoning is the most generally accepted term. Although most human cases of PSP have resulted from the ingestion of bivalve molluscs, the disease has also been associated with gastropod molluscs (spindle-shell molluscs), chitons, starfish, and crustaceans.

Shellfish that serve as vectors of PSP are mainly filter-feeders and ingest large quantities of planktonic organisms, including the toxic dinoflagellates. As a result of the continuous filtration of toxic plankton, large quantities of paralytic shellfish poison are concentrated in the digestive glands of mussels and clams or, in the case of Saxidomus, the Alaska butter clam, in the siphon. Man becomes intoxicated from eating the shellfish.

In recent years, a variety of other dinoflagellate poisons (including tetrodotoxin) that are capable of causing human intoxications has been found in marine molluscs, but these toxins should not be confused with paralytic shellfish poison. Brief mention will be made later of some of these other forms of dinoflagellate intoxications.
3. SIGNIFICANCE OF PARALYTIC SHELLFISH POISONING

3.1 Health aspects

Paralytic shellfish poisoning is one of the more common lethal forms of marine intoxication. In 1974 it was estimated that the worldwide incidence was about 1600 cases with possibly more than 300 deaths per year (Prakash, 1975). Unfortunately, there are no accurate global statistics available on the true incidence of PSP. Sporadic outbreaks occur in North America, Europe, and Japan, and less frequently elsewhere (see Fig. 1). One of the largest outbreaks occurred in a previously unsuspected region, namely the State of Sucre, Venezuela. During 1977 there were three outbreaks there involving 173 people and 10 deaths, and another outbreak in 1979 involved 12 people with no deaths.

Although the overall number of people involved in PSP is relatively small, the biological intoxication is medically and economically significant. The avoidance of PSP within an endemic area is accomplished only by careful monitoring of toxin levels in the shellfish. When lethal outbreaks do take place, they can adversely affect the marketing of shellfish.

Poisoning may result in mild to severe symptoms, which are sometimes followed by death. The morbidity rate among those consuming toxic shellfish is usually high, with a corresponding risk of mortality. The way in which these events are reported in the news media often causes strong emotional reaction in the community. Since there is no known antidote against PSP, the only effective control measure is the closure of the affected source.
FIG. 1. WORLD DISTRIBUTION OF OUTBREAKS OF PARALYTIC SHELLFISH POISONING
(source: Loretta L. Hood)
3.2 Economic and environmental aspects

Economic losses resulting from the occurrence of PSP are considerable for the following reasons:

(a) The closure of harvesting areas leads to severe economic loss to fishermen, processors, and related industries; there is no known way of rendering affected shellfish safe for human consumption other than the closure of affected sources over a period of weeks or months, during which time the toxin is depleted naturally. In some areas, certain species remain toxic for a whole year and therefore cannot be harvested. The toxin is stable and heat treatment, including canning, only partially destroys it. Freezing and other forms of processing or preservation are ineffective in removing or destroying the toxin.

(b) The occurrence of PSP following the consumption of shellfish from one area often leads to the depression of consumer demand for fish and shellfish in other areas. This is due to the sudden onset of outbreaks of PSP and their wide reporting by news media. As an example, in one area of North America the economic loss to the fish and shellfish industries, including secondary effects following a local outbreak, was more than US$ 1 million (Jensen, 1975).

(c) The severity of the effects of PSP on man and the difficulty of predicting its occurrence have led to the introduction by many countries of extensive surveillance and enforcement activities, involving highly qualified personnel in the field, in the laboratory, and in a range of public health functions. The surveillance is maintained for the whole of the period when PSP is likely to occur and may last for six months
of each year. In areas where shellfish are toxic for the whole of the year, permanent surveillance and enforcement activities are required to ensure that professional and recreational fishermen do not harvest the shellfish. It is estimated that the cost of such activities in North America is of the order of US$ 1.2 million per annum.

(d) The occurrence of PSP has severe repercussions on tourism in regions where living in the open air and collecting and eating fish, molluscs, and crustaceans are important tourist activities.

(e) There is evidence that international trade in molluscan shellfish is hampered by the fear that toxic shellfish may be exported to other countries. This factor is likely to become more important as consumption of shellfish is generally increasing and new molluscan resources are being exploited in developing countries. At present, molluscs are the main seafood whose production can be substantially increased by the use of existing cultivation techniques.

In addition to these economic aspects, there are undesirable environmental consequences, including the transfer of dinoflagellate toxins through the food chain with the resultant death of fish and seabirds. Although this mortality has no significant direct or indirect effects on man or commerce, it is highly undesirable. Furthermore, such incidents are sometimes attributed to the indirect effects of industrial pollution and may thus lead to unjustified calls for increased control on the discharge of wastes into the sea.
3.3 Ecological aspects

3.3.1 Plankton (dinoflagellates). The dominant species of dinoflagellate associated with PSP are members of the genera *Gonyaulax* (species: catenella, acatenella, tamarensis-excavata complex), *Pyrodinium* (species: phoneus, bahamense), and *Prorocentrum* spp. (see Plate 1).

These species bloom sporadically in large numbers throughout certain areas of the world, most frequently the north and south temperate zones. *G. catenella* is the predominant toxigenic dinoflagellate along the north Pacific coast of North America from Central California northward along the coasts of Oregon, Washington, British Columbia, Alaska, and westward along the Aleutian Islands to the coasts of Japan, and in the southern hemisphere along the coast of Venezuela. Both *Gonyaulax catenella* and *G. tamarensis-excavata* are present in Japan; *catenella* is predominant in the south and *tamarensis* in the north. *G. tamarensis (excavata)* is the

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Taylor (1979) has proposed that *Gonyaulax catenella*, *G. tamarensis-excavata* complex, and *Pyrodinium phoneus* be transferred to the genus *Protophoxis*. *Pyrodinium bahamense* has been divided into *P. bahamense* var. *bahamense* and *P. bahamense* var. *compressa*. Only *compressa* has been found to be toxic (Harada et al., 1982). *Gymnodinium breve* has recently been transferred to *Pychodiscus breve*. *Prorocentrum* contains several toxic species, but their toxins do not appear to have any relationship to PSP. However, the taxonomic nomenclature used in this guide follows that which appears in the preponderance of the scientific literature dealing with PSP.
predominant poisonous dinoflagellate along the north-east coast of North America (Massachusetts, New Hampshire, and Maine in the USA; Nova Scotia, New Brunswick, and Quebec in Canada), and along the coasts of countries bordering the North Sea (Denmark, Federal Republic of Germany, Netherlands, Norway, United Kingdom).

*Pyrodinium phoneus* has been incriminated in the Netherlands and *P. bahamense* var. *compressa* has been involved in intoxications with vertebrate fish and shellfish in Brunei. PSP outbreaks have been reported in Palau (Western Pacific) Papua New Guinea, Spain and Venezuela. The Palau and Papua New Guinea outbreaks were caused by *P. bahamense* var. *compressa* (Yasumoto, personal communication, 1982).

