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Environmental Health Criteria 37

AQUATIC (MARINE AND FRESHWATER) BIOTOXINS

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The **International Programme on Chemical Safety (IPCS)** is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. The main objective of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment. Supporting activities include the development of epidemiological, experimental laboratory, and risk-assessment methods that could produce internationally comparable results, and the development of manpower in the field of toxicology. Other activities carried out by IPCS include the development of know-how for coping with chemical accidents, coordination of laboratory testing and epidemiological studies, and promotion of research on the mechanisms of the biological action of chemicals.

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NOTE TO READERS OF THE CRITERIA DOCUMENTS

While every effort has been made to present information in the criteria documents as accurately as possible without unduly delaying their publication, mistakes might have occurred and are likely to occur in the future. In the interest of all users of the environmental health criteria documents, readers are kindly requested to communicate any errors found to the Manager of the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda, which will appear in subsequent volumes.

In addition, experts in any particular field dealt with in the criteria documents are kindly requested to make available to the WHO Secretariat any important published information that may have inadvertently been omitted and which may change the evaluation of health risks from exposure to the environmental agent under examination, so that the information may be considered in the event of updating and re-evaluation of the conclusions contained in the criteria documents.

ENVIRONMENTAL HEALTH CRITERIA FOR AQUATIC (MARINE AND FRESHWATER) BIOTOXINS

Following the recommendations of the United Nations Conference on the Human Environment held in Stockholm in 1972, and in response to a number of World Health Assembly Resolutions (WHA23.60, WHA24.47, WHA25.58, WHA26.68), and the recommendation of the Governing Council of the United Nations Environment Programme, (UNEP/GC/10, 3 July 1973), a programme on the integrated assessment of the health effects of environmental pollution was initiated in 1973. The programme, known as the WHO Environmental Health Criteria Programme, has been implemented with the support of the Environment Fund of the United Nations Environment Programme. In 1980, the Environmental Health Criteria Programme was incorporated into the International Programme on Chemical Safety (IPCS). The result of the Environmental Health Criteria Programme is a series of criteria documents.

The draft of the Environmental Health Criteria document on Aquatic (Marine and Freshwater) Biotoxins, prepared by Professor P. Krogh of Copenhagen, Denmark, was sent to focal points in member states and individual experts for comments.

A WHO Task Group on Environmental Health Criteria for Aquatic (Marine and Freshwater) Biotoxins met in Geneva from 12-17 December, 1983. Dr. J. Parizek opened the meeting on behalf of the Director-General. The Task Group reviewed and revised the draft criteria document and made an evaluation of the health risks of exposure to aquatic (marine and freshwater) biotoxins.

The efforts of all who helped in the preparation and finalization of the document are gratefully acknowledged.

* * *

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SUMMARY WITH EVALUATION OF THE HEALTH RISKS OF EXPOSURE TO AQUATIC BIOTOXINS AND RECOMMENDATIONS FOR FURTHER ACTIVITIES

In view of the complex character of the problem of aquatic biotoxins, the evaluation of the risks of adverse health effects made by the Task Group is presented together with the summary of information contained in the document.

Introduction

This document deals with outbreaks of certain human diseases associated with human exposure to compounds produced by algae. Predators feeding on the algae become contaminated by these compounds which, in this way, enter the human food chain. Diseases such as paralytic shellfish poisoning (PSP), ciguatera, and the more recently identified syndromes, neurotoxic shellfish poisoning (NSP) and diarrhoeic shellfish poisoning (DSP), are discussed in the document as well as the evidence of their association with dinoflagellate toxins present in human food. Tetrodotoxin intoxication (puffertish poisoning) is discussed because the compound, which is produced by certain fish in various areas of the world, has a similar action to that of saxitoxin, one of the main components causing PSP. Direct dermal contact with toxins from a marine cyanophyte causes a particular type of acute dermatitis, observed in certain areas of the world. No human disease has been identified as being a result of exposure to toxins from freshwater cyanophytes. However, this topic has been included in the review for completeness.

Significant effects in non-human targets, associated with blooms of PSP-producing and NSP-producing dinoflagellates, and evidence of the involvement of PSP and NSP toxins in these outbreaks are also reviewed.

Paralytic Shellfish Poisons

The PSP toxins constitute a well characterized group of tetrahydropurines. Saxitoxin was the first PSP component identified. Subsequently, 12 other components, closely related in structure to saxitoxin, have been discovered in dinoflagellates and/or in shellfish.

The method used, so far, for the detection and quantitation of PSP components in environmental media such as food, and aimed at the assessment of exposure, is a bioassay using a rather unspecific endpoint (time to death of mice). This method is not suitable for the measurement of the toxins in human tissues and fluids. However, chemical methods such

as those based on spectrophotometry and high pressure liquid chromatography are being introduced for this purpose.

The PSP components are produced by a well-defined group of dinoflagellates (mainly Gonyaulax species), occurring in both tropical and temporate seas. Molluscs, feeding on the algae, accumulate the toxins. There is a report showing that the shellfish species accumulating PSP are resistant to the adverse effects of these compounds. The highest concentrations of PSP in molluscs are found during algal blooms, a phenomenon that may be triggered by meteorological events. However, shellfish also feed on non-motile algal cells (resting cysts) containing PSP, which are not bloom-related. Another algal source of PSP in coral reefs is a red seaweed (Janus sp.), which certain crabs feed on. Finally, two reports have proved that even a freshwater cyanophyte, Aphanizomenon flos-aquae, can produce PSP components. However, at present, there is no evidence that this would be of any significance with regard to human exposure, either directly or through entry of the compounds from freshwater cyanophytes into the food chain.

In marine ecosystems, transfer of PSP components from phytoplankton through zooplankton to fish has been observed, and fish kills and mass death of seabirds have been reported in association with blooms of PSP-producing dinoflagellates. The PSP toxins were found in the zooplankton and in the gut of dead or diseased fish in these outbreaks, but only occasionally, in the muscle tissue of the same fish. The available studies indicate that LD_{50} values for fish and birds are in the same range as those obtained in laboratory studies on mammals.

The only significant pathway of exposure to PSP, known at present for human beings, is the eating of predators contaminated by feeding on marine algal producers of the toxins. The most significant food commodity is bivalve molluscs (mussels, clams, and oysters). In shellfish, the highest concentrations of PSP have been found in the digestive organs (stomach and diverticula), but PSP has also been detected in other soft tissues.

Contamination with PSP is being monitored in shellfish-growing areas in several countries, and areas where the PSP level in the edible portion of the shellfish exceeds a certain value will be closed and will not be reopened before the level of shellfish contamination decreases below the action level.

Human exposure has also occurred through eating crabs contaminated by PSP in the coral reef ecosystems.

In spite of the above-mentioned evidence of PSP presence in dead or dying fish in association with blooms, no report is available, so far, that links consumption of finned fish by human beings with exposure to PSP.

There is no evidence of dermal exposure to PSP or exposure through drinking-water.

No data are available on the absorption, distribution, metabolism, and excretion of PSP toxins in animals, with the exception of limited information on PSP distribution in fish and shellfish.

Most of the studies on the toxicity of PSP have been performed as acute, single-dose experiments, using an extract from Alaskan butter clams containing saxitoxin. In a number of animal species, the LD_{50} values by oral administration range from 100 to 800 µg saxitoxin/kg body weight. When administered parenterally in mice, the LD_{50} is 3-10 µg saxitoxin/kg body weight, compared with 263 µg/kg body weight by oral administration. After parenteral or oral administration, the animals die within a few minutes in dyspnoea.

The systemic action of saxitoxin can be explained by a wide-spread blockade of impulse generation in periferal nerves and skeletal muscles. Saxitoxin affects the excitable membrane of single nerves and muscle fibres by blocking selectively the sodium channel through which the downhill movement of sodium ions accounts for the initiation of the electrical impulse. Most probably saxitoxin (and the other PSP components) occupies a receptor on the outside surface of the membrane very close to the external orifice of the sodium channel.

Among the PSP components, saxitoxin, neosaxitoxin, gonyautoxin I, gonyautoxin III, and decarbamoyl saxitoxin exhibit lethal effects in the same range, whereas the remaining components (gonyautoxin II, IV, V, VI, VIII, VIII-epimer, sulfocarbamoyl gonyautoxin I & IV) are much less toxic.

Human intoxication associated with the consumption of shellfish containing PSP has been observed in many parts of the world. About 2500 cases of paralytic shellfish poisoning have been reported in the available literature. There were 24 deaths among 905 PSP intoxications published in 1969-83. The signs and symptoms in man may range from slight tingling and numbness about the lips to complete paralysis and death from respiratory failure. Signs and symptoms appear rapidly within minutes to hours, and respiratory paralysis leading to death can occur within 2 - 12 h of consumption of the PSP-containing food. The absence of hypotension was noted by the Task Group as being important for differential diagnosis. The results of animal studies indicate the existence of a dose-response relationship. However, the Task Group recognized serious difficulties in establishing the dose associated with the appearance of signs and symptoms and death. These difficulties are based on such factors as the reliability of

the bioassay used, so far, for estimation of the dose and uneven distribution of the toxins in the food consumed. The Task Group noted the efficacy of monitoring the PSP content of shellfish at the production site as a preventive measure, and the need to search for other patients when observing a case of PSP intoxication.

The Task Group recognized also important effects of PSP exposure in non-human targets, represented by fish kill and mass death in seabirds.

Ciguatera Toxins

Consumption of a variety of tropical and subtropical fish has been associated with a human disease (ciguatera) charaterized by neurological, cardiovascular, and gastrointestinal symptoms. Most of the toxicological research has been done using extracts from fish associated with outbreaks of ciguatera. Recent research suggests that a group of toxins produced by the dinoflagellates on which the fish feed, could be transmitted through the fish to human beings as the causative agents. This group produced by several species of dinoflagellates from coral reefs includes ciguatoxin, maitotoxin, and scaritoxin, and as shown very recently, okadaic acid. These compounds have been chemically characterized but the chemical structure is only fully known for okadaic acid. The extremely limited amounts available of these compounds is slowing down chemical and toxicological research as well as studies on the entry of these compounds into the food chain. No chemical methods of analysis for these compounds in food or organisms are available at present. Determinations for ciguatoxicity have so far been carried out by bioassay using mouse, cat, or mongoose. More recently, a radioimmunoassay and a bioassay using mosquitoes have been developed. The use and further development of these methods is limited by the scarcity of reference material.

Ciguatoxin and maitotoxin have been isolated from the biodetritus layer of coral reefs, from the dinoflagellate Gambierdiscus toxicus collected from seawater, and from axenic cultures of G. toxicus. This dinoflagellate is attached to macroalgae in coral reefs. In general, ciguatoxic fish species are limited to fish that feed on dinoflagellates and the detritus of coral reefs, particularly surgeon-fish, parrot-fish, and the larger reef carnivores that prey on these herbivores. Ciguatoxin and maitotoxin have been identified in these fish from chemical and toxicological characteristics.

Purified extracts from ciguatoxic fish administered orally and parenterally to mice and orally to cats and mongooses produced acute effects within 48 h, characterized by diarrhoea, itching, inactivity, and death, after convulsive

spasms. In mice given the most purified ciguatoxin available at present, peritoneally, the LD_{50} value was 0.45 µg/kg body weight.

The only known pathway of human exposure to ciguatera toxins is through food, and with the exception of a marine snail, mainly through coral fish or fish predating on coral fish, such as surgeon- and parrot-fish, snappers, groupers, carrangs, barracuda, Spanish mackerel, and emperor. The probability of exposure seems to be higher when eating large carnivorous fish, particularly the liver and other viscera. Because of the focal entry of the toxins into the food chain, fish of the same species and the same size can be toxic in one place and not in another.

No data are available on the absorption, distribution, retention, and metabolism of the toxins involved in ciguatera.

The clinical picture of human disease (ciguatera) is quite variable. Typically, symptoms occur within 1 - 6 h of ingestion of toxic fish. Initial symptoms usually include nausea, malaise, and numbness and tingling of the lips, tongue, and throat. Patients may later develop some or all of the following signs and symptoms: vomiting, abdominal cramps, diarrhoea, paraesthesia of the extremities, itching, myalgia, and arthralgia. In more severe cases, ataxia, weakness, blurred vision, insomnia, sinus bradycardia, dysrhythmias, and hypotension may develop. A symptom that is particularly suggestive of the diagnosis is the reverse perception of cold and hot. The duration of illness is variable. Most of the patients recover within three days, but malaise, paraesthesia, pruritus, and ataxia may persist for weeks or even years in severe cases. Patients repeatedly poisoned by ciguatoxic fish may develop a resurgence of ciguatera symptoms, even after eating fish containing little or no detectable toxin.

In the most severe cases, death results from circulatory collapse or respiratory failure. Several thousand cases of ciguatera have been reported from tropical areas within the last two decades with case-fatality rates ranging from 0.1 to 4.5%.

Intoxications have also been reported outside the endemic areas and outside the tropical circumglobal belt due to the consumption of fish brought or imported from the endemic areas.

Limited data were available for evaluation of the dose-response relationship. It is generally observed that one meal of toxic fish is sufficient to induce the disease in a human being, and, in one report, it has been suggested, on the basis of direct mouse assay determinations of ciguatoxicity in fish involved in intoxication, that oral intake of as little as 0.1 µg ciguatoxin can cause illness in an adult.

Of special interest are the studies showing an association between ciguatera outbreaks and naturally – occurring or man-made disturbances of coral reef ecosystems.

Tetrodotoxin

Intoxication by tetrodotoxin is different from the previously reported intoxication in several important aspects: (a) the toxin is probably not an algal product, but appears to be produced by certain fish species and a few other animals; (b) human exposure is generally limited to consumption of certain fish species, the identification of which is feasible; with the passage of time, populations in the endemic areas have developed measures to prevent intoxication; (c) on the basis of the number of cases of intoxication, accidental tetrodotoxin poisoning does not appear to be an important public health problem; however, when intoxication does occur, the case fatality rate is high.

Tetrodotoxin is an aminoperhydroquinazoline compound. Though the chemical structure of tetrodotoxin is entirely different from that of saxitoxin, the effects it induces in animals are very similar; the mouse assay developed for PSP has also been used for the detection of tetrodotoxin in the assessment of exposure. Recently, fluorescence spectrometric procedures for tetrodotoxin determination have been developed.

Tetrodotoxin has been found in fish of the family Tetraodontidae (Pufferfish); the ovaries, liver, and intestines contain the highest amount with small amounts in the skin; the toxin has only occasionally been detected in the muscles of these fish. The most toxic pufferfish are caught along the coasts of Japan and China. Tetrodotoxin has also been found in the Japanese ivory shell and in the trumpet shell associated with fatal human cases. In addition, the toxin has been identified in the skin of certain frogs and as the poisonous principal in the venom of the blue-ringed octopuses.

The signs of intoxication induced by tetrodotoxin in experimental animals are comparable with those caused by the PSP compounds. However, for the same degree of neuromuscular paralysis, a systemic, lasting arterial hypotension is produced by tetrodotoxin, which is also a highly potent hypothermic agent. The mode of action of tetrodotoxin is very similar to that of saxitoxin.

