



7. Ciguatera Fish Poisoning (CFP)

Ciguatera fish poisoning (CFP) has been known for centuries. It was reported in the West Indies by Peter Martyr de Anghera in 1511, in the islands of Indian Ocean by Harmansen in 1601 and in the various archipelagos of the Pacific Ocean by De Quiros in 1606. Endemic areas are mainly the tropical and subtropical Pacific and Indian Ocean insular regions and the tropical Caribbean, but continental reef areas are also affected (Legrand, 1998). The name ciguatera was given by Don Antonio Parra in Cuba in 1787 to intoxication following ingestion of the “*cigua*”, the Spanish trivial name of an univalve mollusc, *Turbo pica*, reputed to cause indigestion. The term “*cigua*” was somehow transferred to an intoxication caused by the ingestion of coral reef fishes (De Fouw *et al.*, 2001). The causative toxins, the ciguatoxins, accumulate through the food chain, from small herbivorous fish grazing on the coral reefs into organs of bigger carnivorous fish that feed on them (Angibaud and Rambaud, 1998; Lehane, 2000).

In the past, the ciguatera food poisoning in humans was highly localized to coastal, often island communities of indigenous peoples. However, with the increases in seafood trade, increased worldwide seafood consumption and international tourism, the target populations have become international. At present, ciguatera is the most common type of marine food poisoning worldwide and, with an estimated 10 000 to 50 000 people worldwide suffering from the disease annually, it constitutes a global health problem (De Fouw *et al.*, 2001; Lehane, 2000).

No indicator such as the highly visible surface phenomenon, the so-called “red tide” as seen by shellfish poisonings, has ever been associated with ciguatera. It is this lack of warning signal that has contributed to the dread of ciguatera poisoning (De Fouw *et al.*, 2001).

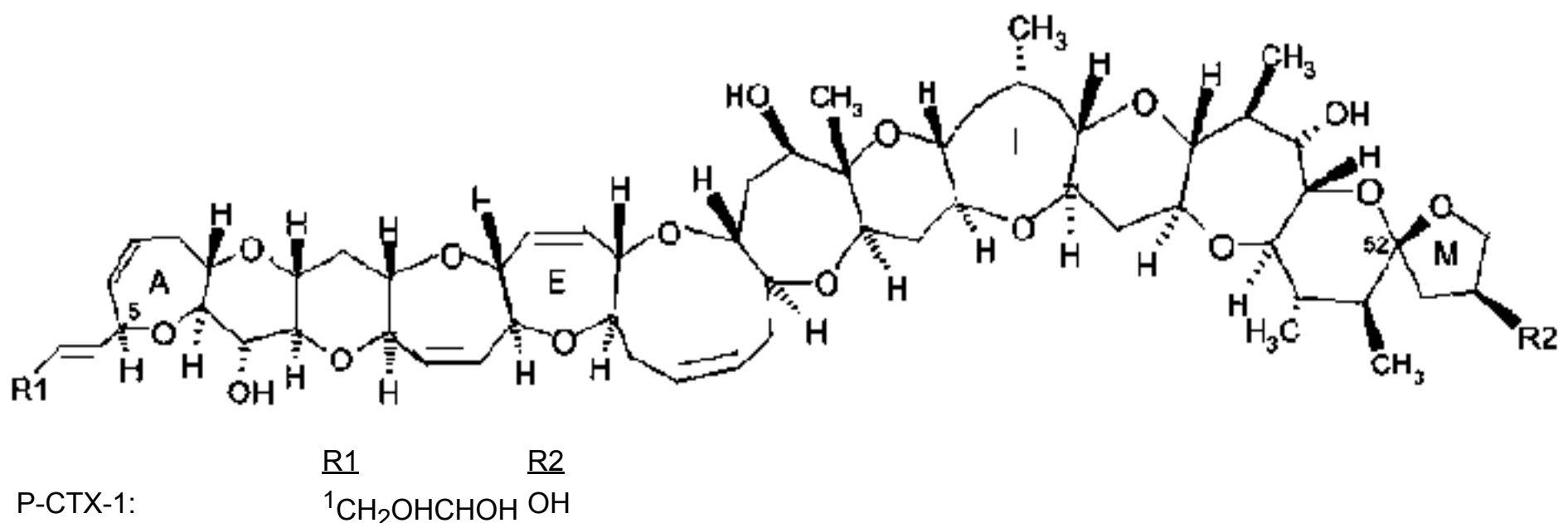
7.1 Chemical structures and properties of ciguatoxins

Ciguatoxins are lipid-soluble polyether compounds consisting of 13 to 14 rings fused by ether linkages into a most rigid ladder-like structure (see Figure 7.1). They are relatively heat-stable molecules that remain toxic after cooking and exposure to mild acidic and basic conditions. Ciguatoxins arise from biotransformation in the fish of precursor gambiertoxins (Lehane and Lewis, 2000; Lehane, 2000).