A large number of species of dinoflagellates have been found to be toxic by laboratory or field tests. The following species have been incriminated in outbreaks involving marine organisms, but have not been incriminated in human intoxications: *Amphidinium carterae (carteri)*, *A. rhynchocephalum*, *Cochlodinium heterolobatum*, *Dinophysis* sp., *Glenodinium foliaceum*, *Gonyaulax fratercula*, *G. grindleyi*, *G. mikimotoi*, *G. monilata*, *G. polyedra*, *G. polygramma*, *G. spirale*, *G. splendens*, *G. veneficum*, *Gyrodinium aureolum*, *G. obtusum*, *Noctiluca scintillans*, *Ostreopsis klebsii*, *O. ovata*, *O. siamensis*, *Oxyrrhis marina*, *Prorocentrum (Exuviaella) balticum*, *P. concavum*, *P. lima*, *P. minimum*, *Protogonyaulax* spp., *Protoperidinium (Peridinium) depressum*, and *Protoperidinium steini*. 
The dinoflagellate Gambierdiscus toxicus has been incriminated in ciguatera fish poisoning\(^a\) (Bagnis et al., 1979; Yasumoto et al., 1979, 1980).

Lassus (1980) also lists the following phytoflagellates, a group of euglena-like protozoans of uncertain phylogenetic position, as toxic: Chattonella subsalsa (Hornellia marina), Eutreptiella sp., Phaeocystis pouchetti, Prymnesium parvum, Pseudopedinella pyriformis, Pyramimonas disomata. These organisms should be considered as potential causes of human biotoxications, but not necessarily of PSP.

The dinoflagellate Gymnodinium breve, which has been the cause of numerous red tides and mass mortalities of fishes in Florida, has also been involved in human intoxications resulting from the ingestion of shellfish, but does not contain paralytic shellfish poison. Although the symptomatology included neurological disturbances, i.e., paraesthesias, the clinical findings were not characteristic of PSP and the intoxications were referred to as "neurotoxic shellfish poisoning" (Hughes, 1979).

\(^a\) Ciguatera is a type of ichthyosarcotoxism caused by the ingestion of warm-water insular marine fishes. Ciguatoxin is a complex poison consisting of both fat- and water-soluble fractions. One of the fat-soluble fractions has been assigned the empirical formula of \((C_35H_65NO_8)_n\) with an estimated relative molecular mass of 1500–1800. The incubation period in humans is within 48 hours. The primary symptoms consist of gastrointestinal disturbances, followed by neurological manifestations consisting of paraesthesias of the lips, tongue, throat, and extremities; reversal of hot and cold sensation; and motor weakness (Halstead, 1965).
Prorocentrum micans and *P. redfieldi* were found to be dominant in culture areas, from which mussels had caused gastrointestinal disturbances (Kat, 1979). However, the causative agent is believed to have been *Dinophysis acuminata*. A similar type of diarrhoeal shellfish poisoning has been reported in Japan caused by *D. fortii* (Yasumoto et al., 1978; Yasumoto et al., 1980). Paralytic symptoms were never observed. *P. minimum* var. *mariae-lebouriae* was involved in an outbreak in which 114 out of 324 victims died after eating short-necked clams (*Venerupis semidecussata*) at Lake Hamana, Shizuoka Prefecture, Japan. Paralytic symptoms were not observed. This has been referred to as venerupin or asari poisoning.

Historically, PSP has been associated with the blooming of dinoflagellates, which may cause a yellowish or reddish discoloration of the water. However, water discoloration may be due to proliferation by many types of planktonic species and does not always result in an outbreak of PSP.

Recent investigations suggest that the presence of toxic dinoflagellates in the sediments of resting cysts appears to be a significant factor in extending the period of toxicity and the area affected (Dale et al., 1978). Toxic cysts sink to the bottom of the water column and accumulate in the flocculent layer at the sediment/water interface, where they may overwinter; transport of cysts may be a significant factor in the spread of PSP. There is some evidence that cysts can render shellfish toxic, casting doubts on the value of counting only motile cells during research and surveillance activities.
The accurate identification of toxic dinoflagellate species is difficult and requires skilled personnel, but for the purposes of routine public health activities, final species determination is not essential during the initial stages of control.

3.3.2 Shellfish. The principal groups of shellfish that have been recorded in PSP outbreaks include species of the following bivalve molluscs: mussels (*Mytilus, Modiolus*), clams (*Saxidomus, Protothaca, Spisula, Mya, Arctica, Numilaria, Mercenaria, Mesodesma, Tresus, Ensis*), and, to a lesser extent, oysters (*Crassostrea, Ostrea*), scallops (*Placopecten, Pecten, Spondylus, Hinnites*), and cockles (*Cardium, Clinocardium*) in temperate zones (see Plate 2). The bivalves *Spondylus butleri* and *Lophus cristagalli* were found to contain paralytic shellfish poison in Palau (Kamiya & Hashimoto, 1978) as were tridacna clams (*Tridacna maxima*) in Okinawa (Kanno et al., 1976). The precise chemical nature of the tridacna poison has not been fully determined. Fatalities from PSP in Magallenes, Chile, involved the mussels *Aulacomya ater* and *Mytilus chilensis* (Avaria, 1979), and in Venezuela *Perna perna* were involved (Reyes-Vasquez et al., 1979). Molluscs become toxic by direct uptake of toxin from planktonic dinoflagellates.

Gastropods have also been involved in PSP outbreaks and/or have been found to contain dinoflagellate toxins. The following gastropods have been incriminated: Atlantic dog winkle (*Thais lapillus*) and moonsnail (*Lunatia heros*) along the New England coast (Tufts, 1979); green turban shell (*Turbo marmoratus, T. argyrostoma*) and top shells (*Tectus pyramis, T. nilotica maximus*) in Okinawa and Palau (Kanno et al., 1976; Yasumoto & Kotaki, 1977; Kotaki et al., 1981); spider conch (*Lambis lambis*) in Okinawa (Yasumoto et al., 1981); rough whelks (*Buccinum undatum*), the ten-banded whelk (*Neptuna decemcostata*), and the spindle shell (*Colus stimpsoni*) of eastern Canada (Medcof, 1972).
Many of these gastropods acquire their toxin as a result of predation on toxic bivalve molluscs.

Most mussels and clams that are used commercially as seafood accumulate the toxin in the hepatopancreas (the so-called digestive or dark gland). They become poisonous when they consume the toxic dinoflagellates and remain so for a considerable period afterwards. Studies have shown that mussels (*Mytilus californianus*) may become toxic even when the dinoflagellate cell counts are as low as 200-400 cells per ml of sea water, a level that does not discolour the water. In Japan, shellfish have been found to be toxic when the dinoflagellate count was as low as 20 cells per litre of sea water (Yasumoto, personal communication, 1982). Studies in England have shown that when mussels (*M. edulis*) are held in dinoflagellate-free salt water at about 15-20°C, the toxicity of the shellfish drops by one half in about 12 days. The detoxification rate appears to be affected by water temperature, and physiological and hydrographic factors that are not fully understood at present. It should be noted that the butter clam (*Saxidomus*) concentrates the poison in the siphon from which it is very slowly eliminated. It has been suggested that the long-term presence of paralytic shellfish poison in the Alaska butter clam may be due to a symbiotic relationship between the toxic dinoflagellate and the body of the clam (Shimizu, 1979). The parts of the shellfish body that are likely to contain paralytic shellfish poison are illustrated in Plate 3.