In human beings, the onset of signs and symptoms of tetrodotoxin intoxication usually occurs from 10 to 45 min after ingestion, but may be delayed by 3 h or more. Paraesthesia appears in the face and extremities and may be followed by sensations of lightness, floating, or numbness. Nausea, vomiting, diarrhoea, and epigastric pain may also be

present. Later, respiratory symptoms become prominent with dyspnoea, shallow, rapid respiration, and the use of auxilliary muscles. Cyanosis and hypotension follow, and convulsions and cardiac arrhythmia may occur. In most instances, the victims retain consciousness until shortly before death, which usually takes place within the first 6 h.

Occasional accidental intoxications including fatal outcome have been found associated with the consumption of pufferfish containing 0.5 - 30 mg tetrodotoxin/kg wet tissue fish. In Japan, about 60 cases with 20 deaths occurred annually in the period 1974-79. Intoxications can occur, occasionally, even in non-endemic areas. Ten cases with three deaths were reported recently in an European country following consumption of imported frozen mislabelled pufferfish containing tetrodotoxin.

Neurotoxic Shellfish Poisons

Two forms of human disease have been reported in association with the red tides of the dinoflagellate Gymnodinium breve around the coast of Florida. In one form associated with consumption of shellfish contaminated with G. breve cells and/or toxins, paraesthesia, alternating hot and cold sensations, nausea, vomiting, diarrhoea, and ataxia occur within 3 h; no paralysis has been observed. The other form is an upper respiratory syndrome that has been reported in association with aerosols of G. breve cells or toxins. The rapidly reversable syndrome is characterized by conjunctival irritation, copious rhinorrhea, and nonproductive cough. Four toxic components were isolated recently from cultured cells of G. breve, and three of them have been determined structurally to be polyethers. However, none of the toxic components has been chemically identified in food, air, or affected organisms. Monitoring of shellfish as a food has been conducted using a mouse bioassay. Fish kills and the mass death of seabirds have been observed in association with blooms of G. breve in this area of Florida.

Diarrhoeic Shellfish Poisons

Very recently, several toxic components have been isolated from shellfish associated with outbreaks of a syndrome in human beings, that is characterized by diarrhoea, nausea, vomiting. Abdominal pain was reported in about half of the patients and chill in a limited number of cases. Time from consumption of shellfish to the onset of illness ranged from 30 min to 12 h. Five of the toxic components have been structurally elucidated as okadaic acid and derivatives, and polyether lactones.

Several species of dinoflagellates have been identified as organisms that produce okadaic acid and are also associated with disease outbreaks. Human outbreaks involving more than 1300 cases have been reported from Japan with smaller outbreaks in Europe and South America.

Dermatitis-Inducing Marine Cyanophyte Toxins

Outbreaks of acute dermatitis in human beings after swimming in the sea during blooms of the filamentous marine cyanophyte Lyngbya majuscula have been reported repeatedly from two areas (Hawaii and Okinawa). Two components that have been isolated from the algae and chemically identified as debromoaplysiatoxin and lyngbyatoxin A have been shown to induce inflammation when applied to the skin of animals.

A gradation of the skin effect with the dose of debromoaplysiatoxin was observed in animal studies. Debromoaplysiatoxin was also shown to induce local skin effects on human volunteers at a concentration as low as 0.5 mg/litre. Histological studies confirmed the similarity between this skin effect and those associated with exposure to L. majuscula.

Freshwater Cyanophyte Toxins

Blooms of certain freshwater cyanophytes (Microcystis aeruginosa, Anabaena flos-aquae, Aphanizomenon flos-aquae) in ponds and lakes have occasionally been observed to be associated with sudden death in farm animals after drinking the water, lesions consisting of either haemorrhages and liver damage, or respiratory failure.

A few toxic components from these algae have been chemically characterized, but no report is available on the occurrence of the components in water. Several studies have reported adverse human health effects associated with the blooms of the same cyanophytes in recreational and municipal water supplies. At present, there is no evidence of the causal involvement of algal toxins in these episodes.

Recommendations

(a) The limited availability of pure aquatic biotoxins, with the exception of saxitoxin and tetrodotoxin, inhibits progress in experimental toxicology, analytical chemistry, phycology, clinical chemistry, and ecotoxicology. As a consequence, very limited quantitative information is available on the exposure of human beings and non-human targets to

algal toxins, and this severely affects monitoring and the establishment of preventive measures.

Internationally coordinated efforts are needed to provide pure algal toxins in quantities to meet these needs;

(b) Methods of analysis for algal toxins in foods, in human and animal tissues and fluids, and in environmental media, should be subjected to international collaborative studies in order to assess the precision and accuracy of the methods;

(c) The surveillance and reporting of human and animal (domestic and wild) cases of algal toxin-related disease should be improved on a world-wide basis; and

(d) As most of the algal toxin-related diseases are associated with blooms, more information on the occurrence of blooms of toxic algae and the conditions producing blooms should be obtained on a world-wide basis. On the basis of this information, attempts should be made to predict the occurrence of algal blooms and to provide early warning systems in affected areas.

INTRODUCTION: AQUATIC BIOTOXINS AND HUMAN HEALTH

It has been known since ancient times that certain fish and shellfish are poisonous and can cause death when eaten. The first Chinese pharmacopoeia, dated 2800 BC, records injunctions against eating pufferfish (Kao, 1966). European settlers in Northern America observed that various taboos and legends of the coastal Indians were associated with eating shellfish. On the east coast, the Indians would not eat mussels, even when starving, and on the west coast, Indians maintained nightly lookouts for bioluminescence in the sea and would not eat shellfish when the sea was "glowing" (Dale & Yentsch, 1978).

The chemical nature and biological basis for these food-borne intoxications have been elucidated over the last fifty years, beginning with the pioneering work of Meyer & Sommer on the etiology of paralytic shellfish poisoning (PSP) in California (Meyer et al., 1928). It is now evident that certain microscopic algae, present in phytoplankton, produce very potent toxins (phycotoxins, or algal toxins), which are chemical compounds mainly of low relative molecular mass. Concentrations of phycotoxins in the sea or in fresh water are highest during an algal bloom or red tide, a phenomenon characterized by a sudden, rapid multiplication of algal cells caused by environmental factors not yet fully understood. The phycotoxins are taken up by predators feeding on plankton, either directly as in the case of bivalve molluscs, or through several trophic levels as in fish. These food items are then consumed by man.

Algal blooms, including those of toxic algae, have become a more frequent phenomenon throughout the world in the last decade or two. The reason is not clear. In some areas, it is believed that climatic and hydrographic factors are important. Man-made pollution of the sea and freshwater and other human activities could change the aquatic environment in ways that provoke proliferation of toxin-producing algae. However, it should be stressed that the occurrence of algal blooms is usually due to natural rather than man-made causes, though anthropogenic inputs are significant in some instances. Furthermore, surveillance, detection, and reporting systems have improved in recent years, resulting in the more efficient accumulation of information concerning algal blooms on a world-wide basis. As fish and shellfish constitute an important part of the world's food supply, and the main source of protein for certain communities, the apparently increasing contamination of food by aquatic biotoxins constitutes a specific chemical hazard deserving appropriate attention.

Though also influenced by algal blooms, toxins from Cyanophytes (blue-green algae) constitute a different problem. In this case, vectors are not known to be involved, and the toxins or microscopic toxic cells are brought into direct contact with human skin during swimming in the sea, or, in the case of freshwater, the toxins or toxic cells may possibly be transferred to the human organism through drinking-water. Thus, the growth of blue-green algae in freshwater reservoirs may add to the difficulties of providing pure drinking-water.

The chemistry of some of the toxins is still only partly known. However, during the last decade, much progress has been made, e.g., the composition of the PSP complex has been elucidated, and some individual components chemically characterized; the structures of some of the neurotoxic and diarrhoeic shellfish poisons have been established. In the absence of sufficient chemical knowledge in the past, most measurements have been made by a bioassay using mice, a procedure that is nonspecific in nature, but is still the only method in practical use for the quality control of seafood. This method is not sensitive enough for the analysis of clinical samples and specific and sensitive methods for detection should be developed on the basis of new knowledge.

Associations between aquatic biotoxins and human intoxication are based not on specific identification of the causal agent in the human body, but on the appearance of certain acute symptoms following the consumption of some food commodities containing the toxic principles. Although the clinical features are variable, the neurological and gastrointestinal systems are commonly involved. Indeed, in some cases, the symptomatology of poisoning due to different biotoxins of this group could be similar and specific analytical methods to aid diagnosis would be desirable.

This document deals with algal toxins and tetrodotoxin. It does not deal with other well-known disease entities involving waterborne agents that infect man directly or contaminate fish and shellfish, producing toxins during food preparation and storage. Thus, scombrotoxin is not dealt with, and shellfish allergens that cause allergic disorders in man, when the shellfish is consumed, have also not been included. Other diseases caused by toxins not yet well defined and of uncertain origin (e.g., clupeotoxism, hallucinatory fish poisoning) are not discussed. The hygienic aspects of fish and shellfish in general have been dealt with in three WHO publications (WHO, 1974, 1979, 1983) and in Wood (1976). The term "aquatic biotoxins" is used, following the example of the working group on aquatic biotoxins of the IUPAC Commission on Food Chemistry, dealing with methods of analysis

for marine biotoxins (dinoflagellate toxins) and freshwater biotoxins (cyanophyte toxins) (Krogh, 1983).

For the purposes of this document, algae are uni- or multicellular organisms able to photosynthesize by means of chloroplasts, cell organelles containing chlorophylls, carotenes and xanthines. Being eukaryotic cells, the algae are members of the Protista, one of the 5 kingdoms (Marqulis & Schwartz, 1982). The unicellular marine algae dealt with in this document all belong to the dinoflagellates. Within several of the dinoflagellate genera there are species in which the cells do not contain chloroplasts, and thus are not true algae. In this context however, all dinoflagellates are considered to be algae, and Dodge's monograph (1982) is used as a reference for dinoflagellate taxonomy. In contrast to the eukaryotic dinoflagellates, the blue-green algae (Cyanophyte) consist of the more primitive (in morphological terms) prokaryotic type of cell, and in the above system are placed in Monera, and often named Cyanobacteria. They are unicellular, colonial, or filamentous organisms, and occur in fresh water and seawater. In this document, Komarek (1958) is used as reference for cyanophyte taxonomy.

1. PARALYTIC SHELLFISH POISONS

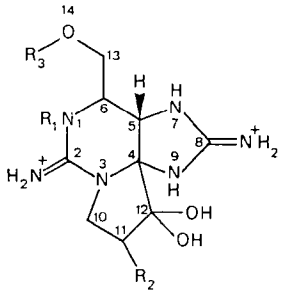
Acute intoxication after consumption of shellfish is a syndrome that has been known for several centuries. The etiology was first elucidated in this century, starting with investigations in California in the 1920s, following several episodes of fatal intoxications related to the consumption of mussels (Meyer et al., 1928; Sommer & Meyer, 1937). The term PSP, which is now widely used in describing this phenomenon, means paralytic shellfish poisons or paralytic shellfish poisoning. A document dealing with paralytic shellfish poisoning has been published recently (Halstead & Schantz, 1984).

1.1 Properties and Analytical Methods

1.1.1 Chemical properties

The chemistry of paralytic shellfish poisons (PSP) has been reviewed by Shimizu (1978) and Schantz (1980). PSP are a group of toxins produced by dinoflagellates of the genus Gonyaulax. The first agent to be chemically characterized was saxitoxin, which, though it was initially discovered in shellfish in California, has since been found in greatest concentrations in the Alaskan butterclam, Saxidomus giganteus, from which the name is derived. Saxitoxin has been shown to be a derivative of tetrahydropurine (Fig. 1) (Bordner et al., 1975; Schantz et al., 1975). It is a white, very hygroscopic solid, soluble in water, slightly soluble in methanol and ethanol, but practically insoluble in most non-polar organic solvents. It is a very basic substance, with two titratable groups, pK_a 8.2 and 11.5, and a relative molecular mass of 299 (Schantz et al., 1961). Subsequently, several other toxins of the PSP group have been characterized chemically, including 1-hydroxy saxitoxin (neosaxitoxin) (Shimizu et al., 1978); 11-hydroxy saxitoxin sulfate and the 11 β-epimer of this compound (Boyer et al., 1978); 11-hydroxy neosaxitoxin sulfate and the 11 β-epimer of this compound (Wichmann et al., 1981; Genenah & Shimizu, 1981) (Fig. 1). The last four compounds, named gonyautoxins II, III, I, and IV, respectively, by Shimizu et al. (1976) and Alam et al. (1982), are slightly basic, but otherwise have properties similar to those of saxitoxin. In general, PSP toxins are heat stable at acidic pH, but very unstable and easily oxidized under alkaline conditions.

Recently, a novel group of PSP compounds with a sulfocarbomoyl group has been isolated from both dinoflagellates and shellfish (Kobayashi & Shimizu, 1981;



WHO 84534

		R_1	R_2	R_3
1)	saxitoxin	-H	-H	$-C(=O)-NH_2$
2)	neosaxitoxin	-OH	-H	$-C(=O)-NH_2$
3)	gonyautoxin-I	-OH	$-\alpha OSO_3^-$	$-C(=O)-NH_2$
4)	gonyautoxin-II	-H	$-\alpha OSO_3^-$	$-C(=O)-NH_2$
5)	gonyautoxin-III	-H	$-\beta OSO_3^-$	$-C(=O)-NH_2$
6)	gonyautoxin-IV	-H	$-\beta OSO_3^-$	$-C(=O)-NH_2$
7)	gonyautoxin-V	-H	-H	$-C(=O)-N(H)-SO_3^-$
8)	gonyautoxin-VI	-OH	-H	$-C(=O)-N(H)-SO_3^-$
9)	gonyautoxin-VIII	-H	$-\beta OSO_3^-$	$-C(=O)-N(H)-SO_3^-$
10)	gonyautoxin-VIII epimer	-H	$-\alpha OSO_3^-$	$-C(=O)-N(H)-SO_3^-$
11)	sulfocarbamoyl gonyautoxin-I	-OH	$-\beta OSO_3^-$	$-C(=O)-N(H)-SO_3^-$
12)	sulfocarbamoyl gonyautoxin-IV	-OH	$-\alpha OSO_3^-$	$-C(=O)-N(H)-SO_3^-$
13)	decarbamoyl-saxitoxin	-H	-H	-H

Fig. 1. Structure of paralytic shellfish poisons. Adapted from: Genenah & Shimizu (1981), Wichmann et al. (1981), Hall (1982), Harada et al. (1982), and Harada et al. (1983).

Hall, 1982; Harada et al., 1982a). These toxins have a low toxicity until hydrolysed to more potent forms (nos. 7 - 12, Fig. 1). In addition, decarbamoylsaxitoxin, which previously had been made only in the laboratory, has been found in nature (Harada et al., 1983; Sullivan et al., 1983a). Thus, 13 PSP compounds are now known.

1.1.2 Methods of analysis for PSP in foods

This subject has been reviewed by Krogh (1979). The most commonly used procedure for PSP determination is a bioassay using mice, but this assay is not completely satisfactory, because of lack of sensitivity and pronounced variations. However, several other alternative chemical procedures are being developed, some of which may be applicable to PSP monitoring programmes. Despite the shortcomings of the mouse assay, the method is the only one suitable for regulatory purposes, where these limitations are of less significance.