In areas in the Pacific, the principal and most potent ciguatoxin is Pacific ciguatoxin-1 (P-CTX-1, mol. wt. 1112). Its likely precursor is gambiertoxin-4B (GTX-4B). The main ciguatoxins in the Pacific, P-CTX-1, P-CTX-2 and P-CTX-3, are present in fish in different relative amounts (Lehane and Lewis, 2000; Lehane, 2000). The structures of more than 20 congeners of ciguatoxin were elucidated. Structural modifications were mainly seen in the both termini of the toxin molecules and mostly by oxidation (Naoki *et al.*, 2001; Yasumoto *et al.*, 2000). Caribbean (and Indian Ocean) ciguatoxins differ from Pacific ciguatoxins. Caribbean CTX-1 (C-CTX-1) is less polar than P-CTX-1. Structures of two Caribbean ciguatoxins (C-CTX-1 and C-CTX-2) were elucidated in 1998. Multiple forms of ciguatoxin with minor molecular differences and pathogenicity were described. CTX-1 is the major toxin found in carnivorous fish and poses a human health risk at levels above 0.1 µg/kg fish (de Fouw *et al.*, 2001).

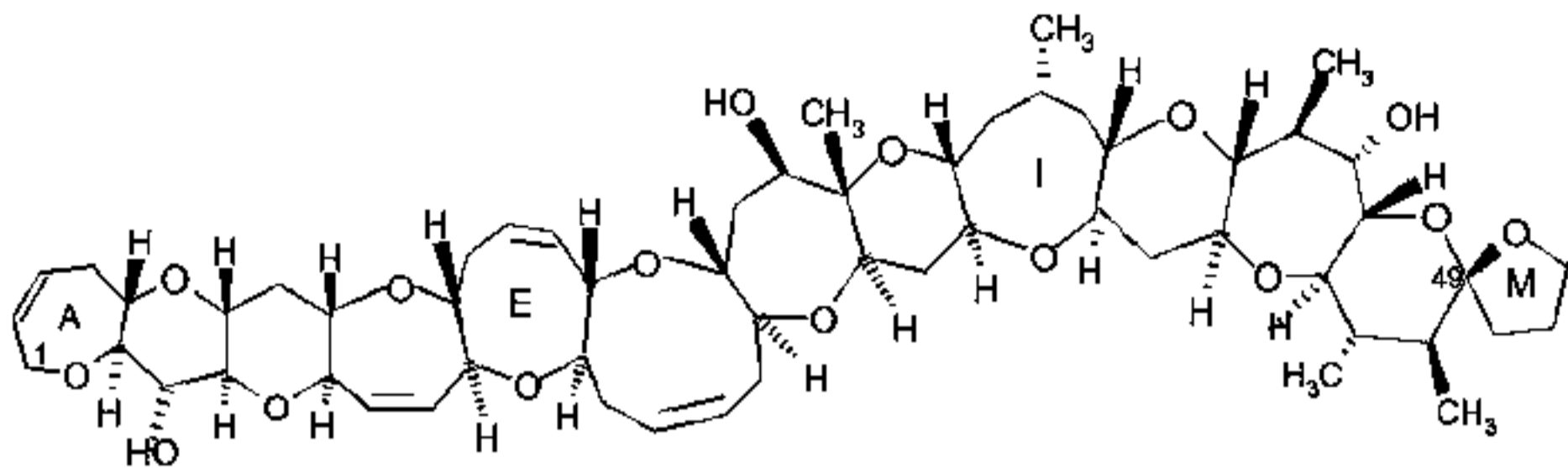
Various species of parrotfish have previously been reported to contain a toxin less polar than CTX-1, named scaritoxin. Judging from the reported chromatographic properties, scaritoxin seems to correspond to a mixture of CTX-4A and CTX-4B (De Fouw *et al.*, 2001).

Figure 7.1 Structure of Pacific (P) and Caribbean (C) ciguatoxins (CTXs)