It may require a year or more for affected shellfish to become safe for human consumption. In the case of scallops, the adductor muscle, the only part usually eaten in North America, does not become toxic although other tissues may contain high concentrations of toxin. Consequently, a large industry is based upon the utilization of adductor muscles, even during periods when shellfish contain toxin.
EXPLANATION OF PLATES

PLATE 1. Scanning electron micrographs of dinoflagellates commonly involved in marine biological intoxications.
   A. *Gonyaulax catenella* (Source: A. R. Loeblich, III and L. A. Loeblich)
   B. *Gonyaulax excavata* (Source: A. R. Loeblich, III and L. A. Loeblich)
   C. *Pyrodinium bahamense* (Source: Karen A. Steidinger)
   D. *Gymnodinium breve* (Source: Karen A. Steidinger)

PLATE 2. Representative species of shellfish that have frequently been incriminated as vectors of paralytic shellfish poisoning.
   A. *Modiolus modiolus*
   B. *Mytilus californianus*
   C. *Protocerata staminea*
   D. *Saxidomus giganteus*
   E. *Mercenaria mercenaria* (Source: Richard Murphy)
   F. *Mya arenaria*
   G. *Spisula solidissima*
   H. *Crassostrea gigas*

(Photographs A-D and F-H are from the files of the International BiotoxicoIogical Center, World Life Research Institute, Colton, CA, USA.)

PLATE 3. Anatomy of the butter clam (*Saxidomus*), showing parts of the body likely to contain paralytic shellfish poison. The poison is concentrated primarily in the siphon.

(Source: International BiotoxicoIogical Center, World Life Research Institute, Colton, CA, USA.)
3.3.3 Relationship of paralytic shellfish poison to other marine organisms. For many years it was believed that dinoflagellate poisons were vectored only by certain species of shellfish, and this remains true for Europe and North America. However, recent studies have shown that these poisons may occur in a wide range of phylogenetically unrelated marine animals in other parts of the world. Fatalities have resulted from eating chub mackerel (*Rastrelliger* sp.) and scads (*Sellar* sp.) that had been feeding on toxic dinoflagellates (*Pyrodinium bahamense* var. *compressa*) at Brunei and Sabah (Beales, 1976; MacLean, 1979). Fatalities have been reported from PSP due to eating "fish" and "shellfish" (species unknown) in Talasea, New Britain, and Madang, Papua New Guinea (MacLean, 1979). Paralytic shellfish poison has been found in coral reef crabs (*Atergatis floridus*, *Platypodia granulosa*, *Zosimus aeneus*) in Okinawa (Yasumoto et al., 1981; Hashimoto, 1979) and the sand crab (*Emerita analoga*) in California (Sommer & Meyer, 1937). Crab intoxications and fatalities have occurred in the Philippines (*Demanía toxica*) (Alcala & Halstead, 1970), New Hebrides (*Zosimus aeneus*) (Hashimoto, 1979), and from Asiatic horseshoe crabs (*Carcinoscorpius rotundicauda*, *Tachypleus gigas*) in Thailand (Halstead, 1965). The chemical nature of the poisons involved in these crabs has not been determined, but they resemble paralytic shellfish poison.

The toxic dinoflagellate (*Gambierdiscus toxicus*) has been incriminated in ciguatera fish poisoning both in the West Indies and in the Indo-Pacific region (Yasumoto et al., 1979; Adachi & Fukuyo, 1979). Ciguatera fish poisoning is of importance because of its widespread and unpredictable involvement in reef fishes throughout the West Indies, and the Indo-Pacific and Indian Oceans.
3.4 Environmental conditions

Dinoflagellates are widely distributed, but PSP is usually endemic in specific geographic areas. The occurrence of dinoflagellate blooms is unpredictable and they fluctuate greatly in their population density. They constitute a public health hazard because of their unpredictability and the rapidity with which toxic concentrations may develop. The conditions that allow formation of blooms are unknown, but may include: specific nutritional requirements, water temperature, solar radiation, weather patterns that bring about movements in water masses, upwelling and tidal mixing, and development of a thermocline (an upper layer of marine water that does not mix with underlying water). Toxic blooms may develop in polluted as well as unpolluted waters.

3.4.1 Seasonal incidence. The seasons in which toxicity levels are highest vary somewhat according to geographical location. Along the Pacific coast of the USA and Canada most cases of PSP have occurred during the period May to October. Generally, the dangerous period lasts only a few days, but shellfish may remain toxic for a month. Toxic shellfish have not been detected on the north Pacific coast between November and January, so there is some foundation to the adage that warns against the consumption of shellfish during the months without the letter "r" in their spelling. European and South African outbreaks of PSP have taken place from May to November. The highest toxicity levels in shellfish in eastern Canada and New England have occurred between July and September. The outbreaks in Sabah and Brunei occurred during March, in Papua New Guinea (including New Britain) from December to February, and in Chile during October and November. Water temperatures and solar radiation appear to be the common denominator in every instance.
3.4.2 **Habitat.** There is no evidence that the toxicity of shellfish can be determined from the nature of the terrain. On the Pacific coast of North America, toxic shellfish are found along open, unprotected coastlines. Samples of shellfish taken from the mouths of bays have been found to have a lower toxicity than those taken from the heads of bays or in protected areas. In Alaska most of the toxic clams have been taken from beaches near the open waters along the wide straits characteristic of south-eastern Alaska, rather than from the beaches of the outside waters. In the Bay of Fundy, eastern Canada, toxic shellfish have come from areas where extreme tidal conditions bring an ocean environment close to shore. In the Massachusetts outbreaks, the toxic shellfish were reported in estuarine or near-estuarine locations and not along the open coastline. Most of the European outbreaks have occurred from eating shellfish taken from open coasts, although some incidents have been associated with harbour, estuarine, or brackish water areas. The Venezuelan outbreaks occurred from shellfish taken from the north-west tip of Margarita Island and along the northern coast of the State of Sucre. Thus, the type of habitat in which outbreaks of PSP have occurred is quite variable, but the hydrographic conditions are probably similar. The barrier produced by a thermocline appears to be the most important hydrographic factor.