1.1.2.1 Biological methods

(a) Mouse bioassay

During the investigations that established the association between toxic shellfish and the toxin-producing dinoflagellates (Gonyaulax catenella), Sommer & Meyer (1937) developed a bioassay for the PSP toxin. It consisted of the intraperitoneal injection of mice with an acidified extract of shellfish tissues, and the determination of the rapidity of death following the injection. By standardizing the conditions for the bioassay (mouse weight, pH of extract, and salt concentration), and introducing a purified saxitoxin standard (Schantz et al., 1958), a fairly reliable routine procedure was established. When the assay was tested collaboratively (McFarren, 1959), a standard error of about 20% was observed. The procedure has the status of an AOAC official final action method (Association of Official Analytical Chemists, 1980), and is, so far, the only method for assaying PSP that is in routine use by regulatory agencies all over the world (Adams & Miescier, 1980).

Because different strains of mice differ in their susceptibility to the PSP toxins, the sensitivity of the mouse colony used in the assay must be determined by calculating a correction factor (CF value) after intraperitoneal injection of the saxitoxin standard. The acidified extracts of shellfish are screen-tested in a few mice, in order to determine the dilution of the extract that will kill mice of 19 - 21 g body weight within 5 - 7 min, the conditions under which the assay is most sensitive. In the main test, the time

to death is converted into mouse units (MU), from which the concentration of toxin can be calculated using the CF value, assuming that the PSP toxins are saxitoxin or its derivatives. Saxitoxin levels as low as about 400 µg/kg can be detected by the procedure, and the sensitivity is reduced with increased salt (NaCl) concentrations in the extract. Near the detection limit, the toxin concentration may be underestimated by as much as 60% (Schantz et al., 1958).

Although Sommer & Meyer (1937) suggested that characteristic PSP symptoms, such as dyspnoea, could be used in the mouse test, these symptoms are subject to individual variations, and other factors, such as the rate of absorption, the site of injection, etc. (Kao, 1966). The principle of the mouse bioassay is a measurement of the time to the last gasping breath, which is a clearer end-point. The result of the mouse bioassay is non-specific: other agents can also cause death within 5 - 10 min following intraperitoneal administration. Thus, the mouse bioassay cannot distinguish PSP from tetrodotoxin (Johnson & Mulberry, 1966, section 3), but confusion between PSP and other toxins is unlikely in cases where the origin of the sample is known.

(b) Immunological assay

Johnson & Mulberry (1966) developed an assay in which purified PSP (saxitoxin) was conjugated with proteins by formaldehyde condensation, and the antitoxin to the conjugate was produced in rabbits. The antisera was used in haemagglutination and bentonite flocculation tests, with PSP extracted from spiked samples of butterclams, causing variable inhibition in the tests. The haemagglutination-inhibition test was slightly more sensitive than the mouse assay, whereas the detection limit of the bentonite flocculation-inhibition test was comparable to that of the mouse assay. Puffer fish poison (tetrodotoxin), which is detectable by the mouse assay, did not react in the tests. The method suffers from some saturation phenomenon and is not useful for a quantitative determination of PSP in shellfish, because increasing amounts of PSP in extracts caused almost identical reactions.

1.1.2.2 Chemical methods

Several spectrophotometric procedures involving various colour reactions of the PSP toxins have been developed. The earliest procedure (McFarren et al., 1958, 1959) was based on the Jaffe reaction, which involved a colorimetric reaction with the guanidine moeity. This procedure was inadequate, with a limit of detection of 1000 - 1500 µg/kg, and suffered

from interference by other naturally occurring guanidine compounds.

A fluorimetric method has been developed for the determination of saxitoxin (Bates & Rapoport, 1975; Gershey et al., 1977; Bates et al., 1978) comprising acid extraction, clean-up on a weakly acidic resin column, and the alkaline oxidation of the eluate with hydrogen peroxide. The fluorescent purine derivative of saxitoxin thus obtained was measured spectrophotometrically. Levels as low as 4.0 µg saxitoxin/kg were measured in saxitoxin-contaminated shellfish. Subsequent attempts to use this method in combination with chromatographic separation of various PSP toxins failed, because the N-1 hydroxy compounds, such as neosaxitoxin, and gonyautoxins I and IV, did not yield fluorescent products (Bates et al., 1978; Buckley et al., 1978). In some contaminated shellfish, the latter compounds may comprise the major portion of the toxic material. In a study comparing the mouse bioassay with a modified Bates-Rapoport procedure, the results of the latter were 11 - 22% higher than those of the mouse bioassay (Shoptaugh et al., 1981).

Recently, a high pressure liquid chromatographic (HPLC) procedure has been reported, using separation of the toxins on a bonded phase cyano column and detection by fluorescence following alkaline oxidation with periodate (Sullivan & Iwaoka, 1983). Six PSP components (saxitoxin, neosaxitoxin,, gonyautoxin I-IV) were identified and quantificted, with a good correlation with the mouse-bioassay method (Sullivan et al., 1983b).

1.2 Sources and Occurrence

1.2.1 Algal formation of toxins

The PSP toxins occur in, and are produced by, certain unicellular marine algae known as dinoflagellates, members of the phylum Dinophyta. Most of the PSP-producing dinoflagellates are found in the genus Gonyaulax, including: G. tamarensis, G. catenella, G. acatenella, G. monilata, and G. polyedra (Prakash,1967; Schmidt & Loeblich, 1979). G. excavata is considered to be a variety of G. tamarensis (Taylor, 1975). Other thecate toxin producers occur in the genus Pyrodinium, such as P. bahamense (Wall, 1975; Harada et al., 1982a) and P. phoneus, though the latter organism was probably G. tamarensis (Taylor, 1975). Toxic P. bahamense has been raised to varietal status, as P. bahamense var. compressa, compared to the non-toxic P. bahamense var. bahamense (Steidinger et al., 1980).

Dinoflagellates are among the major components of the marine phytoplankton. They are single-celled organisms, 40 - 50 µm in diameter, and propelled by two flagellae; some are bioluminescent. In addition to the motile form, some dinoflagellates, such as G. excavata, produce resting cysts (zygotes), as a result of sexual reproduction (Dale, 1977). Lacking flagella, these cysts sink and accumulate at the sediment-water interface, where they overwinter. Under laboratory conditions, the transformation of motile cells into another type of cyst (temporary cysts) has been observed, resulting from environmental stress, such as low temperature (Fig. 2). Motile cells reproduce asexually by binary fission.

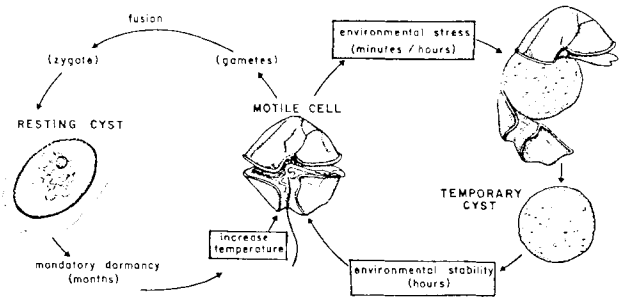


Fig. 2. Cyst development in Gonyaulax excavata: (A) resting cyst; (B) motile cell; (C) temporary cyst. Changes in environmental factos which stimulate the formation and conversion of cysts (encystment and excystment) are indicated (Adapted from: Yentsch & Incze, 1980).

The toxin-producing species of the genus Gonyaulax vary in toxic potential, as indicated in Table 1.

The toxic potential varies not only between species, but also between strains within species (Schmidt & Loeblich, 1979). Toxic and non-toxic strains of G. tamarensis have been encountered, even in the same locality (Yentsch et al., 1978). From the biochemical point of view, these observations might indicate that PSP toxins are secondary metabolites similar to toxins produced by microscopic fungi (mycotoxins), but not much is known about the pathways by which PSP toxins are produced. Thus, in a study of saxitoxin production by G. catenella in axenic culture using a number of ^{14}C-labelled compounds likely to be precursors, such as guanidine and propionate, no clue to the biosynthesis was obtained, and it

Table 1. Relative toxicity of dinoflagellates of the genus Gonyaulax[a]

Species	Minimum number of cells required to produce 1 mouse unit of PSP (about 0.18 µg PSP)
G. polyedra	$1.7 \cdot 10^5$
G. catenella	$7 \cdot 10^4$
G. catenella	$5 \cdot 10^4$
G. catenella	$1.0 \cdot 10^4$
G. acatenella	$6 \cdot 10^3$
G. tamarensis	$4.5 \cdot 10^3$

[a] Adapted from: Prakash (1967).

was concluded that, apparently, a highly specific pathway was in operation, not involving pathways for active C-1 and C-2 compounds (Proctor et al., 1975). The highest yield of saxitoxin was obtained at a temperature of 12 - 13 °C during continuous illumination.

PSP toxins are found not only in the motile cells of Gonyaulax species, but also in the resting cysts, where levels 10 - 1000 times higher than those in motile cells have been found (Dale et al., 1978). In contrast, identical levels of PSP in cysts and motile cells of G. tamarensis were measured by two other research groups (Oshima et al., 1982; White & Lewis, 1982).

Saxitoxin and neosaxitoxin have also been isolated from strains of a cyanophyte organism, Aphanizomenon flos-aquae (Ikawa et al., 1982) (section 6.2). In addition, PSP components (gonyautoxin I, II, III) have been detected in a macroalga, Jania sp., belonging to the red algae (Rodophyta) (Kotaki et al., 1983). These red algae are eaten by crabs and snails, and PSP has been detected in these molluscs (sections 1.2.2.1, 1.2.2.2).

1.2.1.1 Oceanographic conditions associated with blooms (red tide)

The topic has been reviewed by Yentsch & Incze (1980). Contamination of shellfish with PSP toxins has traditionally been associated with the appearance of algal blooms, the so-called red tide. Dinoflagellates are able to reproduce asexually at high rates, under the influence of environmental conditions, which have not yet been fully elucidated. When a population of dinoflagellates develops quickly forming dense concentrations of from 10^4 cell/litre to 10^6 cell/litre, it is termed a bloom. At levels of 10^6 cell/litre, the

water can become discoloured depending on the participating algal species, hence the name "red tide", but it is important to realize that not all algal blooms are red coloured.

It is, however, also important to note that not all red tides are associated with toxic blooms and contamination of shellfish; they can also result from concentrations of non-toxic dinoflagellates or ciliates (McAlice, 1968). Conversely, shellfish can still accumulate PSP when Gonyaulax concentrations in the sea are below those found in algal blooms.

As red tides are essentially a coastal phenomenon, it has been suggested that land drainage might play a role in their initiation (Prakash, 1975). Concentrations of chelators and trace metals may be involved. Thus, Anderson & Morel (1978) reported that G. tamarensis was more sensitive to Cu(II) ions than other members of the phytoplankton, and that it grew well, when the concentrations of Cu(II) ions were exceedingly low. It has also been suggested that, by binding the copper ion, chelators could decrease its toxicity, whereas binding zinc and iron could increase the availability of these nutrients for growth (Anderson & Corbett, 1979). Field studies involving measurements of copper and iron during blooms support this hypothesis (Dale & Yentsch, 1978).

While blooms may result directly from the rapid growth of algal populations, physical (hydrographic) factors may transport existing populations to specific areas, where biological behaviour, such as positive phototaxis, can result in the formation of dense concentrations (Mulligan, 1975; Margalef et al., 1979; Seliger et al., 1979). These phenomena may be triggered by meteorological events, such as rainfall and wind (Hartwell, 1975; Yentsch & Glover, 1977). Recent data suggest that frontal zones, or discontinuities between water masses, are the factors most likely to influence the development of red tides. These frontal zones may result from tide - or wind-generated convergences, or discontinuities. They are frequently marked by pronounced differences in the vertical stability of the two water masses (Pingree et al., 1975; Tyler & Seeliger, 1978; Yentsch & Mague, 1979).

1.2.2 Occurrence in seafood

1.2.2.1 Accumulation in molluscs

(a) Bivalves

The topic has been reviewed by Yentsch & Incze (1980). The PSP components are transferred to shellfish (mussels, clams, scallops) during filter-feeding, a characteristic feature of bivalves. During this process, food organisms in

the seawater, such as Gonyaulax cells, are transported from the gills in the mantle cavity to the oesophagus and stomach. Digestion takes place in the stomach and its associated diverticula, often erroneously termed "liver" (Russel-Hunter, 1972). The highest concentrations of PSP have been found in these digestive organs, apparently bound to melanin, but PSP is also found in other soft tissues of the bivalves. The rate of PSP accumulation varies among shellfish species, as indicated in Table 2.

Table 2. Rate of PSP accumulation in two species of shellfish fed Gonyaulax excavata under laboratory conditions[a]

Shellfish species	Days after feeding	PSP concentration (µg/kg)[b]
Mya arenaria	0	ND[c]
	7	3110
	14	1350
Mytilus edulis	0	ND
	7	5370
	14	3110

[a] Adapted from: White & Maranda (1978).
[b] 340 µg/kg was the limit of detection using the mouse bioassay.
[c] ND = Not detected.

Shellfish are generally not harmed by the presence of PSP toxins, though Gilfillan & Hansen (1975) noted some depression in the filtration rate of bivalves exposed to dense concentrations of Gonyaulax cells. Black mussels (Choromytilus meridionalis) and white mussels (Donax serra) in South Africa have often been found paralysed during red tides associated with G. catenella (Popkiss et al., 1979).

PSP toxins may be transferred to shellfish not only through the motile cells, but also through the resting cysts, which may contain PSP (section 1.2.1). Resting cysts have been identified in the digestive tract of molluscs (Ayres & Cullum, 1978).

(b) Gastropods

Amounts of PSP have been detected in the digestive glands of carnivorous gastropods, such as the rough whelk (Buccinum undulatum) under natural conditions as well as during studies in which PSP-containing digestive glands of scallops were fed to the gastropods (Caddy & Chandler, 1968).

Two species of turban shells (Turbo argyrostoma, T. marmorata) and two species of top shells (Toctus nilotica maxima, T. pyramis), inhabiting coral reefs, have been found to contain PSP toxins in the visceral regions (Kotaki et al., 1983). Saxitoxin, neosaxitoxin, and a new toxin tentatively code-named TST were predominant in the toxin profile. The highest toxicity recorded was 4000 µg PSP/kg, although a marked regional and individual variation existed among specimens tested. As the PSP-containing alga Jania sp. was present in the stomach of the gastropods, this alga was presumed to be the source of the toxins.

1.2.2.2 Accumulation in crustacea

PSP was not found in lobsters (Homarus americanus) during red tide episodes; however, when lobsters were fed PSP-containing clams under experimental conditions, PSP was found in the contents of the gut, but not in tissues (Yentsch & Balch, 1975). PSP may accumulate in crabs (Schantz et al., 1975; Foxall et al., 1979). Xanthid crabs inhabiting coral reefs have been found to cause intoxication with a high fatality rate in Fiji, Japan, Palau, and the Philippines (Hashimoto, 1979; Alcala, 1983; Raj et al., 1983). The species most frequently implicated in poisoning is Zosimus aenus, which accumulates high levels of neosaxitoxin and saxitoxin. The source of toxin appears to be the PSP-containing alga Jania sp. The marked regional and individual variation in toxicity was explained by the abundance of this alga in the habitat of the crabs. Other species of coral-reef crabs, although too small to be regarded as food, also accumulate PSP toxins if Jania sp. grows in their vicinity (Kotaki et al., 1983).