3.4.3 **Environmental requirements for toxic dinoflagellates.** The production of a toxic dinoflagellate bloom requires the proper blend of a number of physical and biological factors. Initiation of the bloom is dependent upon the presence of benthic-resting thick-walled cysts (hynocysts). Dispersion of the cysts is by currents, tides, storms, upwellings, and possibly by such man-caused operations as dredging and transplantation of shellfish. The results of these water movements may be a resuspension of sediments, involving nutrients and cysts. Excystment is temperature-dependent and not correlated with light, salinity, or nutritional factors.
In addition to the physical factors, growth and development of blooms are largely dependent upon the biological value of the water and the nutritional requirements of the organism. Nutrients include available trace minerals, chelators, vitamins, and particulate and dissolved organic matter, which meet the nutritional needs of the organism. In many places, "red tides" tend to be coastal phenomena that involve a lowering of salinity due to river discharge, rainfall, and land drainage (Steidinger, 1975; Wall, 1975; Provasoli, 1979; Barber, 1973; Dale et al., 1978; Prakash, 1967), but outbreaks often occur in areas of relatively high salinity.
4. CHEMICAL AND PHYSICAL PROPERTIES

Paralytic shellfish poisoning is caused by a group of related, substituted tetrahydropurine bases produced by dinoflagellates. One of these poisons, called saxitoxin, is produced by *Gonyaulax catenella*, *G. tamarensis* var. *excavata*, *Pyrodinium bahamense* var. *compressa*, and possibly other species of dinoflagellates. Saxitoxin is classed as a neurotoxin and is a substituted tetrahydropurine base, $pK_a$ 8.2 and 11.5. It is a white, hygroscopic solid, very soluble in water, slightly soluble in methanol and ethanol, but insoluble in most non-polar organic solvents. The molecular formula of the free base is $\text{C}_{10}\text{H}_{17}\text{N}_7\text{O}_4$, with a relative molecular mass of 299. The structural formula is shown in Fig. 2 (Schantz et al., 1975). The dihydroxy or hydrated ketone group on the five-membered ring (position 12) is essential for its poisonous activity, which consists of specifically blocking the sodium channels in nerve and muscle cell membranes. Catalytic reduction of this group with hydrogen at atmospheric pressure and 20°C to a monohydroxy group eliminates the activity. Removal of the carbamyl group side-chain on the six-membered ring with 3 mol/litre hydrochloric acid at 100°C, leaving a hydroxy group in its place, produces a molecule with about 60% of the original activity. The presence of this active hydroxy group establishes a means for the preparation of various derivatives of saxitoxin, including those containing radioactive elements.

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*The chemical data presented in this section were prepared by Dr. E. J. Schantz, Department of Food Microbiology and Technology, Food Research Institute, University of Wisconsin, USA.*
FIG. 2. CHEMICAL STRUCTURE OF THE POISONS PRODUCED BY DINOFLAGELLATES THAT CAUSE PARALYTIC SHELLFISH POISONING

Poison                  | R₁         | R₂         | R₃         
Saxitoxin (STX)         | -H         | -H         | -H         
Neo STX                 | -H         | -OH        | -H         
11-(OSO₃) STX           | -OSO₃⁻     | -H         | -H         
N-Carbamyl-1-OSO₃      | -H         | -H         | -OSO₃⁻     

Any derivatives of saxitoxin with a substitution of one of the two H atoms at position 11 exist as α and β epimers, all 12 of which are derivatives of saxitoxin. The sulfated toxins are sometimes called gonyautoxins instead of saxitoxin derivatives.
Another poison that is a derivative of saxitoxin and causes paralytic shellfish poisoning in exactly the same manner is produced by *Gonyaulax tamarensis* var. *excavata*, which occurs along the Atlantic coasts of north-east North America and countries bordering the North Sea. The major poison produced by this organism is a sulfonic acid ester of saxitoxin (11-hydroxysaxitoxin sulfate) (Boyer et al., 1978). 11-hydroxysaxitoxin is also produced by *G. catenella* (Oshima et al., 1976; Onoue et al., 1981). This poison, in contrast to saxitoxin, is a neutral or slightly basic substance. Its solubility properties are similar to those of saxitoxin. Its molecular formula is $C_{10}H_{16}N_7O_8S$, with a relative molecular mass of 394. The sulfonic acid group neutralizes the strongly basic character of the guanidinium group, resulting in the neutral character of the molecule. This particular property of the molecule allows this poison to be separated from the basic saxitoxin on cation exchange resins. If the sulfonic acid ester is hydrolysed from the molecule, a hydroxyl group remains and the molecule assumes basic properties like saxitoxin.

In addition to 11-hydroxysaxitoxin sulfate, *G. tamarensis* var. *excavata* produces small amounts (5-10% of the total poison) of neosaxitoxin (Shimizu et al., 1978). In some strains of *G. tamarensis* this is the dominant toxin, and *G. catenella* may also produce it (Yasumoto, 1982). Neosaxitoxin has a hydroxyl group on the nitrogen at position 1 (see Fig. 2) and it has also been isolated from extracts of the dinoflagellates and from scallops as the sulfonic acid ester (Wichmann et al., 1981). Derivatives of saxitoxin that possess a functional group replacing one of the hydrogens at position 11 occur as α and β epimers. The total number of poisons including these epimers is six, which comprise all the naturally occurring poisons isolated so far. However, laboratory studies at the University of Wisconsin have produced epimers of 11-hydroxysaxitoxin,
which may occur naturally in trace amounts as a hydrolysis product of the sulfonic acid ester. If these epimers are included, a total of nine poisons have been identified (Wichmann et al., 1981). Some of these derivatives are called gonyautoxins (I-VIII) (Shimizu et al., 1975, 1976, 1977, 1978, 1981; Alam et al., 1982). It has recently been demonstrated that gonyautoxin-VIII is a carbamoyl-N-sulfo-gonyautoxin-III (Kobayashi & Shimizu, 1981). The relative abundances of these toxins appear to vary among the various species and strains of dinoflagellates.

On a molar or weight basis, saxitoxin is somewhat more effective in blocking the sodium channels of nerve and muscle cell membranes than any of its derivatives isolated or synthetically produced in laboratory work at the University of Wisconsin. The specific toxicities of six paralytic shellfish toxins - neosaxitoxin and gonyautoxin-I, -II, -III, -IV, and -V - in mice have been established in an unequivocal manner and their toxicities relative to saxitoxin calculated (Genenah & Shimizu, 1981).

4.1 Toxicology

4.1.1 Detection and assay. Paralytic shellfish toxins acquired from marine dinoflagellates are among the most potent non-protein poisons known. Therefore, adequate detection and assay methods are of the utmost importance. Several physical and chemical methods for the detection and assay of dinoflagellate toxins have been reported in the literature (Bates & Rapoport, 1975; Buckley et al., 1976; Shoptaugh et al., 1978, 1981). Although these chemical methods appear to offer promise for the future, they have not yet been adopted by most public health agencies. Some of the chemical methods are able to detect the presence of some of the dinoflagellate toxins, but they do not correlate well with the mouse bioassay test, which generally gives a more accurate
estimate of the concentration of toxin in biological materials. The current mouse assay method requires a consistent supply of mice of uniform weight, which at times may present a logistic problem. The need for a quicker, simpler, and less expensive assay method is recognized. Krogh (1979) has prepared a fairly comprehensive review of biological and chemical procedures for the measurement of paralytic shellfish poisons, but at present the mouse bioassay technique is considered to be the only reliable method of evaluating the presence of paralytic shellfish poison.

The standard mouse assay technique is the most suitable method of measuring dinoflagellate toxins in biological materials. The method was evolved by Sommer & Meyer (1937), then modified by Schantz et al. (1957), as a nonspecific test for dinoflagellate toxins. One mouse unit (MU) is defined as the amount of toxin injected intraperitoneally that would kill a 20-g mouse in 15 minutes. One mouse unit of saxitoxin is equivalent to 0.18 g of active poison. It is important that the assay as described by the United States Food and Drug Administration be used with the standard saxitoxin solution prepared from pure saxitoxin. A standardized solution is available from the United States Food and Drug Administration, Bureau of Foods, Microbiology Division, 200 C Street, Washington, DC, USA. The procedure (Horwitz, 1980) is described below.

4.1.2 Materials. The person testing for paralytic shellfish poison is cautioned to use rubber gloves when handling materials that may contain the poison.

(a) Paralytic shellfish poison standard solution: 100 mg/litre. Available as acidified 20% alcoholic solution. The standard is stable indefinitely in a cool place.
(b) Paralytic shellfish poison working standard solution: 1 mg/litre. Dilute 1 ml standard solution to 100 ml with water. The solution is stable for several weeks at 3-4°C.

(c) Mice: Healthy mice, 19-21 g from stock colony used for routine assay. If the mice weigh less than 19 g or more than 21 g, apply correction factor to obtain true death time (see Table 2). Do not use mice weighing more than 23 g and do not re-use mice.

4.1.3 Standardization of bioassay. Dilute 10-ml aliquots of 1 mg/litre standard solution with 10, 15, 20, 25, and 30 ml of water, respectively, until intraperitoneal injection of 1-ml doses into a few test mice causes a median death time of 5-7 minutes. The pH of the dilutions should be 2-4 and must not be more than 4.5. Test additional dilutions in 1-ml increments of water - e.g., if 10 ml diluted with 25 ml of water kills mice in 5-7 minutes, test solutions of 10 ml plus 24 ml and 10 ml plus 26 ml.

Inject a group of 10 mice with each of two, or preferably three, dilutions that fall within a median death time of 5-7 minutes. Give a 1-ml dose to each mouse by intraperitoneal injection and determine the death time as the time elapsed from completion of the injection to the last gasping breath of the mouse.

Repeat the assay 1 or 2 days later, using dilutions prepared as above that differ by 1-ml increments of water. Then repeat the entire test, starting with the testing of dilutions prepared from a newly prepared working standard solution.

Calculate the median death time for each group of 10 mice used on each dilution. If all groups of 10 mice injected with any one dilution gave a median death time of less than 5 or more than 7 minutes,
disregard the results from this dilution in subsequent calculations. On the other hand, if any groups of 10 mice injected with one dilution gave a median death time falling between 5 and 7 minutes, include all groups of 10 mice used on that dilution, even though some of the median death times may be less than 5 or more than 7 minutes. From the median death time for each group of 10 mice in each of the selected dilutions, determine the number of mouse units/ml from Table 1. Divide the calculated concentration of poison (mg/litre) by the number of mouse units per ml to obtain the conversion factor (CF) expressing μg poison equivalent to one mouse unit. Calculate the average of the individual CF values, and use this average value as a reference point to check routine assays. Individual CF values may vary significantly within a laboratory if the techniques and mice are not rigidly controlled. This situation will require continued use of the working standard or secondary standard, depending on the volume of assay work performed.

4.1.4 Use of standard with routine assays of shellfish. Check the CF value periodically as follows: if shellfish products are assayed less than once a week, determine the CF value on each day assays are performed by injecting 5 mice with the appropriate dilution of the working standard. If assays are made on several days during the week, only one check need be made each week on dilution of the standard such that the median death time falls within 5-7 minutes. The CF value thus determined should check with the average CF value within ± 20%. If it does not check within this range, complete the group of 10 mice by adding 5 mice to the 5 mice already injected, and inject a second group of 10 mice with the same dilution of standard.
<table>
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<th>Death time&lt;sup&gt;a&lt;/sup&gt;</th>
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</table>

\( ^a \) Minutes:seconds.

Average the CF value determined for the second group with that of the first group. Take the resulting value as the new CF value. A variation of more than 20% represents a significant change in the response of the mice to the poison, or in the technique of the assay. Changes of this type require a change in CF value.

Repeated checks of the CF value ordinarily produce consistent results within \( \pm 20\% \). If wider variations are found frequently, the possibility of uncontrolled or unrecognized variables in method should be investigated before proceeding with routine assays.
4.1.5 Preparation of sample

(a) Clams, oysters, and mussels: Thoroughly clean the outside of the shellfish with fresh water. Open by cutting the adductor muscles. Rinse inside with fresh water to remove sand and other foreign material. Remove the meat from the shell by separating the adductor muscles and tissue connecting at the hinge. Do not use heat or anaesthetics before opening the shell, and do not cut or damage the body of the mollusc at this stage. Collect approximately 100-150 g of meats in a glazed dish. As soon as possible, transfer the meats to a No. 10 sieve without layering, and let them drain for 5 minutes. Pick out pieces of shell and discard the drainings. Grind in a household-type grinder with 3-mm to 6-mm holes or in a blender until homogeneous.

(b) Scallops: Separate the edible portion (adductor muscle) and apply the test to this portion. Drain and grind as for clams.

(c) Canned shellfish: Prepare by blending as for clams.

4.1.6 Extraction. Weigh 100 g of well-mixed material into a tared beaker. Add 100 ml of 0.1 mol/litre HCl, stir thoroughly, and check the pH (this should be less than 4.0, preferably about 3.0; if necessary, adjust the pH as indicated below). Heat the mixture, boil gently for 5 minutes, and let it cool to room temperature. Adjust the cooled mixture to pH 2.0-4.0 (never more than 4.5) as determined by BDH Universal Indicator, phenol blue, Congo red paper, or pH meter. To lower the pH, add 5 mol/litre HCl dropwise with stirring; to raise the pH, add 0.1 mol/litre NaOH dropwise with constant stirring to prevent local alkalinization and consequent destruction of poison. Transfer the mixture to a graduated cylinder and dilute to 200 ml.
Return the mixture to the beaker, stir to homogeneity, and let it settle until a portion of supernate is translucent and can be decanted free from solid particles large enough to block a 26-gauge hypodermic needle. If necessary, centrifuge the mixture or supernate for 5 minutes at 3000 rev/min or filter through paper. Only enough liquid to perform the bioassay is necessary.

4.1.7 Mouse test. Intraperitoneally inoculate each test mouse with 1 ml of acid extract. Note the time of inoculation and observe the mice carefully for time of death as indicated by the last gasping breath. Record the death time from a stopwatch or clock with a sweep second hand. One mouse may be used for the initial determination, but 2 or 3 are preferred. If the death time or median death time of several mice is less than 5 minutes, make a dilution to obtain a death time of 5-7 minutes. If the death time of 1 or 2 mice injected with the undiluted sample is less than 7 minutes, a total of three mice must be inoculated to establish the toxicity of the sample. If large dilutions are necessary, adjust the pH of dilution by dropwise addition of dilution HCl (0.1 or 0.01 mol/litre) to pH 2.0-4.0 (never more than 4.5). Inoculate 3 mice with the dilution that gives death times of 5-7 minutes.

4.1.8 Calculation of toxicity. Determine the median death times of the mice, including survivors, and from Table 1 determine the corresponding number of mouse units. If test animals weigh less than 19 g or more than 21 g make a correction for each mouse by multiplying the number of mouse units corresponding to the death time for that mouse by the weight correction factor for the mouse obtained from Table 2, then determine the number of median mouse units for the group. (Consider the death time of survivors as more than 60 minutes, or equivalent to less than 0.875 MU
TABLE 2. CORRECTION TABLE FOR WEIGHT OF MICE
(from Horwitz, 1980)

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when calculating the median.) Convert the number of mouse units to µg of poison per ml by multiplying by the CF value: µg poison per 100 g of meat equals (mg/litre) x dilution factor x 200. Consider any value more than 80 µg/100 g as hazardous and indicating that the meat is unsafe for human consumption (Horwitz, 1980).