1.2.2.3 Transmission through zooplankton to fish

Kill episodes have been observed in fish (herring, sand lance) spatially and temporally associated with blooms of toxic G. excavata in the North Sea (Adams et al., 1968) and in the Bay of Fundy, Canada (White, 1977). As these fish feed on zooplankton but not on dinoflagellates, it has been suggested that the zooplankton, feeding on the dinoflagellates, may act as vectors of PSP. In the stomach of herrings from the Bay of Fundy kill, White (1977) was able to identify thecosomatous pteropods (Limacina retroversa), and the stomach contents contained PSP. In an experimental study, a similar amount of PSP (21 µg/fish) caused paralysis and death in herrings. Zooplankton collected during a bloom of toxic G. excavata contained PSP, even 3 weeks after the bloom peak, when Gonyaulax cells had disappeared, indicating accumulation in

zooplankton (White, 1979). This observation was subsequently confirmed in an experimental study using Acartia clausii and Balanys sp. (barnacle nauplii) as representatives of zooplankton grazing on PSP-producing G. excavata (White, 1981a). The PSP levels measured in the zooplankton were comparable with maximum levels commonly encountered in filter-feeding molluscs, e.g., 10 000 - 50 000 µg PSP/kg. In a recent herring kill occurring during a bloom of toxic G. excavata, another zooplankton organism, Evadne nordmanni, was identified as the vector of PSP (White, 1980). The PSP found in the dead or diseased herrings are listed in Table 3.

Table 3. Contents of PSP in Atlantic herrings from a kill during G. excavata blooms[a]

Number of fish sampled	Mean length (cm)	Mean weight (g)	Mean µg/kg content[b] gut	muscle
17	21.6	110	1100	ND[c]
17	21.8	116	2450	ND
24	14.9	34	660	ND
14	16.4	39	950	330
11	15.6	35	2180	590
40	21.5	108	14 140	ND

[a] Adapted from: White (1980).
[b] 300 µg/kg was the limit of detection using the mouse bioassay.
[c] ND = not detected.

PSP was not detected in muscle tissue in most of the cases. According to the author, this was consistent with experimental data, which also showed that PSP was not found in muscle tissue of herrings killed by the oral administration of PSP.

1.2.2.4 Accumulation in fish

PSP, as measured by the mouse bioassay, has been detected in sand-launce (970 µg/kg), involved in a mass death of sea birds (Nisbet, 1983) (section 1.5.1.2). Pufferfish, collected from areas with occasional PSP episodes, have been found to contain saxitoxin in the liver and roe, amounting to 0.2% of total toxicity, the main part being tetrodotoxin (Yasumoto, 1980).

1.3 Exposure

All reported cases of PSP intoxication in free-living animals (section 1.5.1) and in human beings (section 1.6) have been associated with alimentary exposure to contaminated food. The shellfish most often reported to contain PSP are clams and mussels and include members of the families Mactridae (Spisula solidissima), Myacidae (Mya arenaria), Mytilidae (Mytilus californianus, Mytilus edulis, Modiolus modiolus), and Veneridae (Protatheca staminea, Saxidomus giganteus, Saxidomus nuttalli) (Halstead, 1978). Occasionally, Spondylus butleri (Harada et al., 1982), scallops, and oysters may be involved. The contamination of these species can be focal and temporal. In the Pacific area, some toxic crabs have been mentioned as being responsible for PSP-type outbreaks. There have not been any reports on PSP cases associated with other routes of exposure, such as dermal exposure to seawater containing toxic algae, or respiratory exposure to droplets of such seawater. Several countries have developed surveillance programmes for PSP contamination of shellfish (WHO, 1979). In the USA, shellfish-growing areas are closed if the concentration of PSP in the edible portion of the shellfish equals or exceeds 800 µg PSP/kg, as measured by the mouse assay, until the concentration has decreased to below 800 µg PSP/kg (Anon., 1965). The action level of 800 µg PSP/kg has been established on the basis of exposure data from earlier PSP outbreaks in Canada (Tennant et al., 1955; Anon., 1957). The 800 µg PSP/kg level is more than 10 times lower than the lowest level that has caused intoxication in these outbreaks.

1.4 Metabolism

Data are not available on PSP absorption, distribution, metabolism, and excretion, probably because sensitive chemical methods for quantification have only recently been developed. However, there is one old study stating that the poison is quickly eliminated in the urine (Prinzmetal et al., 1932).

1.5 Effects in Animals

1.5.1 Field observations

1.5.1.1 Fish

Fish kills by PSP, produced during G. excavata blooms, have been reported from Europe (North Sea) (Adams et al., 1968) and from the north-east coast of North America (White,

1977, 1980), as mentioned in section 1.2.2.2. The fish involved, sand eel (Ammodytes sp.) and herrings (Clupea harengus harengus), do not feed on dinoflagellates, and zooplankton appears to have acted as a vector of PSP. No reports describing pathological and microbiological findings from these fish kills seem to have been published, but, experimentally, oral administration of PSP to herrings was rapidly fatal, with oral LD_{50} values for herring, pollock, flounder, salmon, and cod in the range of 400 - 755 µg PSP/kg (White, 1977, 1981b).

1.5.1.2 Sea birds

Twice in the last decade, mass death of sea-birds associated with an algal bloom has occurred in the North Sea, off the north-east coast of England. In May 1968, a bloom of G. excavata was associated with mass death, particularly of shags (Phalacrocorax aristotelis), but also of terns (Sterna spp.) and cormorants (Phalacrocorax carbo) (Coulson et al., 1968). The pathological lesions observed in the dead birds included extensive inflammation of the alimentary tract and often haemorrhages at the base of the brain and elsewhere in the body, symptoms typical of PSP-induced death in birds. As sea birds do not eat mussels, fish (e.g., the sand eel) appear to have been the vector for PSP. In the second episode, in 1975, the loss of sea birds was recorded in detail by the monitoring of colour-ringed birds (Armstrong et al., 1978). Thus, a 62 - 64% mortality rate of shags was associated with the G. excavata bloom in the spring of 1975, compared with an average annual mortality rate of 16%. Increased mortality rates were also observed in the herring gull (Larus argentatus), the cormorant, and the fulmar (Fulmarus glacialis). A monitoring programme for PSP in mussels was introduced in the United Kingdom (for the north-east coast) following the 1968, G. excavata bloom (Ayres & Cullum, 1978). The maximum annual values for PSP in mussels over a 9-year period are listed in Table 4. Thus, mass deaths in sea birds associated with PSP, never observed before in England, were encountered in 2 out of the 3 years with high maximal PSP levels in mussels, within the period 1968-76. PSP were not actually detected in the sea birds and the sand lance, using the mouse assay, presumably because the levels were below the detection limit of the assay. Consequently, Armstrong et al. (1978) recommended that a more sensitive chemical procedure for the determination of PSP should be introduced so that analyses for PSP could be performed directly on the birds. Mass death in common terns (Sterna

Table 4. Maximum values of PSP in mussels (M. edulis) from the north-east coast of England, 1968-1976[a]

Year	PSP level[b] (µg/kg)
1968	100 000
1969	12 250
1970	8000
1971	1000
1972	400
1973	400
1974	5500
1975	12 300
1976	1750

[a] Adapted from: Ayres & Cullum (1978).
[b] Determined by the mouse assay.

hirundo) was observed off the Massachusetts coast in June 1978 (Nisbet, 1983). PSP was detected in the sand-launce, the tern's principal food, and, at the same time, high levels of PSP were found in shellfish in this area.

1.5.2 Experimental studies

1.5.2.1 Acute toxicity

As mentioned in section 1.1.2.1, the principle of the mouse assay developed by Sommer & Meyer (1937) is measurement of time to death. In the same paper, the authors suggested that signs charateristic of PSP intoxication, such as dyspnoea, could be observed after the intraperitoneal administration of toxin. Hypotensive effects have been observed to accompany the respiratory depression, implicating both central and peripheral actions (Watts et al., 1966). In a study using a PSP preparation extracted from Alaskan butter clams, Wiberg & Stephenson (1960) determined the LD_{50} values for mice using three routes of administration (intravenous, intraperitoneal, and oral). The determination was conducted on groups of 130 - 160 male mice per route, with 4 dose levels per route. In addition, the intraperitoneal LD_{50} was determined in female mice, using groups of 70 animals and 2 dose levels. The observation time was 4 h. As indicated in Table 5, PSP is much less toxic when administered by the oral route than parenterally.

Increasing the pH of the injection medium or the addition of sodium ions reduced intraperitoneal toxicity. The sodium ion did not influence the oral or intravenous toxicity.

Table 5. LD_{50} following a single dose of PSP in the mouse in relation to the route of administration[a]

Route of administration	LD_{50} (µg PSP/kg body weight) (The 95% confidence limit in parenthesis)	
	male	female
intravenous	3.4 (3.2 - 3.6)	
intraperitoneal	10.0 (9.7 - 10.5)	8.0 (7.6 - 8.6)
oral	263 (251 - 267)	

[a] Adapted from: Wiberg & Stephenson (1960).

A similar dependence of LD_{50} values on the route of administration was observed in rats of different ages (Table 6) (Watts et al., 1966). A PSP extract from Alaskan butter clams was used in this study, with 2 routes of administration (oral and intraperitoneal). Sixteen Osborne-Mendel rats (equal number of males and females in each group) were used for each dose level, and 4 dose levels were used per age level and per route of administration. The effect on respiration was studied in the rats administered PSP orally. The newborns and weanlings responded with dyspnoea and a marked decrease in respiration rate throughout the study period, whereas the adult rats exhibited laboured breathing followed by a profound reduction in respiration rate within 5 min of treatment. In addition, convulsions were observed in weanlings and adult rats, but not in newborns.

Table 6. LD_{50} following oral or intraperitoneal administration of a single dose of PSP to rats of different ages[a]

Age	LD_{50} (µg PSP/kg body weight) (The 95% confidence limits are in parenthesis)	
	oral	intraperitoneal
newborn (24 h) (51 - 80)	64 (4.7 - 6.5)	5.5
weanling (21 days) (204 - 356)	270 (7.7 - 9.0)	8.3
adult (60 - 70 days) (490 - 576)	531 (8.5 - 11.8)	10.0

[a] Adapted from: Watts et al. (1966).

Prior exposure to non-lethal doses of PSP seeems to lower the susceptibility of rats to lethal doses of PSP. In a study using Sprague-Dawley rats (sex not indicated), the oral LD_{50} value for a purified PSP material was determined (McFarren et al., 1960). One group of rats was given a non-lethal dose of PSP (about one-third of the LD_{50}), 14 days before the test. The LD_{50} for the pretreated rats was about 50% higher than that for untreated rats. No explanation was presented of the mechanism involved, and this observation has not been repeated by others.

Comparative data on LD_{50} values for various species of animals have been obtained following oral administration to animals of extracts of clams containing 1.6 - 4.0 mg PSP/kg, determined by the mouse assay (Table 7).

Table 7. Comparison of LD_{50} values following a single oral dose of PSP in various species of animals[a]

Animal	LD_{50} (µg PSP/kg body weight)
mouse	420
rat	212
monkey	400 - 800
cat	280
rabbit	200
dog	200
guinea-pig	128
pigeon	100

[a] Adapted from: McFarren et al. (1960).

All the above-mentioned toxicological studies conducted until recently have been carried out using as PSP material the same extract from Alaskan butter clam prepared by Schantz et al. (1958). According to a more recent study (Genenah & Shimizu, 1981), it can be assumed, that on the basis of chemical analysis, the toxic component in this extract was saxitoxin.

The toxicity of the various PSP components has been compared, using freshly-isolated compounds and testing their toxicity by means of the AOAC mouse bioassay (section 1.1.2.1) (Table 8).

The effects of saxitoxin on the nerves of bivalve molluscs have been studied (Twarog et al., 1972) and it appears that species known to accumulate PSP, such as Mytilus edulis, M. californianus, Placopecten magellanicus, Saxidomus nuttalli,

Table 8. Comparison of the lethal effects of the various PSP components, based on a single dose intraperitoneally administered to mice[a]

PSP component	Lethality (MU/μ mol)
saxitoxin	2045
neosaxitoxin	1617
gonyautoxin I	1638
gonyautoxin II	793
gonyautoxin III	2234
gonyautoxin IV	673
gynyautoxin V	136
gynyautoxin VI	108
gynyautoxin VIII	277
gynyautoxin VIII epimer	20
sulfocarbamoyl gonyautoxin I	ND
sulfocarbamoyl gonyautoxin IV	ND
decarbamoyl saxitoxin	1378

[a] Adapted from: Genenah & Shimizu (1981), Wichmann et al. (1981), Harada et al. (1982), and Harada et al. (1983).
ND = No data available.

and Mya arenaria are resistant to saxitoxin, whereas many other species are up to 100 times more sensitive than the resistant species.

1.5.2.2 Mode of action

Both saxitoxin and tetrodotoxin (section 3) have been used extensively as experimental tools in neurobiology. Of the various PSP components, only saxitoxin has been studied in detail as far as pharmacological effects are concerned, in part because the other components are usually not available in sufficient quantities for such studies. The mechanism of the cellular and systemic actions of saxitoxin have been reviewed by Kao (1966, 1972, 1983), Evans (1975), and Narahashi (1972).

Nearly all the systemic actions of saxitoxin can be explained by a wide-spread blockade of impulse-generation in peripheral nerves and skeletal muscles. Direct cardiac effects are usually minimal. In mammals, these effects lead to paralysis, respiratory depression and circulatory failure. In contrast to tetrodotoxin, saxitoxin typically induces less hypotension for the same degree of muscular paralysis, and the hypotension tends to be more transitory (Kao, 1972). A depressant effect of saxitoxin on both the central vasomotor and respiratory centres was observed when the toxin was administered either directly into the cerebral ventricles or intravenously (Borison et al., 1980a). However, under

conditions of distribution equilibrium, such as those occurring in human poison victims who had ingested saxitoxin, the peripheral effects were the more important in accounting for the symptomatology (Borison et al., 1980a).

Extensive experiments on single nerves and muscle fibres have shown that saxitoxin, like tetrodotoxin, affects the excitable membrane by blocking selectively the sodium channel through which the downhill movement of sodium ions accounts for the initiation of the electrical impulse (Narahashi, 1972). Recent studies with several other PSP compounds have shown that they act with a similar mechanism (Kao, 1983). It had been suggested that saxitoxin blocks the sodium influx simply by plugging the sodium channel with one of the guanidinium moieties (Kao & Nishiyama, 1965; Hille, 1975). However, recent studies of some structure-activity relationships of saxitoxin and several of its analogues clearly demonstrate this postulate to be untenable. Most probably, saxitoxin and its analogues occupy a receptor on the outside surface of the membrane very close to the external orifice of the sodium channel. Saxitoxin binds to the receptor site in part by electrostatic attraction between the cationic 7,8,9 guanidinium group and fixed anionic sites of the membrane, and by hydrogen-bonding involving the C-12 hydroxyl groups (Kao, 1983).

1.6 Effects on Man

1.6.1 Clinical studies

The signs and symptoms of PSP in man may range from a slight tingling and numbness about the lips to complete paralysis and death from respiratory failure (Meyer et al., 1928; Medcof et al., 1947; McFarren et al., 1960). Typically, the tingling sensation around the lips, gums, and tongue develops within 5 - 30 min of consumption. In moderate and severe cases, this is regularly followed by a feeling of numbness in the finger tips and toes, and, within 4 - 6 h the same sensation may progress to the arms, legs, and neck, so that voluntary movements can be made only with great difficulty. In fatal cases, death is usually caused by respiratory paralysis within 2 - 12 h of consumption of the PSP-containing food. Typical symptoms, which may help in distinguishing the cases as mild, severe, or extreme, are (Prakash et al., 1971):

Mild

Tingling sensation or numbness around lips, gradually spreading to face and neck; prickly sensation in fingertips and toes; headache, dizziness, nausea;

Moderate

Incoherent speech; progression of prickly sensation to arms and legs; stiffness and incoordination of limbs; general weakness and feeling of lightness; slight respiratory difficulty; rapid pulse;

Severe

Muscular paralysis; pronounced respiratory difficulty; choking sensation; high probability of death in absence of ventilatory support.

Sensitivity to PSP is so variable that estimates of the human dose resulting in death range from 500 µg to 1000 µg (Tennant et al., 1955) to 12 400 µg (Meyer, 1953).

There are no reports of late effects in survivors or of the effects of long-term, low-level exposure to PSP.

1.6.2 Epidemiological studies

Cases of human intoxication, associated with the consumption of shellfish and supposed to be caused by PSP, have been known for a long time; according to Prakash et al. (1971) about 1600 cases have been reported, on a world-wide basis, up to 1970 most occurring in Europe, Japan, and North America. In the last decade, however, a changing pattern of PSP distribution has emerged, with cases also being reported from developing countries (Table 9). Whether this indicates a real increase in the number of annual PSP cases, or is the result of improved surveillance and reporting is not known. Data are available, from the USA, comparing outbreaks of PSP with those due to other chemical agents of food-borne disease. In the period 1970-74, PSP constituted 4.3% of all reported outbreaks (Hughes et al., 1977). When food-borne infectious agents (bacteria, viruses, and parasites) were considered in addition to chemical agents in 1978-81, PSP made up 1.1% of all outbreaks of food-borne diseases with known etiology (Anon, 1981a,b; Anon, 1983b,c).

Table 9. More recent reports on the occurrence of PSP

Country	Number of of people affected	Number of deaths	Species of shellfish	Origin of shellfish	Concentration of PSP	Dinoflagellate involved	Reference
Canada	2	0	Mussels	local	430 000 µg/kg		Acres & Gray (1978)
Canada	5	1	Mussels, clams	local	21 000 µg/kg		Anon (1982)
Germany, Federal Republic of	19	0	Mussels (*Mytilus edulis*)	Vigo (Spain)	12 000-40 000 µg/kg		Simon et al. (1977)
India	98	1	Mussels	local			Bhat (1981)[a]
Malaysia	201	4	Clams	local		*P. bahamense*	Roy (1977)[a]
Mexico	20	3	Mussels	local			Anon (1979)[a]
Norway	4	0	Mussels (*M. edulis*)	local	400-4000 µg ingested		Gulbrandsen & Aalvik (1981)
South Africa	6	2	Black mussels (*Chloromytilus meridionalis*)	local	84 000 µg/kg	*G. catenella*	Grindley & Sapeika (1969)
South Africa	17	0	Black mussels	local	up to 72 830 µg/kg	*G. catenella*	Popkiss et al. (1979)
Switzerland	23	0	Mussels (*M. edulis*)	Vigo (Spain)	20 000 µg/kg		Zwahlen et al. (1977)
Thailand	62	1	Mussels (*Mytilus* sp.)	local	9400 µg/kg		Anon (1983)

Table 9 (contd).

United Kingdom	78	0	Mussels (M. edulis)	local	600-6000 µg ingested	G. tamarensis	McCollum et al. (1968)
USA (California)	51	1	Mussels Oysters	local	3000-40 000 µg/kg		Anon (1980)
USA (Maine, New Hampshire, Massachusetts)	33	0	NS[b]	local	NS	NS	Anon (1972)
USA (Massachusetts)	26	0	Mussels, Clams scallops	local	30 000-50 000 µg/kg	G. tamarensis	Anon (1973)
Venezuela	171	10	Mussels	local	790-33 000 µg/kg	G. tamarensis Cochlodinium sp.	Reyes-Vasquez et al. (1979)
Venezuela	9	1	Mussels (Perma perma)	local			Anon (1981c)[a]
West Europe (including Federal Republic of Germany and Switzerland mentioned above)	120	0	Mussels	Vigo (Spain)	12 000-40 000 µg/kg		Zwahlen et al., (1977)

[a] The diagnosis of the human cases has not been aetiologically confirmed, as no data were reported on the presence of PSP components in the food associated with the disease.
[b] NS = Not specified.

2. CIGUATERA TOXINS

A variety of fish inhabiting tropical and subtropical seas may become toxic, and, by ingestion, cause an intoxication in human beings named "ciguatera", which is characterized by neurological and gastrointestinal symptoms. The term ciguatera is of Spanish origin, derived from cigua, which is a Carribean trivial name for a marine snail, Turbo pica that, when eaten, is said to cause indigestion. The principal toxin is ciguatoxin, but other toxic components have recently been identified. The subject has been reviewed by Bagnis (1981a) and Withers (1982). The disease ciguatera has been known since the 17th century and appears to be the most commonly occurring disease associated with seafood toxins (Hughes et al., 1977).

2.1 Properties and Analytical Methods

2.1.1 Chemical properties

The chemical properties of ciguatoxin have been reviewed by Scheuer (1982). The chemical structure of ciguatoxin is still largely unknown. The toxin has been extracted from the the moray eel liver and, after elaborate purification, has been obtained in a pure crystalline form as a white solid (Scheuer, 1982). Ciguatoxin is a highly-oxygenated lipid, soluble in polar organic solvents but insoluble in water. The relative molecular mass is estimated to be 1111.7 ± 0.3, and possible molecular formulae are $C_{53}H_{77}NO_{24}$ or $C_{54}H_{78}O_{24}$, but other combinations cannot be excluded.[a]

Other toxic components isolated from ciguatoxic fish are maitotoxin (Yasumoto et al., 1971) and scaritoxin (Chungue et al., 1976). The chemical structure of the two components is unknown. However, scaritoxin resembles ciguatoxin in chemical and some chromatographic properties, but is distinguishable from it by DEAE-cellulose column and by thin layer chromatography (Chungue et al., 1977). Maitotoxin is a highly oxygenated water-soluble compound with a large relative

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[a] Recent results of spectral and chemical studies of the crystalline material has shown that ciguatoxin is a molecule with a structure resembling that of such polyethers as okadaic acid and brevetoxin C (Nukina et al., 1983).

molecular mass and has no structural relationship with ciguatoxin and scaritoxin.[a]

2.1.2 Methods of analysis for foodstuffs

All analytical results for ciguatoxin and related components referred to in this document were obtained by biological methods. According to published information, the more recently developed radioimmunoassay, mentioned in 2.1.2.2, has not been applied under practical conditions (Laigret et al., 1981; Parc et al., 1981).

2.1.2.1 Biological methods

This topic has been recently reviewed by Yasumoto et al. (1984). For ciguatoxin, a mouse injection test, first mentioned by Banner et al. (1961), has since been modified by Kimura et al. (1982) and Yasumoto et al. (1984). The method consists of injecting serially-diluted semipurified toxin extracts into mice and observing the mortality ratio for 24 h. The results are obtained as mouse units, and one mouse unit is defined as the amount of toxin that kills a mouse (20 g body weight) in 24 h. The method does not distinguish between ciguatoxin and scaritoxin.

A bioassay for ciguatoxin in fish has been developed on the basis of feeding cats or mongooses a ration containing 100 g of the fish to be tested per kg ration (Bagnis & Fevai, 1971; Banner, 1975). The cat is less satisfactory, because it may regurgitate part of the test meal. Test animals were observed for 48 h, with the response rated from 0 (no response) to 5 (death within 48 h). Recently, a bioassay using mosquitoes (Aedes aegypti) has been developed (Chungue et al., 1984; Pompon et al., 1984). The procedure involves intrathoracic injection in mosquitoes of serially- dilated extract from fish, and the toxicity of the fish is expressed as mosquito LD_{50}. A good correlation between the mosquito bioassay and the mouse bioassay was observed. All the tests described above appear to be non-specific and only semiquantitative at best.

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[a] In a recent study of dinoflagellates, isolated from ciguatera areas in the Carribean using unialgal cultures, at least 5 different toxins were identified: ciguatoxin, maitoxin, okadaic acid, scaritoxin, and an unnamed toxin. All the compounds are thought to contribute to the ciguatera syndrome in the Carribean (Tindall, 1983).

2.1.2.2 Chemical methods

A radioimmunoassay for ciguatoxin has been developed, using antibodies produced against a conjugate of human serum albumin and ciguatoxin isolated from toxic moray eel (Hokama et al., 1977). Results of the assay were correlated with those of the assays on mongoose, mouse, and guinea-pig atrium. All the three assay procedures showed good correlation when ciguatoxin was present in fish tissues in high concentrations (Kimura et al., 1982).

2.2 Sources, Occurrence, and Exposure

2.2.1 Algae

A dinoflagellate, Gambierdiscus toxicus, has been identified as the source of ciguatoxin and maitotoxin (Bagnis et al., 1977; Yasumoto et al., 1977a; Adachi & Fukuyo, 1979). G. toxicus is an armoured dinoflagellate with two flagella, living around coral reefs, closely attached to macroalgae (Bagnis et al., 1979b), such as Turbinaria ornata, Amphiroa sp., and Jania sp. Ciguatoxin and maitotoxin have been isolated from the biodetritus layer on coral reefs, from G. toxicus collected from sea water, and from axenic cultures of G. toxicus, using the mouse assay and some biochemical characteristics as identification procedures (Bagnis et al., 1979b). Fish, such as parrot-fish (Scarus gibbus) and surgeon-fish (Ctenchaetus striatus), representatives of fish species likely to contain ciguatoxin and maitotoxin, feed on the layers of microorganisms and detritus colonizing coral beds, and thereby accummulate the toxins (Bagnis et al., 1980).

Subsequently, strains of G. toxicus, able to produce ciguatoxin and maitotoxin, have repeatedly been isolated from macroalgae such as Halimeda sp., Penicillus sp., Acetabularia sp. and Gracilaria sp., and from coral reef off the coast of Florida, USA. These findings elucidate the origin of toxicity of Florida Barracuda (Sphyranea barracuda), a fish species often associated with cases of ciguatera in USA (Bergmann & Alam, 1981; Bergman, 1982).[a]

Surveys of coastal sea water in the Pacific (French Polynesia) have demonstrated that G. toxicus is associated with the occurrence of ciguatoxic fish (Chanteau, 1978;

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[a] Recently it has been reported that three dinoflagellate species (Gambierdiscus toxicus, Prorocentrum concavum, and Prorocentrum rhathymum) are producers of toxins that contribute to the ciguatera syndrome in the Carribean (Tindall, 1983).

Bagnis, 1981c). A temporal fluctuation in the concentration of G. toxicus cells in the sea water was observed, apparently associated with the death of corals caused by constructions in the lagoon, whereas no association was found with a number of trace elements in the sea water (Bagnis, 1977; Yasumoto et al., 1980a).

2.2.2 Occurrence in fish

The occurrence of ciguatoxin in fish has been reviewed by Banner (1975) and WHO (1983). More than 400 species of bony fish have been reported in the literature to have caused ciguatera (Halstead, 1978). In general, ciguatoxic species are limited to fish that feed on algae and the detritus of coral reefs, particularly the surgeon-fish (Ctenochaetus striatus), parrot-fish (Scarus gibbus), and the larger reef carnivores that prey on these herbivores (Bagnis, 1981b). Thus, the larger carnivores such as moray eels, snappers, groupers, carrangs, Spanish mackerels, emperors, certain in-shore tunas, and barracuda are most toxic. Ciguatoxin has been detected in the contents of the gut, the liver, and the flesh (muscle tissue) of surgeon-fish (Yasumoto et al., 1971), and parrot-fish, groupers, and snappers (Bagnis & Letourneux, 1974), by means of the mouse assay and chromatography (Chanteau et al., 1976; Yasumoto et al., 1977b). The highest concentrations of ciguatoxin were found in the liver and other viscera (Helfrich et al., 1968; Chungue & Bagnis, 1976). Not all the fish in a single population contained equal levels of toxin. Furthermore, even when the flesh did not contain detectable levels of ciguatoxin, the liver contained an appreciable amount, as demonstrated in the moray eel (Yasumoto & Scheuer, 1969). Ciguatoxin has also been found in the viscera of a turban shell (Turbo argyrostoma, a marine snail), a food item that has occasionally caused ciguatera-like intoxication in man (Yasumoto & Kanno, 1976).

2.2.3 Environmental factors influencing the growth of causative dinoflagellates

Randall, in a review in 1958, had already suggested that the occurrence of ciguatera might have an environmental background. Randall also refined an earlier hypothesis that the disease agent was transmitted from herbivorous to carnivorous fish, and he suggested, without any direct proof, however, that the causative organism was a benthic blue-green alga. He further mentioned that disturbances of the coral reef caused creation of new surfaces to support vigorous growth of the hypothetically toxic cyanophyte organism. Evidence supporting the hypothesis of an environmental

influence on ciguatera has subsequently been provided in a series of investigations in French Polynesia by Bagnis and co-workers (Bagnis, 1969, 1974, 1977, 1980, 1981b; Bagnis et al., 1973, 1974, 1980). Thus, natural disturbances of the coral reefs, such as hurricanes and storms, or man-made disturbances, such as blasting of reefs, crashing of ship anchors, and building of piers or wharfs, provide conditions for growth of the macroalgae to which G. toxicus cells are attached, resulting in increased dinoflagellate populations. These disturbances, which cause increased numbers of ciguatoxic fish and increased toxin levels in the affected fish resulting in increased incidence rates of ciguatera, may have long-lasting effects, up to 10 - 15 years after the disturbance took place. At this point, no information is available on the environmental influence on the other dinoflagellates (P. concavum and P. rhathymum) associated with ciguatoxic fish.

2.2.4 Human exposure

The only known pathway of human exposure is through the consumption of contaminated fish, with the exception of a marine snail. In the past, this exposure was limited to the circumglobal tropical and subtropical belt shown in Fig. 3. However, recent evidence (section 2.5) has shown that interregional transport of fish can result in human exposure in other parts of the world.