The following standards have been established in the USA for tolerable toxin titres:

Whole mussels, raw, frozen or tinned, can be offered for sale provided that their average content is less than 400 MU/100 g mussel meat, and that no single packet contains more than 2000 MU/100 g.

Comminuted mussels, where the toxicity is liable to slight variation, shall contain an average of less than 2000 MU/100 g and no single packet shall exceed this value.

In 1958 the Shellfish Sanitation Workshop laid down for United States interstate trade in mussels 80 µg/100 g (or 400 MU/100 g) as a quarantine limit for mussel fishery.\(^{a}\)

4.1.9 Shortened bioassay tests. For routine control purposes, and particularly where extensive laboratory facilities including laboratory animals are not available, much useful information can be obtained from the use of a shortened test. For research or legal purposes, more precise estimates of toxin concentration need to be made according to the protocols described above. A shortened test may be useful for screening samples, but will also provide rough estimates of the

levels of toxin present, and whether concentrations are falling or rising.

In the shortened test, toxin extraction is carried out in the usual way. A pair of mice (or a single mouse) between 18 and 21 g weight are then injected with 1 ml of extract. If the animals survive 60 minutes, it can be assumed that either no toxin or negligible amounts of toxin are present (i.e. less than about 175 MU/100 g). If the animals die in 5 minutes or less following injection, it can be assumed that the extract contains more than the maximum safe concentration of toxin (400 MU or 80 μg toxin/100 g tissue). In these circumstances, the test should be repeated using 1 or 2 mice and, where necessary, the extract diluted to produce an expected survival time of 5 minutes. Where deaths occur between 5 and 60 minutes following the injection of undiluted extracts, toxin concentrations are likely to be in the range of 175 and 380 MU/100 g tissue, and the tests should be repeated with 1 or 2 mice.

An experienced scientist using these procedures can determine levels of toxin with sufficient accuracy to advise regulatory authorities. Providing the weight range of the mice is maintained, and a uniform source of mice is used, standardization of the bioassay need be carried out only once. In emergencies, a screening test may be carried out without standardization.

4.1.10 Chemical assay methods. The mouse bioassay method is the most accurate method for toxicity assessment purposes. However, in Scandinavia and other parts of Europe animal assay methods are sometimes prohibited; in these instances a chemical method may be required. The fluorimetric method developed by Bates & Rapoport (1975) has been used for several years in Norway with reputedly satisfactory results. The technique involves alkaline hydrogen peroxide oxidation of saxitoxin to
8-amino-6-hydroxymethyl-2-iminopurine-3(2H)-propionic acid (later referred to as AHIP). The chemical assay procedure is as follows:

1. Remove the meat from the frozen shellfish, cut into chunks, and grind in a blender for about 1 minute until a homogeneous consistency results. (2) Add 2.0 ml of 0.5 mol/litre trichloroacetic acid (freshly diluted from 2 mol/litre acid) to 2.0 g of ground shellfish meat and mix with a glass rod. (3) Heat to an internal temperature of 85-90°C for 10 minutes. Mix after 5 and 10 minutes. (4) Cool in an ice-bath to 20°C. Add about 0.2 ml of 100 g/litre NaOH with stirring until a constant pH of 5.0-5.5 is reached. (5) Centrifuge at 12,000 g for 10 minutes. (6) Apply supernatant to a 6 × 75 mm column of 2 ml of Bio-Rex 70, 50-100 mesh resin, previously equilibrated with 0.2 mol/litre, pH 5, sodium acetate buffer and discard the eluent. Prepare the resin by rinsing several times with HCl and NaOH, rinse with water and suspend in several volumes 0.2 mol/litre acetic acid, adjusting the pH to 5.0 with HCl. The resin may be recycled after use. (7) Elute with 30 ml of 0.2 mol/litre, pH 5.0, sodium acetate buffer, 25 ml of water, and 1.0 ml of 0.5 mol/litre HCl. Discard the eluents. (8) Elute with 4.0 ml of 0.5 mol/litre HCl and collect the eluent in a centrifuge tube. Mix, then divide into two equal volumes in centrifuge tubes. (9) Add 2.0 ml of 1.2 mol/litre NaOH and 0.05 ml of 10% H2O2 to one portion and mix. Substitute H2O for H2O2 in the other. (10) Centrifuge at 1000 g for one minute and transfer the supernatants into cuvettes. (11) Forty minutes after the H2O2 was added, neutralize to pH 5 with about 0.15 ml of glacial acetic acid. (12) Measure the fluorescence of the oxidized portion and subtract that of the unoxidized blank, using excitation at 330 nm and reading the emission at 380 nm. The Raman peak of pure water (excitation 330 nm, emission 371 nm) is useful for routine calibration and corresponds to approximately
0.017 µg of saxitoxin per gram of shellfish, or 7 x 10^{-9} \text{ mol/litre}. The relationship between the saxitoxin concentration and the fluorescence is completely linear. The instrument is conveniently calibrated with a solution of AHIP, freshly prepared by oxidation of purified saxitoxin. The concentration of AHIP is determined by ultraviolet absorption spectrophotometry, then this solution is appropriately diluted (pH 5, 1 g/litre H_{2}O_{2}) and its fluorescence is measured against the Raman peak. (13) An abbreviated assay, useful for routine applications, is possible if the Bio-Rex 70 chromatography and elution (steps (6), (7), and (8)) are omitted. A significant amount of time is saved, and limited experience shows that the blank increases by only 50%.

4.2 Effects in animals

Toxins responsible for PSP are capable of affecting a variety of animals, including fish, birds, and mammals. Information on the toxic effects has been gathered both from field cases involving large-scale mortality of seabirds and fish and from numerous experimental studies in various species of animal (e.g., frog, chicken, mouse, rat, rabbit, cat, and dog). Pharmacological studies show that the primary mechanism of action of paralytic shellfish poison is blockage of the sodium channels of the excitable cell membranes of nerves, thereby blocking the generation of nerve impulses. It is believed that there are about 10, or possibly more, toxins in the paralytic shellfish poison complex, but only saxitoxin has been studied pharmacologically to any extent. The route of administration has a major influence on the toxicity; intravenous or intraperitoneal injection requires 20-40 times less toxin than oral ingestion to produce the same effect. The LD_{50} values in mice for saxitoxin administered by these three routes have been recorded: intravenous, 2.4 µg/kg; intraperitoneal, 10.0 µg/kg; per os, 263 µg/kg.
Studies with rats indicate that the sensitivity to saxitoxin decreases with age, and interspecies differences in sensitivity are generally within an order of magnitude, at least for warm-blooded animals (e.g., mouse, rat, rabbit, cat, and dog). Excellent reviews on the pharmacological properties of saxitoxin have been written by Kao (1966), Kao & Nishiyama (1965), Kao & Fuhrman (1967), and Ritchie & Bogart (1977).