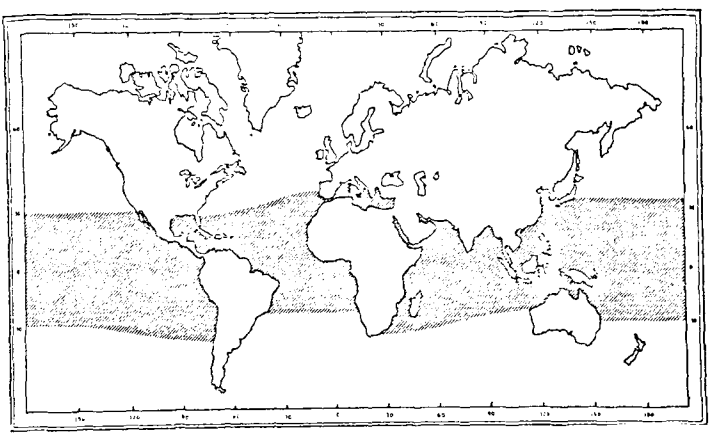


Fig. 3. The circumglobal belt (shaded area) in which ciguatera intoxication is known to occur (From: Hessel et al., 1960).

2.3 Metabolism

Data on absorption, retention, distribution, and metabolism of ciguatoxin in human beings or animals are not available because reliable chemical methods of analysis are lacking.

2.4 Effects on Animals

2.4.1 Experimental studies

Ciguatoxin, extracted from fish, purified to some extent by solvent extraction, and injected intravenously or intraperitoneally into mice, produced acute effects characterized by diarrhoea, retching, inactivity, and death after convulsive spasms (Bagnis, 1970; Banner, 1975). Similar effects were observed within 48 h, when cats or mangooses were fed a ration containing ciguatoxin. The effects on mice, cats, and mongooses were used as a basis for the biological determination of ciguatoxin, decribed in section 2.1.2.1. The pronounced toxicity of ciguatera toxins is noteworthy. Thus, ciguatoxin has an LD_{50} (ip) in mice of 0.45 µg/kg body weight (Scheuer, 1982), while the MLD (ip) in mice for maitotoxin is 0.15 µg/kg body weight (Yasumoto et al., 1984).

2.4.2 Mode of action

Few relevant data on the mode of action are available because of the restricted availability of purified toxins of this group. The pharmacological action of ciguatoxin is related to its direct effects on excitable membranes rather than to its antichlolinesterase properties. Ciguatoxin has a potent depolarising action due to a selective increase in sodium permeability in the nerve cells and striated muscle, which can be counteracted by calcium ions (Rayner, 1972). The effect of ciguatoxin on smooth muscle can be explained by a potent releasing action of the toxin on endogenous norepinephrine from adrenergic nerve terminals and a potentiating effect on the postsynaptic membrane (Ohizumi et al., 1981).

2.5 Effects on man

2.5.1 Clinical Studies

The clinical picture is quite variable. Typically, symptoms occur within 1 - 6 h of ingestion of toxic fish. Initial symptoms usually include nausea, malaise, and numbness

and tingling of the lips, tongue, and throat. Patients may later develop some or all of the following signs and symptoms: vomiting, abdominal cramps, diarrhoea, paraesthesia of the extremities, itching myalgia, and arthralgia. In more severe cases, ataxia, weakness, blurred vision, insomnia, sinus bradycardia, dysrhythmias, and hypotension may develop (Bagnis 1968; Morris et al., 1982a). A symptom that particularly suggests the diagnosis is alternating sensations of cold and hot (Bagnis, 1967). The duration of illness is variable. Most of the patients recover within three days, but malaise, paraesthesia, pruritus, and ataxia may persist for weeks or even years in severe cases (Hughes & Merson, 1976; Bagnis et al., 1979d). Patients repeatedly poisoned by ciguatoxic fish may develop a resurgence of ciguatera symptoms even after eating fish containing little or no detectable toxin (Bagnis, 1984a).

On the basis of mouse-assay analyses of toxic fish recovered from patients meals, Yasumoto suggested that the oral intake of as little as 0.1 µg (10 MU) of ciguatoxin can cause illness in an adult (Yasumoto, 1980; Yasumoto et al., 1984).

In the most severe cases, death results from circulatory collapse or respiratory failure. Halstead (1978) reported a case-fatality rate of about 12%, but mentioned that limited inability statistics were available. This publication includes an intensive review of case reports on ciguatera, going back to the beginning of the last century. However, 3 deaths due to ciguatera occurred among 3009 cases (corresponding to a case-fatality rate of 0.1%) in French Polynesia (Bagnis et al., 1979a). No deaths occurred among 184 cases reported to the Centers for Disease Control, USA, in 1970-74 (Hughes & Merson, 1976) or among 33 patients in the US Virgin Islands in 1980 (Morris et al., 1982a); 3 out of 67 patients died in outbreaks that were reported from Puerto Rico in 1981, corresponding to a case fatality rate of 4.5% (Anon, in press).

2.5.2 Epidemiological studies

Cases of ciguatera are commonly encountered throughout the Caribbean area and much of the Pacific area, in the zones between latitudes 35° N and 35° S (Fig. 3). The location and other characteristics of recently-reported outbreaks are summarized in Table 10.

The annual incidence of ciguatera intoxication on the Virgin Islands on the basis of emergency room admissions for 3 years was 3.6 cases per 1000 (Morris et al., 1982b). Results of a household survey suggested that the true annual incidence was actually 7.3 per 1000. These cases were diagnosed for the

Table 10. Recent reports on the occurrence of outbreaks of ciguatera

Country	Number of people affected	Number of deaths	Species of fish	Origin of fish	Reference
Bahamas	14	0	barracuda	Local	Anon (1982)
Canada	2	0	barracuda	Jamaica	Anon (1983b)
Cuba	100	0	moray eel Spanish mackerel	Local	Bagnis (1978)
Fiji	925	1	snapper barracuda grouper emperor (mainly)	local	Yasumoto et al. (1984)
France	2	0	not specified (frozen fish)	China (Province of Taiwan)	Baylet et al. (1978)
French Polynesia New Caledonia (South Pacific)	3009	3	surgeon-fish parrot-fish grouper snapper carrang emperor barracuda (mainly)	local	Bagnis et al. (1979)

Table 10 (contd).

Country	Number of people affected	Number of deaths	Species of fish	Origin of fish	Reference
Jamaica	250	0	grouper barracuda	local	Bagnis (1978)
La Reunion (Indian Ocean)	367	0	snapper	Salya de Malha	
USA (Florida)	129	0	grouper snapper (mainly)	local	Lawrence et al. (1980)
USA (Maryland)	12	0	grouper	Florida	Anon (1980)
USA (Shipboard)	24	0	barracuda	Gulf of Mexico	Barkin (1974)
US Virgin Islands	51	0	snapper	local	Engleberg et al. (1983)
US Virgin Islands	33	0	carrang snapper	local	Morris et al. (1982a,b)

characteristic combination of gastrointestinal and neurological symptoms. Hanno (1981) estimated that the true incidence on the Virgin Islands might be as high as 30 per 1000. In the South Pacific, incidence rates vary from 1 case per 10 000 in Wallis, Futuna, Naury, Guam, Salomons, and Cook Islands to 4 - 5 cases per 1000 in Tuvalu and French Polynesia (Bagnis, 1984a). In the USA, ciguatera accounted for 22% of all outbreaks of food-borne chemical diseases reported in the period 1970-74 (Hughes et al., 1977). When food-borne infectious agents (bacteria, viruses, and parasites) were considered in addition to chemical agents in 1978-81, ciguatera made up 8.4% of all reported outbreaks of food-borne diseases of known etiology (Anon, 1981c; Anon 1983b,c). Of the 67 ciguatera outbreaks reported during these 4 years, 34 (51%) occurred in Hawaii, 11 (16%) in Puerto Rico, 5 (7%) in the US Virgin Islands, and 5 (7%) in Florida (Anon., 1981b,c; Anon., 1983c,d).

Cases of ciguatera have also been encountered outside the circumglobal belt, where the organism G. toxicus is present and where ciguatoxic fish are traditionally caught. Thus an outbreak of ciguatera occurred in Maryland, USA, involving 12 persons showing symptoms, and two persons who were hospitalized because of hypotension. The intoxication was due to a fish (grouper) that had been transported from Florida to the restaurant in Maryland, where the episode took place (Anon, 1980). In France, ciguatera has been diagnosed in association with the consumption of ciguatoxic fish imported frozen from China, Province of Taiwan (Baylet et al., 1978). In Canada, an outbreak of ciguatera was observed that involved two persons and was associated with the consumption of barracuda brought by tourists from Jamaica (Anon, in press).

3. TETRODOTOXIN (PUFFERFISH POISON)

In contrast to other biotoxins included in this document, tetrodotoxin, according to present knowledge, is probably not produced by algae, but by certain fishes and a few other animals. On the basis of the number of human victims involved yearly, tetrodotoxin poisoning is not an important public-health problem. However, in contrast with most other algal intoxications discussed below, the illness in tetrodotoxin intoxication is severe and the mortality rate is high. Furthermore, increase in world trade has led to cases of the shipping and sale of misbranded toxic fish to countries where tetrodotoxin poisoning had previously been unknown (Pocchiari, 1977). Thus, the magnitude of tetrodotoxin poisoning as a public-health problem is influenced less by the number of human victims involved than by its potential threat to human life and health.

The history of tetrodotoxin poisoning has been reviewed in some detail by Kao (1966). In recent years, considerable interest in tetrodotoxin has developed among natural-product chemists and neurobiologists. For the former, there are challenging problems related to its isolation and purification as well as to its structure. As regards the latter, tetrodotoxin remains the most important and most widely-used tool for selectively blocking the sodium channel.

3.1 Properties and Analytical Methods

3.1.1 Chemical properties

Schantz (1973) and Scheuer (1977) have reviewed the chemistry of tetrodotoxin. The compound has been obtained from an extract of pufferfish viscera in the form of colourless crystal prisms that are slightly soluble in water. It is an aminoperhydroquinazoline compound (Fig. 4), with a relative molecular mass of 319. It has a guanidinium group with a pKa of 11.6, and a unique intramolecular hemilactal bond. The toxin is unstable at pH levels above 8.5 and below 3.

3.1.2 Methods of analysis for tetrodotoxin in foods

Though of entirely different chemical structure, tetrodotoxin induces toxic effects very similar to those of saxitoxin (section 1.5), and the mouse bioassay developed for PSP has also been used for tetrodotoxin (Kao, 1966; Schantz, 1973). In Japan, a modification of the mouse bioassay is now in operation as an official method (Kawabata, 1978), and a

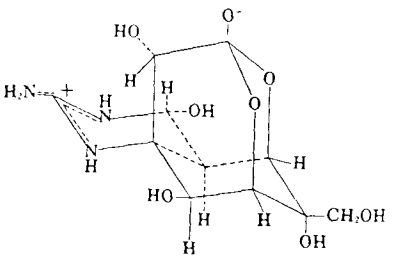


Fig. 4. Structure of tetrodotoxin.

chemical assay has been developed (Nunez et al., 1976) on the basis of the production of fluorescent compounds of tetrodotoxin by alkali treatment. In this method, there is a linear relation between the fluorescence-intensity and the concentration of tetrodotoxin in the range of 0.34 - 10 µg/ml. A continuous analyser, using the same reaction, was constructed by Yasumoto et al (1982). In this method, which is more sensitive and specific than the previous method, the toxin, after separation from contaminants on an ion-exchange column, is converted to fluorescent compounds by heating in a solution of 2N sodium hydroxide. Both the retention time of the toxin and the intensity of fluorescence are recorded automatically on a fluorimeter. A linear relation exists between the intensity of fluorescence and the concentration of tetrodotoxin in the range of 0.02 - 4 µg toxin/mg. The results are reproducible within a variation of 3%.

Another continuous analyser was constructed by Onoue et al. (1983) in which 1-phthalaldehyde was used as the reagent to produce a fluorescent derivative from tetrodotoxin. This method has the advantage of differential detection of tetrodotoxin from PSP toxins. It suffers from the need for laborious pretreatment of the extract, because the reagent is more reactive with amino acids, which are present in an overwhelming abundance in the extracts.

3.2 Occurrence and Human Exposure

The occurrence of tetrodotoxin has been reviewed by Kao (1966) and Blankenship (1976). It is mainly found in the ovaries, liver, and intestines of various species of pufferfish, lesser amounts being found in the skin; the body muscle is usually free of the toxin, with the exception of Lagocephalus lunaris lunaris, which often contains fatal amounts of tetrodotoxin in the muscle tissue (Tabeta & Kumagai, 1980). The most toxic pufferfish are members of the

family Tetraodontidae, but not all the species in this family contain the toxin. The most toxic ones are caught along the coasts of China and Japan, and the meat of these species is considered a delicacy. The amount of toxin in the roe is related to the reproductive cycle, and is greatest just before spawning (early summer). Tetrodotoxin has also been found in the skin of a group of newts of the genus Taricha, native to northern California and southern Oregon, in the USA. It has been detected in the skin of Central American frogs of the genus Atelopus. In addition, tetrodotoxin has been identified as the poisonous principal in the venom of the blue-ringed octopuses, Hapalochlaena maculosa and H. lunata of southern Australia, involved in human fatalities through bites (Freeman & Turner, 1970; Sheumack et al., 1978). Tetrodotoxin has also been found in the Japanese ivory shell, Babylonia japonica, and in the trumpet shell, associated with fatal human cases following consumption (Narita et al., 1981; Noguchi et al., 1981).

There has not been any report linking the presence of tetrodotoxin in these animals with algae or microbes, but it is noteworthy that pufferfish raised artificially in ponds do not contain tetrodotoxin (Matsui et al., 1981, 1982).

Human exposure is generally limited to consumption of certain fish species, the identification of which is feasible. This is more difficult with frozen fish flesh. In international trade with frozen fish from areas where tetrodotoxin-containing fish are caught, special care should be taken to avoid transport of contaminated fish flesh (section 3.4).

3.3 Mode of Action

The mode of action of tetrodotoxin is very similar to that of saxitoxin and is dealt with in section 1.5.2.2.

3.4 Effects on Animals

The effects of tetrodotoxin, either in contaminated fish or in a purified form, have been tested experimentally on a large variety of animal species (Table 11). In all animals, with few exceptions, the signs of intoxication are generally the same, and comparable with those caused by the PSP compounds. These effects involve primarily the peripheral neuromuscular system, which is paralysed to different extents because of interference with the generation and conduction of electrical impulses (section 1.5). There are 3 manifestations of tetrodotoxin intoxication that appear to be somewhat different from those due to PSP compounds. Tetrodotoxin is a highly potent emetic agent, so that vomiting is frequently

Table 11. Comparative lethality of tetrodotoxin in various animals[a]

	Minimum lethal dose (µg tetrodotoxin/kg body weight)
Plaice (Paralichthys olivaceus)	0.5
Dragonfly	1.3
Carp	2.0
Pigeon	2.7
Rat	2.7
Sparrow	4.0
Guinea-pig	4.5
Frog	5
Hen	6
Rabbit	8
Mouse	8
Dog	9
Cat	10
Turtle	46
Eel	80
Toad (Bufo)	200
Snake (non-poisonous, species not given)	450

[a] Adapted from: Kao (1966).

observed in both cats and dogs and also in man. For the same degree of neuromuscular paralysis, the systemic arterial hypotension produced by tetrodotoxin is significantly greater and lasts appreciably longer than that produced by the PSP toxins. Lastly, tetrodotoxin, acting through a central mechanism, is a highly potent hypothermic agent.

Pufferfish and taricha newts containing tetrodotoxin are resistant to the action of tetrodotoxin.