The toxin of Gymnodinium breve is quite distinct from that of paralytic shellfish poison. G. breve toxin has been studied quite extensively (Abbott & Paster, 1972; Alam et al., 1979; Risk et al., 1979; Sasner, 1973; Spiegelstein et al., 1973; Steidinger et al., 1973). Three toxins have been isolated (GB-I, -II, and -III). All three toxins have neurotoxic activity; GB-I also has haemolytic activity. GB-II is the principal toxin; it depolarizes membranes, but has no anticholinesterase activity. The LD_{50} of GB-II is 0.5 mg/kg (mice, intraperitoneal). The structures of these toxins are not completely known, and there is no complete agreement as to their pharmacological and chemical properties.

4.3 Effects in man

Symptoms of PSP in man include paraesthesia affecting the oral region and the limbs. This is noticed as a tingling or burning sensation of the lips, tongue, and face, with gradual progression to the neck, arms, fingertips, legs, and toes. The paraesthesia later changes to numbness, such that voluntary movements are made with difficulty. Symptoms usually develop within 30 minutes of consumption. Paralysis in upper and lower limbs may follow, manifesting itself in ataxia, loss of motor coordination, constrictive sensation in the throat, and incoherent speech. Other symptoms sometimes observed are a sensation of lightness ("floating in the air"), weakness, dizziness, headache, salivation, intense
thirst, and temporary blindness. Gastrointestinal disturbances are less common. Mental symptoms vary, but most victims are calm and conscious of their condition throughout their illness. Muscular twitches and convulsions are rare. Following high toxin intake, paralysis of respiratory muscles may occasionally progress to respiratory arrest and consequent death. In major outbreaks, deaths following the consumption of toxic shellfish have been found to occur after intake of amounts estimated between 5000 MU and more than 30,000 MU. A specific antidote or therapy is not available; symptomatic treatment (artificial respiration) has been used successfully in some cases. The case-fatality rate is about 8.5%.

There is an allergic form of shellfish poisoning, which manifests itself by a severe allergic reaction. The incubation period is usually short, within a period of several hours. The symptoms consist of a diffuse erythema, swelling, and urticaria of the face and neck, but may involve the entire body (the rash may be accompanied by a severe itching); headache, sensation of warmth, conjunctivitis, coryza, gastric distress, dryness of the throat, swelling of the tongue, and respiratory distress may be present. The patient may be helped by the use of antihistaminic drugs. Generally the patient recovers within a period of a few hours, but death may occur.

The allergic form of shellfish poisoning should not be confused with scombroid poisoning, which is a form of ichthyosarcotoxism, caused largely by fishes of the suborder Scombroideae, all of which are members of the single family Scombridae - the tunas and related species. Scombrotoxism is generally caused by the improper preservation of scombroid fishes, which results in certain bacteria acting on histidine in the muscle of the fish and converting it to saurine, a histamine-like
substance. This is the only known form of ichthysosarcotoxism in which bacteria play an active role in toxin production within the body of the fish. The symptoms of scombroid poisoning resemble those of histamine intoxication. Symptoms usually develop within a few minutes after ingestion of the toxic fish and include intense headache, dizziness, throbbing of the carotid and temporal vessels, epigastric pain, burning of the throat, cardiac palpitation, rapid weak pulse, dryness of the mouth, thirst, inability to swallow, gastrointestinal upset, diarrhoea, abdominal pain, generalized erythema, urticarial eruptions, severe pruritus, swelling and flushing of the face, bronchospasm, suffocation and severe respiratory distress. There is danger of shock, and deaths have been reported. In rare instances, scombroid fishes have been involved in both scombroid and ciguatera poisoning in the same individual. The victim usually recovers within a period of one or several days. Treatment requires the use of antihistaminic drugs. On a worldwide scale, scombrototoxism accounts for the greatest overall morbidity rate of any single type of ichthysosarcotoxism. There are no public health control measures.

There is no evidence to indicate that people living in areas where PSP is endemic and who have been exposed to low-level toxicity are more resistant to paralytic shellfish toxin than those in other areas. Further studies need to be undertaken to establish the relationship between toxin intake and effects on man.
5. SURVEILLANCE, PREVENTION, AND CONTROL

The primary principle employed in the establishment and operation of preventive programmes is that control must be exercised at the source. This is necessary because of the sudden and sporadic onset of PSP and of the diffuse nature of the shellfish business itself, such that effective control cannot be exercised at the processing and marketing levels.

Several shellfish controlling authorities in North America and Europe have considerable experience in the management of areas affected by paralytic shellfish poison. The extensive North American experience and the control programmes that have evolved provide a sound basis for the formulation of the strategy, control, and methods that may be applied in other countries. For general guidance on legislation and the establishment of the control service, reference should be made to the WHO Guide to Shellfish Hygiene (Wood, 1976).

5.1 Definition of the problem

Recent experience has shown that countries having substantial mollusc resources in temperate zones in the northern and southern hemispheres should be aware of the potential risks of paralytic shellfish poisoning. Even in areas where there is no previous history, a careful study of historical marine science documents and public health records should be made. Consultation with local fishing authorities, and even local fishermen, may also provide information on the incidence of PSP. In addition, epidemiological surveys of the coastal populations should be conducted to determine if unreported cases of paralytic shellfish poisoning have occurred. This is particularly important where a new shellfish industry is being developed or a new species is to be exploited. To define further the scope of the potential problem, the quantity of shellfish that it is
intended to produce and the areas to be harvested should be determined. In addition, attention should be given to shellfish that are harvested by individuals for their own consumption. These people pose a difficult public health problem because their protection usually requires intensive public education measures.

5.2 Responsibilities of the regulatory authority

The establishment of national control measures should be vested in one agency that has the legal authority to make regulations and to take effective regulatory action. In the development of control measures, the regulatory authorities should fully inform the shellfish industry of the nature and scope of the potential problem and actions that may be taken. Methods should be clearly established for informing shellfish harvesters and others when areas are to be closed. The regulatory authorities should ensure that samples of shellfish are delivered to the laboratory by the fastest possible means and that laboratory results are rapidly communicated to the responsible official by a clearly established route. As an aid to control, regulatory authorities should make initial surveys of areas where new industries are being developed, and carry out routine surveillance of established shellfish growing areas.

5.3 Initial surveys

Where new industries are about to be established, an initial surveillance programme should consist of the collection of shellfish samples for toxin assay from stations representative of the major harvesting areas. Sampling should be continued for a period of one year, at intervals of a month, with particular emphasis on the period of maximum solar radiation and highest water temperatures. Sampling stations should be located so as to detect the first occurrence of paralytic shellfish
poison in the area. Such factors as the configuration of the coastline, hydrographic features involving current speeds and direction, areas of upwelling, and the shellfish species to be exploited need to be carefully considered. Sampling should always include the shellfish taken from the major areas of production. Mussels concentrate paralytic shellfish poison comparatively rapidly and may be used as early warning indicators of toxin development. Local information can provide a useful guide to sampling.

5.4 Routine surveillance

In areas where there is a long history of shellfish consumption, but no epidemiological evidence of paralytic shellfish poisoning, nothing more than a minimum surveillance programme of growing areas is indicated. Where PSP is known to occur regularly, weekly samples of shellfish should be taken before the expected earliest onset of toxicity. In addition, public health officials should ensure that any epidemiological information indicating that shellfish may have become toxic should be made available to those responsible for surveillance of the growing areas so that additional sampling can be carried out.