Two field cases of tetrodotoxin intoxication have been reported in which cats had been fed a diet of pufferfish containing an unknown level of tetrodotoxin (Atwell & Stutchbury, 1978). The cases were characterized by paralysis, ataxia, and respiratory depression, and the symptoms could be reproduced in cats by feeding liver and flesh contaminated with tetrodotoxin.

3.5 Effects on Man

In man, the onset of symptoms of tetrodotoxin intoxication usually occurs from 10 to 45 min after ingestion, but may be delayed by 3 h or more. Paraesthesia appears in the face and extremities and may be followed by sensations of lightness, floating, or numbness. Nausea, vomiting, diarrhoea, and epigastric pain may also be present. Later, respiratory symptoms become prominent with dyspnoea, shallow, rapid

respiration, and the use of auxilliary muscles. Cyanosis and hypotension follow, and convulsions and cardiac arrhythmia may occur. In most instances, the victims retain consciousness until shortly before death, which usually takes place within the first 6 h (Torda et al., 1973). In Japan, the average annual number of tetrodotoxin cases for the period 1974-79 was 60, with 20 deaths (Kainuma, 1981). In the USA, two non-fatal outbreaks were reported in the period 1970-74 (Hughes et al., 1977). In Italy, 10 cases of tetrodotoxin intoxication were observed, with 3 deaths, following the consumption of frozen pufferfish imported from China, Province of Taiwan, mislabelled as angler fish (Pocchiari, 1977). Samples of the pufferfish contained from 0.5 to 30 mg tetrodotoxin per kg wet tissue.

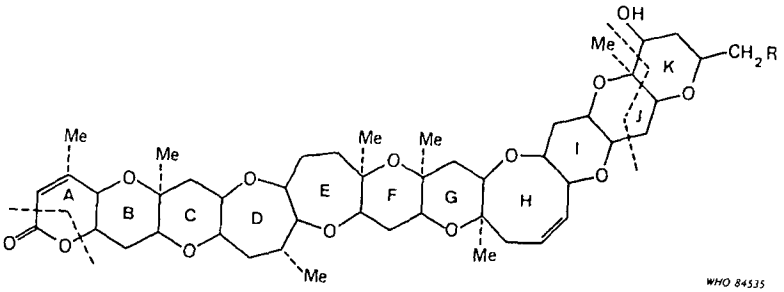
4. NEUROTOXIC SHELLFISH POISONS

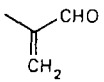
A disease in human beings associated with red tides involving the dinoflagellate Gymnodinium breve has been encountered around the coasts of Florida, USA, named neurotoxic shellfish poisoning (NSP). According to symptoms and mode of exposure, two syndromes can be identified: (a) NSP associated with the consumption of shellfish containing cells or metabolites of toxic G. breve. The symptoms are predominantly neurotoxic in nature and resemble PSP, except that paralysis does not occur; (b) NSP characterized by respiratory symptoms and associated with exposure to aerosols of G. breve cells (Hughes & Merson, 1976).

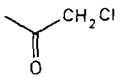
There is however much less data available for this disease, compared with the other diseases caused by dinoflagellate toxins and tetrodotoxin. Thus, the G. breve toxins have never been chemically identified in food (and air) in episodes involving human beings, and only a limited number of toxicity studies on animals have been conducted so far.

4.1 Properties and Analytical Methods

The chemical properties of toxins obtained in earlier investigations have been reviewed by Shimizu (1978). More recently, 4 toxic components have been isolated from cultured cells of G. breve and the stucture was determined for three of them, named brevetoxin B, brevetoxin C, and GB-3 (Lin et al., 1981; Chou & Shimizu, 1982; Golik et al, 1982). These components share the same skeleton made up of a single carbon chain locked into a rigid ladder-like novel structure consisting of 11 continous transfused ether rings (Fig. 5). The compounds are soluble in organic solvents but are unstable in chloroform; this has caused difficulties in isolating the toxins in earlier investigations. The toxins are not fluorescent and do not have properties that make detection and quantification easy. Hence, no chemical method for analysis exists. During the purification procedure, a fish bioassay has been employed (Lin et al., 1981). A mouse bioassay has been developed comparable to the assay used for PSP (section 1.1.2.1), but involving more elaborate extraction and clean-up procedures and an observation time of 6 - 24 h (Subcommittee on laboratory methods for the examination of shellfish, 1970; Spiegelstein et al., 1973). The disadvantage is that no standard preparation of G. breve toxins is available for standardization of the mouse bioassay.



Brevetoxin B R = 

Brevetoxin C R = 

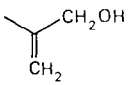
GB-3 toxin R = 

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Fig. 5. Structure of Gymnodinium breve toxins.

4.2 Sources and Occurrence

The NSP toxic compounds have been isolated exclusively from G. breve, a non-thecate (naked) dinoflagellate, encountered around the coasts of Florida, USA, particularly during red tides, which are initiated in offshore waters primarily in the late summer and autumn months (Steidinger, 1975). The iron content of the water might be used as a predictive guide, as a maximum of iron has been observed immediately preceding red tides (Kim & Martin, 1974). Taxonomically, the organism has recently been transferred to Ptychodiscus brevis (Steidinger, 1979).

4.3 Effects on Animals

4.3.1 Field observations

A disease has been observed in fish and birds that is thought to be caused by G. breve toxins, because the episodes

have occurred in close association with blooms of G. breve cells, and because similar symptoms were observed after feeding G. breve cells to birds.

Every 3 - 4 years, blooms of G. breve occur on the west coast of Florida, causing massive fish kills. The fish species involved are mainly tomtate fish (Haemulon aurolineatum) and striped mullet (Mugil cephalus) (Forrester et al., 1977). The fragile naked cells of G. breve rupture on passage through the gill processes of the fish, releasing the toxins, which readily pass through the gill surfaces with lethal effect, if the G. breve cell concentration is sufficiently high. Fish that swim into a red-tide area will continue actively for a while, then will suddenly lose balance, gasping at the surface before becoming passive on the bottom followed by a terminal struggle. Death occurs without pathologic lesions (Abbott et al., 1975).

Mass death of sea birds and mass fish kills have been observed, associated with red tides, off the west coast of Florida (Forrester et al., 1977). The birds involved were double-crested cormorants (Phalacrocorax auritus), redbreasted mergansers (Mergus merganser), and lesser scaup (Aythya affinis). The signs shown by the affected birds included weakness, reluctance to fly, clear nasal discharge, viscous oral discharge, oil gland dysfunction, diarrhoea, dyspnoea, tachypnoea, tachycardia, and hypotension.

4.3.2 Experimental animal studies

In a study using white Pekin ducklings, force-fed with tissues of clams that had been filter-feeding on toxic G. breve cells and with sea water containing G. breve cells, the birds showed ataxia and spastic movements within 3 days, and died within 5 days (Forrester et al., 1977). Similar signs, including death within 6 - 22 h, were observed in male white Leghorn chicks fed tissues of oysters (Crassostrea virginica) that had been filter-fed in the laboratory on toxic G. breve cells (Ray & Aldrich, 1965).

Mice are susceptible to G. breve toxin preparations administered intravenously, intraperitoneally, or subcutaneously, showing signs similar to those observed in mice administered PSP (section 1.4.2.1). A bioassay was also developed using the mosquito fish (Bambusia affinis), which seems to be very susceptible to toxic G. breve (Spiegelstein et al., 1973).

During in vitro experiments, spasmogenic effects of G. breve toxin preparations through the stimulation of the post-ganglionic cholinergic nerve fibre have been elucidated in muscle preparations of guinea-pig ileum (Grunfeld & Spiegelstein, 1974). The results of further in vitro

experiments have shown that G. breve toxin preparations depolarize the resting membrane potential by increasing sodium permeability in rat phrenic nerve diaphragm preparations (Gallagher & Shinnick-Gallagher, 1980; Shinnick-Gallagher, 1980). In cats, administered G. breve toxin preparations intravenously (after vagotomy) and intracerebroventricularly, regular breathholding and hypertension with tachycardia was observed, leading ultimately to respiratory and circulatory failure (Borison et al., 1980b).

The acute effects of a crystalline preparation of brevetoxin B (named T34) have been observed during in vivo and in vitro studies, summarized in Table 12 (Baden et al., 1981).

Table 12. Comparative toxicity of brevetoxin B[a]

Test organism	LC_{50} or LD_{50} [b] (24 h)	EC_{50} [b,c] (mg/litre)	Endpoint measured
Mosquito fish (Gambusia affinis)	0.011 mg/litre (0.005 - 0.023)		death
Mouse (ip)	0.20 mg/kg body weight (0.15 - 0.27)		death
Tissue culture			
KB tumour		0.26 (0.23 - 0.29)	cell growth (protein determination)
B 388 lymphocytic leukaemia		0.32 (0.12 - 0.89)	cell growth (cell number)
L 1210 lymphoid leukaemia		0.42 (0.17 - 1.03)	cell growth (cell number)
Sea urchin egg		8.9 (6.5 - 12.2)	division of fertilized eggs

[a] Adapted from: Baden et al. (1981).
[b] The 95% confidence limits are in parenthesis.
[c] EC_{50} = concentration in the median causing 50% inhibition.

Crystalline preparations of 2 toxic components from G. breve, named T17 and T34, the latter being identical to brevetoxin B, were injected intratracheally into guinea-pigs. T17, at doses ranging from 0.001 to 0.080 mg/kg body weight increased the resistance to pulmonary inflation at all doses. The pulmonary response to T17 differed slightly from those to histamine and acetylcholine in its longer persistence at peak

levels. The rate of onset was, however, equally rapid in all cases. T17, (0.02%) administered at 0.01 mg/kg body weight, caused bronchoconstriction approximately equivalent to that caused by 0.05 µg acetylcholine/kg. The studies using T34 (0.20%) were not pursued because the concentration necessary to produce bronchoconstriction equivalent to that of 0.050 µg atropine/kg body weight was 0.05 mg/kg (Baden et al., 1982).

4.4 Effects on Man

In human beings, consuming shellfish contaminated with G. breve cells, paraesthesia, alternating sensations of hot and cold, nausea, vomiting, diarrhoea, and ataxia occur within 3 h (McFarren et al., 1965). Paralysis has not been observed, and the disease (NSP) appears to be milder than PSP (Hughes & Merson, 1976). In the USA, in the period 1970-74, 2 outbreaks (a total of 5 cases) of NSP were recorded, both associated with the consumption of clams; no deaths occurred. The concentrations of NSP in the clams were in the range 30 - 118 MU/100 g (Hughes et al., 1977; Hughes, 1979). In USA, shellfish containing any detectable level of NSP per 100 g, as determined by the mouse assay, is considered potentially unsafe for human consumption (Subcommittee on laboratory methods for the examination of shellfish, 1970).

An upper respiratory syndrome of NSP has been reported, associated with aerosols of G. breve cells and/or toxins, in coastal areas of Florida, USA (Hughes & Merson, 1976). The rapidly reversible syndrome is characterized by conjunctival irritation, copius rhinorrhoea, and nonproductive cough.

5. DIARRHOEIC SHELLFISH POISON

An intoxication characterized by gastrointestinal disturbances, often occurring as outbreaks associated with the consumption of shellfish, and consequently named diarrhoeic shellfish poisoning (DSP), has been reported from several parts of the world, including the Far East, Europe, and South America. The identification of the toxin-producing algal organisms, and the characterization of the chemical structure of some of the algal toxins present in the shellfish involved have been achieved very recently. Information on such aspects as analytical procedures and toxicology is therefore limited at present. However, as many hundreds of DSP cases have been reported, it is included in the document for completeness.

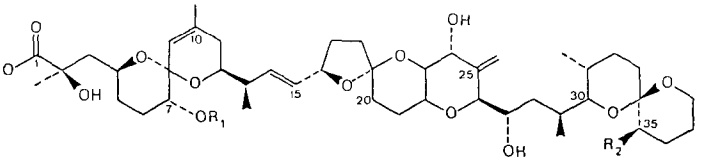
5.1 Sources and Occurrence

Dinophysis fortii, an armoured marine dinoflagellate, has been identified as a producer of DSP in Japan (Yasumoto et al., 1980b), whereas D. acuminata is suspected of being the toxin producer in recent outbreaks in the Netherlands, based on epidemiological evidence. DSP has not been detected in cells of D. acuminata because attempts to cultivate the organism isolated from Dutch waters have been unsuccessful (Kat, 1983a,b). Cases in Chile were associated with the occurrence of D. acuta, though detailed information is not available (Guzman & Compodonico, 1975). Occurrence of one of the DSP toxins, okadaic acid, has been confirmed in a benthic dinoflagellate, Prorocentrum lima (Murakami et al., 1982), though involvement of this species in DSP has never been known.

Species of Dinophysis are distributed widely but seldom form red tides. It has been noted that in the presence of D. fortii at a low cell density of 200 cells/litre, mussels and scallops become toxic enough to affect man. The infestation period in Japan ranges from April to September (Yasumoto et al., 1978).

5.2 Chemical Properties

The presence in shellfish of 9 toxic components has been recognized and the chemical structures of 5 components have been established (Murata et al., 1982; Yasumoto et al., 1984). These toxins are classified into two groups: okadaic acid and its derivatives named dinophysistoxins, and the novel polyether lactones named pectenotoxins (Fig. 6). The chemical structure for dinophysistoxin-2 is not yet known because of its limited availability, while pectenotoxin-3, -4 and -5 are closely related to pectenotoxin-1, in chemical structure.



Okadaic acid : R_1 = H, R_2 = H
Dinophysistoxin - 1 : R_1 = H, R_2 = CH_3
Dinophysistoxin - 3 : R_1 = acyl, R_2 = CH_3

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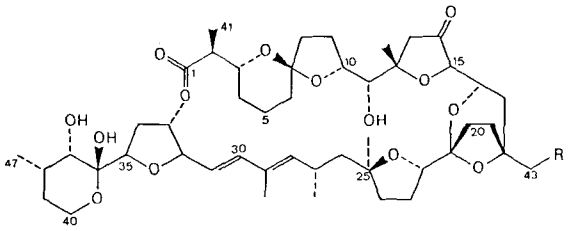


Pectenotoxin - 1 : R = OH
Pectenotoxin - 2 : R = H

WHO 84538

Fig. 6. Structure of diarrhoeic shellfish poisons.

5.3 Analytical Method

A mouse bioassay, using intraperitoneal injection of toxin extracts and a 24-h observation period, is being used as a regulatory measure to monitor shellfish toxicity in Japan, and shellfish with a DSP toxin level exceeding 50 MU/kg are banned from harvesting or sale (Anon, 1981d, personal communication, Yasumoto, 1983). In a rat bioassay used in the Netherlands for monitoring purpose, the material to be tested is included in the diet and observations of diarrhoeal symptoms and reduced feed intake are recorded (Kat, 1983a,b).

5.4 Effects on Animals - Experimental Studies

Mice injected with toxic extracts of DSP shellfish intraperitoneally show inactivation and general weakness, and

die within 30 min - 48 h, depending on the dose given. On the basis of intraperitoneal administration, chicks are less sensitive. Vomiting was observed by Yasumoto et al. (1980b) in cats fed toxic mussels and scallops. When rats (Rattus norvegicus) were fed DSP toxic shellfish as part of the diet, diarrhoea and reduced feed intake were observed (Kat, 1983b).

5.5 Effects on Man

During the period 1976-82, more than 1300 people were diagnosed as DSP cases in Japan. Frequency of signs and symptoms were: diarrhoea (92%), nausea (80%), vomiting (79%), abdominal pain (53%), and chill (10%). The time from consumption of shellfish to the onset of illness ranged from 30 min to several hours, but seldom exceeded 12 h. About 70% of patients developed symptoms within 4 h. Suffering may last for 3 days but leaves few after-effects. In the Netherlands, more than 30 cases were encountered in the DSP outbreak in 1981 (Kat, 1983b). Cases of gastrointestinal disorders have been observed in Chile in 1970 and 1971, apparently associated with blooms of Dinophysis sp. (Avaria, 1979).

6. CYANOPHYTE TOXINS

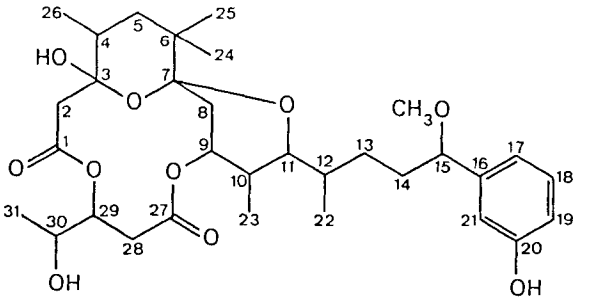
6.1 Dermatitis-Inducing Marine Cyanophyte Toxins

6.1.1 Sources and properties

The subject of cyanophyte toxins has been reviewed by Moore (1981). Contact with the filamentous cyanophyte Lyngbya majuscula, when swimming in the sea, can result in a type of dermatitis called "swimmers itch" or "seaweed dermatitis", as reported from Hawaii (Grauer & Arnold, 1961) and Japan (Okinawa) (Hashimoto, 1979), Two skin-toxic components have been isolated from L. majuscula, i.e., debromoaplysiatoxin and lyngbyatoxin A. In crystalline form, debromoaplysiatoxin consists of colourless needles and has a melting point of 105.5° - 107.0 °C and a relative molecular mass of 592 (Mynderse et al., 1977) (Fig. 7). Lyngbyatoxin A is a tan-coloured gummy solid in crystalline form, with a relative molecular mass of 437 (Cardellina et al., 1979) (Fig. 7). Debromoaplysiatoxin has also been isolated from two other species within the cyanophyte family Oscillatoriaceae, i.e., Oscillatoria nigroviridis and Schizothrix calcicola (Mynderse et al., 1977).

6.1.2 Effects on animals

The toxicity of debromoaplysiatoxin has been studied on the skin of mice and rabbits (Solomon & Stoughton, 1978). The toxin was dissolved in 100% ethanol to make 0.5%, 0.05%, 0.005%, 0.0005%, and 0.00005% solutions. Groups of hairless, female mice (strain HRS/J) were used, with 3 animals per group; 10 µl of solution was applied to the back of each mouse. Each solution (10 µl) was applied to separate areas of the shaved back of a New Zealand white rabbit. In the mice, there were petechial haemorrhages within 1 h of application of 0.5% debromoaplysiatoxin; by 24 h, the area was pale and oedematous, and by 5 days there was a firm crust, which took 2 - 3 weeks to heal. A dose-effect relationship was observed, and even the smallest dose (0.00005%) induced mild oedema. Histologically, the changes over 24 h of application of 0.5% solution included almost complete destruction of the epidermis, oedema, coagulation of collagen, infiltration throughout the dermis and deep into the subdermal muscle of polymorphonuclear leukocytes and erythrocytes. The dermal and subdermal blood vessels showed mural fibrinoid necrosis with polymorphonuclear infiltration and leukocytoclasis. The histological changes seen in mice treated with smaller doses were similar but less notable. In



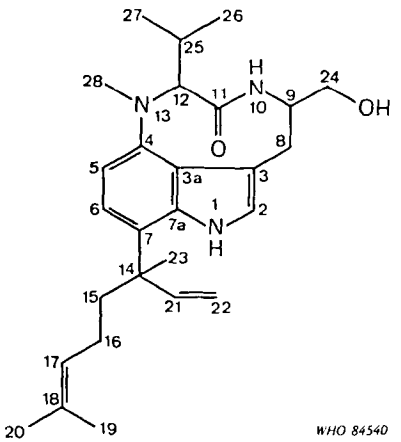


Fig. 7. Structure of dermatitis-inducing cyanophyte toxins, debromoaplysiatoxin (top) and lyngbyatoxin A (bottom).

mice treated twice with the 0.005% solution with a 2-week interval, the macroscopic reaction was similar to that seen in mice receiving only one treatment. In the skin of the rabbit, there was also a dose-effect relationship, and the lowest dose induced a reaction consisting of mild erythema. Histologically, the changes were similar to those seen in the mice.

Thus, 10 µl of the 0.00005% solution (5 pg debromoaplysiatoxin) induced skin inflammation in 2 animal species, and the authors concluded that no other agent is known that will induce inflammation when applied in such a small amount. No details of the skin testing of lyngbyatoxin A appear to have been published.

6.1.3 Effects on man

Cases of acute dermatitis after contact with L. majuscula have been reported from Hawaii (Grauer & Arnold, 1961) and Japan (Hashimoto, 1979). In Hawaii, more than 125 cases were received for treatment and hundreds of mild cases were suspected in the period July-August 1958. The clinical picture is characterized by the gradual onset of itching and burning within a few min to a few h after swimming in the sea, where fragments of the alga are suspended. Visible dermatitis and redness develops after 3 - 8 h, followed by blisters and deep desquamation. The eruption affects the region of the body not covered by the swimming trunks. Histologically, the lesions were described as acute, vesicular dermatitis, characterized by superficial desquamation, oedema of the epidermis with vesicles within the epidermis. Occasionally, the vesicles contained polymorphonuclear leukocytes and erythrocytes, and the deepest portion of the epidermis was infiltrated by polymorphonuclear leukocytes (Grauer & Arnold, 1961).

These lesions have been reproduced by applying solutions of debromoaplysiatoxin on the skin (Solomon & Stoughton, 1978). The compound was dissolved in 100% ethanol to obtain 0.5%, 0.05%, 0.005%, 0.0005%, and 0.00005% solutions, which were applied on the skin of the two investigators. The lowest concentration with which dermatitis developed in 6 - 12 h was the 0.05% solution. Histological studies confirmed the similarity between the dermatitis induced experimentally and that associated with L. majuscula, mentioned above.

6.2 Freshwater Cyanophyte Toxins

Although reports on disease induced in farm animals by toxic cyanophytes in drinking-water are known from the last century, the elucidation of the chemical nature of these cyanophyte toxins has been progressing very slowly. The first full documentation of the chemical structure of a freshwater cyanophyte toxin (anatoxin-a) was published in 1977 (Devlin et al., 1977), and a chemical method of analysis for this toxin was subsequently reported (Astrachan & Archer, 1981). There are indications of intoxications induced in animals, as field cases in farm animals, and as experimentally-induced disease

in a variety of animal species, which can be related to certain species of cyanophytes living in freshwater. There are adverse effects in human beings, which may be related to drinking water containing high concentrations of cyanophytes. For these reasons, freshwater cyanophyte toxins have been included in this monograph. It can be anticipated that more information on the chemistry and occurrence of the cyanophyte toxins will be made available in the near future, as much research is in progress this field (Carmichael, 1981).

Most of the available information on toxic freshwater cyanophytes is concerned with the following 3 species: Microcystis aeruginosa, Anabaena flos-aquae, and Aphanizomenon flos-aquae. M. aeruginosa is a coccoid blue-green alga, and the onset of Microcystis blooms in lakes is correlated with temperature, with blooms occurring when the water temperature reaches 19 - 20 °C, an observation supported by experimental data (Krüger & Eloff, 1978). Anabaena flos-aquae and Aphanizomenon flos-aquae are filamentous blue-green algae.

The cells of blue-green algae are embedded in mucilage containing bacteria, mainly gram-negative, which may play a role as producers of vitamins and metal chelators. Zoogloea bacteria have been found closely associated with Anabaena flos-aquae preceeding the peak of a bloom (Caldwell & Caldwell, 1978), whereas members of the Enterobacteriaceae have been shown to depress toxin production by Anabaena flos-aquae (Carmichael & Gorham, 1977). All 3 algal species occur ubiquitously (Kondrateva & Kovalenko, 1975) and toxic blooms of these algae have been reported from many countries.

The topic has recently been reviewed by Goryunova & Demina (1974), Kirpenko et al. (1977), Gorham & Carmichael (1980), and Carmichael (1981).

6.2.1 Sources, properties, analytical methods, and exposure

Microcystis aeruginosa toxin

Several toxic preparations have been isolated from M. aeruginosa, which contain peptides, carbohydrates, and other compounds, and have relative molecular masses ranging from 1300 to 19 400. Two compounds of low relative molecular mass have recently been isolated, one a peptide with hepatotoxic properties, the other causing respiratory arrest in mice (Carmichael, 1981).

Anabaena flos-aquae toxin

A toxin, anatoxin-a, which when administered to mice orally or intraperitoneally caused acute effects including signs of paralysis and death, was isolated from Anabaena

flos-aquae and chemically characterized as an alkaloid (Fig. 8), with a relative molecular mass of 165 (Devlin et al., 1977). A method of analysis for anatoxin-a has been developed, involving high performance liquid chromatography, with 90% recovery in the concentration range 1 - 500 mg/kg, and a limit of detection of 0.1 mg/kg (Astrachan & Archer, 1981). Furthermore, three toxic preparations, namely anatoxin b, c, and d, have also been isolated (Carmichael & Gorham, 1977). Anatoxin-a acts as a potent nicotinic agonist paralysing peripheral muscles by a depolarizing neuromuscular blockade (Carmichael, 1981).

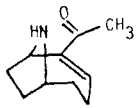


Fig. 8. Structure of anatoxin-a.

Aphanizomenon flos-aquae toxins

Purification of a toxic factor from Aphanizomenon flos-aquae, possessing electrophysiological properties similar to those of saxitoxin, resulted in several closely interrelated fractions, which were similar to saxitoxin on the basis of chromatography and infrared spectroscopy (Jackim & Gentile, 1968). Recently, two toxic compounds, identical to saxitoxin and neosaxitoxin on the basis of paper electrophoretic and thin layer chromatographic properties, were isolated from strains of A. flos-aquae (Ikawa et al., 1982).

No studies are available on the possibility of the passing of freshwater cyanophyte toxins into the human food chain or of their bioconcentration by predators similar to dinoflagellate toxins. The possibility of human exposure to cyanophyte toxins through recreational and municipal water supplies has been considered in association with cyanophyte blooms (Carmichael, 1981; section 6.2.3), however, no reports are available on the chemical identificaton and quantification of cyanophyte toxins in recreational and municipal water supplies.

6.2.2 Effects on animals

Microcystis aeruginosa cells and toxins

Field cases of Microcystis intoxication in farm animals, particularly in cattle, have been reported, as a result of

drinking water from lakes containing blooms of M. aeruginosa (Hammer, 1968; Skulberg, 1979). The cases were acute, characterized by haemorrhages, photosensitization, and liver damage including necrosis of hepatocytes and moderate proliferation of bile duct epithelia. Liver damage characterized by panlobular hepatocytic necrosis, superimposed haemorrhage, connective tissue proliferation, and pleomorphic hepatocytes developed in laboratory studies on vervet monkeys given lyophilised M. aeruginosa cells orally for 6 - 7 months (Tustin et al., 1973). Gonadotoxic and embryotoxic effects, as well as mutagenic effects in the bone marrow, were reported in rats orally administered an extract of M. aeruginosa collected from a bloom (Kirpenko et al., 1981).

Anabaena flos-aquae cells and toxins

Field cases of sudden death in cattle have been reported, associated with drinking water from lakes containing blooms of Anabaena flos-aquae (Hammer, 1968; Carmichael et al., 1977). Death occurred within a few hours of ingestion of a lethal bolus, and the signs observed were characteristic of respiratory failure. Toxic Anabaena flos-aquae cells administered orally to calves, rats, ducks, and goldfish caused death as a result of respiratory arrest (Carmichael et al., 1975). Using a toxic extract from the cells, it was concluded that the main effect was the production of a sustained post-synaptic depolarizing neuromuscular blockade. No adverse effects on food consumption, growth, blood cells, serum enzymes, hepatic mixed function oxidase and morphological changes, were observed, when anatoxin-a was administered to female Sprague-Dawley rats (20 animals per group) in the drinking-water at 5.1 and 0.5 mg/litre for 7 weeks, or when anatoxin-a was injected intraperitoneally into 18 female rats (0.016 mg/rat) for 21 days. Female golden hamsters (5 - 6 animals per injected group, 7 - 9 animals per control group) were injected intraperitoneally with anatoxin-a (0.125 and 0.2 mg/kg body weight) 3 times per day on day 8 - 11, or on day 8 - 14, or on day 12 - 14 of the gestation period. Decreased fetal weights were observed in most groups compared with the controls, and hydrocephaly was observed in one litter from the group administered 0.125 mg anatoxin-a/kg body weight on day 12 - 14 (Astrachan et al., 1980).

Aphanizomenon flos-aquae cells and toxins

The following lethal doses were observed after peritoneal injection of a toxic extract from Aphanizomenon flos-aquae cells: in killifish (Fundulus heteroclitus), 0.5 mg/kg body weight; in sheepshead minnows (Cyprinodon variegatus), 0.5

mg/kg; and in mice, 8 mg/kg (Gentile & Maloney, 1969). Copepods, ostracods, and cladocerans were unaffected by toxin concentrations of 2 g/litre in the water environment.

6.2.3 Episodes of adverse effects reported in association with human exposure to toxic cyanophytes

During blooms of cyanophytes (Microcystis sp., Anabaena sp. and Aphanizomenon flos-aquae) in Canadian lakes in June-July 1959, many cases of acute death in domestic animals (dogs, cattle, horses) were encountered associated with the drinking of lake water (Dillenberg & Dehnel, 1960). In addition, 12 people became ill after swimming in the lakes, with headache, nausea, and gastrointestinal upsets. In the vomitus and stools of one of the patients, cyanophyte cells (Microcystis sp., Anabaena circinalis) were identified; no other microbial causative agents were found in this patient.

In August 1975, a water-borne outbreak of gastrointestinal disease occurred in Sewickley in Pennsylvania, USA, affecting 62% of the population (size 8000). Bacterial agents could be excluded, and it was assumed that blue-green algae present in the uncovered drinking-water reservoir were the cause, although a viral etiology could not be completely excluded. The water contained more than 100 000 blue-green algae cells per ml, dominated by Schizothrix calcicola and Lyngbya spp; no test for algal toxicity was conducted (Lippy & Erb, 1976). Elevation of gamma-glutamyl transpeptidase and of alanine aminotransferase, indicating toxic liver injury, as measured in a community in Australia, was found to be associated with blooms of toxic M. aeruginosa in the drinking-water reservoir. No such enzyme changes were measured in an adjacent control population (Falconer et al., 1983).

7. EVALUATION OF HEALTH RISKS OF EXPOSURE TO AQUATIC BIOTOXINS

In view of the character of the problem, the health risk evaluation is presented together with the summary at the beginning of the document.

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