Any human intoxication by paralytic shellfish poison should be thoroughly investigated, documented, and reported to the appropriate authorities. An example of an epidemiological report form used in Canada and in the USA for this purpose is presented in Annex 1.

After sufficient data have been collected in a particular area, the frequency of sampling may be adjusted to reflect the expected occurrence. In areas where toxicity is known to occur, weekly sampling of key stations during danger periods is recommended.
The most widely accepted proven method for detecting paralytic shellfish poison is the mouse bioassay procedure (see section 4.1). This requires simple laboratory facilities, trained personnel, and the availability of a supply of laboratory mice. Where these facilities are not available close to the sampling area, samples may be safely transported under chilled conditions without loss of toxin.

Small amounts of toxin are often detected in shellfish and, for efficient control, the officials need to establish a concentration at which action should be taken. For more than 20 years, regulatory bodies in Canada and the USA, and more recently in the United Kingdom, have found a concentration of 800 μg (4000 MU)/kg of wet flesh to be a satisfactory working level. When this level is exceeded, action is taken to close affected areas and stop further shellfish harvesting. This level provides a wide margin of safety, but is necessary to give the regulatory authorities time to take action before levels in shellfish become unacceptable for human consumption; it also takes into account the variation of the bioassay test method, the extreme natural variation of toxin levels in shellfish, and the practical difficulties of frequent sampling. Furthermore, no paralytic shellfish poisoning has ever been recorded when control activities were carried out on this basis.

5.5 Classification of areas

Shellfish harvesting areas may be divided into the following three categories on the basis of previous investigations and records, anticipated harvesting operations, and initial survey results:

Nontoxic areas - areas that have no known history of producing toxic shellfish and where sampling results have all been negative.
Seasonally toxic areas - areas that produce samples of shellfish occasionally containing levels of paralytic shellfish poison above the accepted public health level.

Chronically toxic areas - areas where shellfish are frequently or continually found to contain levels of paralytic shellfish poison above the accepted public health level.

Formulation of a national management plan for the control of seasonally and chronically toxic areas should be governed by the following criteria, having due regard to the size and scope of the shellfish production and the population at risk. Key sampling stations are those where shellfish samples are most likely to give the earliest warning of paralytic shellfish poison accumulation in shellfish.

Arrangements need to be made for sufficiently frequent sampling of key stations to detect the occurrence of toxic shellfish. Countries already having established PSP management plans have found that it is adequate to undertake sampling once a month during periods when shellfish are rarely toxic and once a week during periods when shellfish above the acceptance level are expected. Experience has indicated that this frequency is adequate to provide information for effective control.

In general, PSP management programmes operate as follows:

(1) Key shellfish sampling stations are located.

(2) Seasonal sampling plans are developed.

(3) The most sensitive species, usually mussels, are used as indicators.
(4) As paralytic shellfish poison levels rise at key stations, satellite stations are established and monitored.

(5) Areas exceeding quarantine levels are closed to harvesting, posted, and patrolled.

(6) Market sampling may be conducted as warranted, and an embargo placed on affected shellfish.

(7) Public education measures are taken to alert recreational harvesters.

(8) Areas are reopened to harvesting when toxin levels in shellfish meats fall consistently below quarantine levels.

On the east coast of North America, visible "red tide" slicks, dead ducks or gulls, and "weakened shellfish" are sometimes used as early warning indicators of PSP. However, visual evidence cannot always be relied upon. During extended periods of freedom from toxicity, surveillance tends to decrease, resulting in an increased health hazard when massive unexpected blooms occur.

5.6 Detoxification of paralytic shellfish poison by ozone

Ozone gas added to seawater has been found effective in inactivating paralytic shellfish poison under certain experimental conditions. It has been suggested that passing ozonized seawater over toxic shellfish beds could reduce their toxicity. Preliminary studies have been encouraging, but are still experimental in nature. The drawbacks to ozonation are cost-effectiveness and reduction of flavour, thereby reducing consumer acceptability of the shellfish (Blogoslawski & Stewart, 1977; Blogoslawski & Neve, 1978; Blogoslawski, 1979).
6. INTERNATIONAL PROGRAMMES, COORDINATION, AND COOPERATION

International cooperation and coordination are needed because of the widespread geographical distribution of PSP throughout the world, expansion of international trade in shellfish, and the increasing risks associated with the transport of pathogens by vectors. This need is accentuated by the recent expansion of shellfish production in new areas, particularly in developing countries. The WHO meeting on paralytic shellfish poisoning held from 5 to 8 December 1978 recommended that international organizations, and in particular FAO and WHO, should continue to provide technical advice to their Member States on matters related to health risks associated with shellfish production. This is particularly important when new areas are being exploited.

Successful programmes already exist in some countries and could be adopted by others. However, gaps still exist in our knowledge of PSP and research should continue. For efficient use of scientific and technical information, a worldwide system for the collection, evaluation, and dissemination of data would be desirable. In planning such a system, consideration should be given to the existing activities of international organizations, including the dissemination of appropriate information through such journals as the Weekly Epidemiological Record. It has been suggested that the international exchange of PSP data could be undertaken in part through the WHO Surveillance Programme for Control of Foodborne Diseases in Europe, which aims, among other things, at the prompt dissemination of information in order to assist control actions. In view of the high attack rate, the rapid exchange of epidemiological information would be of considerable usefulness in the prevention and control of outbreaks caused by exported shellfish. PSP comes within the programmes of the WHO
zoonoses centres, whose activities include the control of foodborne diseases. Data on PSP outbreaks and other forms of marine biological intoxications are being collected by the International Bioticoxicological Centre, World Life Research Institute, Colton, CA 92324, USA.

Education and training in all aspects of the prevention and control of PSP also needs to be coordinated at the international level.

Standardization of laboratory methods is a prerequisite for comparison of data received from different countries, and for this purpose a supply of the purified toxin for the standardization of bioassay techniques is essential (see section 4.1). In assessing the importance of PSP, consideration has not only to be given to the medical aspects, but also to the variety of related socioeconomic consequences of this disease. Attention should be given to such aspects as the cost of illness, maintenance of surveillance and enforcement programmes, losses of high-quality food, and the effect of PSP on the marketing and trading of shellfish. Past experience has shown that a single outbreak of PSP may have devastating effects on national shellfisheries, in addition to the socioeconomic repercussions encountered in the area concerned.
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Dr J. D. Clem, Chief, Shellfish Sanitation Branch, Bureau of Foods, Food and Drug Administration, Washington, DC, USA

Dr B. C. Dazo, World Health Organization, Regional Office for the Western Pacific, Manila, Philippines

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Professor D. Grossklaus, Director, FAO/WHO Collaborating Centre for Research and Training in Food Hygiene and Zoonoses, Institute of Veterinary Medicine (Robert von Ostertag Institute), Berlin (West)

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REPORT FORM TO BE USED FOR CASES OF
SUSPECTED PARALYTIC SHELLFISH POISONING

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<th>How much shellfish broth or bouillon ingested?</th>
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