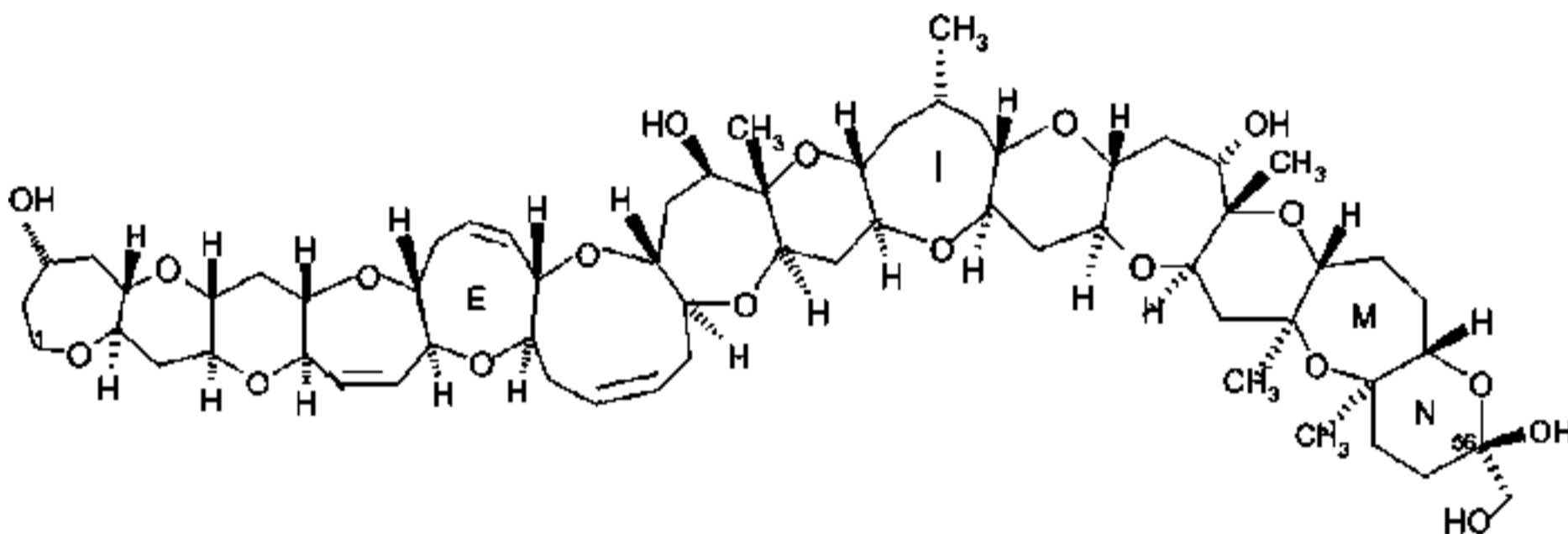


P-CTX-3 (P-CTX-2): $^1\text{CH}_2\text{OHCHOH H}$

P-CTX-4B (P-CTX-4A); $^1\text{CH}_2\text{CH H}$



P-CTX-3C



C-CTX-1 (C-CTX-2)

The energetically less favoured epimers, P-CTX-2 (52-epi P-CTX-3), P-CTX-4A (52-epi P-CTX-4B) and C-CTX-2 (56-epi C-CTX-1) are indicated in parenthesis. 2,3-Dihydroxy P-CTX-3C and 51-hydroxy P-CTX-3C have also been isolated from Pacific fish (Lewis, 2001).

7.2 Methods of analysis

7.2.1 In general

Ciguatoxins are odourless, tasteless and generally undetectable by any simple test. Therefore, bioassays have traditionally been used to monitor suspected fish. Many native tests for toxicity of fish have been examined including the discolouration of silver coins or copper wire, or the repulsion of flies and ants, but all of these were rejected as invalid (Park, 1994).

Feeding tests to cat or mongoose are simple and relatively sensitive but they are cumbersome and non-quantitative. The mouse bioassay requires purification of fish extracts since the mouse is not very sensitive to ciguatoxin. The alternative mosquito bioassay correlates well with cat and mouse bioassay. Other bioassays that have been developed have used chicken, brine shrimp and guinea pig atrium. All traditional bioassays have one common disadvantage, the lack of specificity for individual toxins. Recent studies have also focused on the development of chemical methods, such as TLC and LC for the detection and quantification of ciguatera-related toxins. Alternative assays based on immunochemical technology have been developed and show greatest promise for use in seafood safety monitoring programmes (Park, 1994).

7.2.2 Bioassays

All the mentioned bioassays have the limited chemical specificity for individual toxins in common (Jurancic and Park, 1991), although for a broad screening this property can be advantageous detecting a poisoning. The bioassays are semi-quantitative and sensitive. Ciguatoxin induces characteristic signs of toxicity but the use of some animal species can be problematic in terms of cost and ethical difficulties.

in vivo assays

mouse bioassay

The mouse bioassay, based on the method described by Banner *et al.* (1960) is presently the most widely used assay for the detection of ciguatoxins in fish. The method consists of injecting i.p. (intraperitoneal) serially diluted semi-purified or crude toxic extracts into mice and observing the symptoms for 24 hours. The procedure of the assay is described in detail by Yasumoto *et al.* (1984b). This assay has been described for the detection of ciguatoxins in up to 20 mg of ether extract from the flesh of fish. The diethyl ether fraction containing ciguatoxin is suspended in 0.5 ml 1-5% Tween 60/0.9% saline solution and injected intraperitoneally into mice (20 ± 2 g) of either sex. Mice are observed continuously for the first two hours, after that regularly checks are performed. Two mice are tested for each fraction. Mice are housed at 23 ± 2 °C and observed over seven days and signs and times to death recorded. Rectal body temperature is intermittently measured. The relationship between dose and time to death is used to quantify each fraction. Total lethality is expressed in mouse units (MU). For the mix of ciguatoxins found in carnivorous fish (Lewis and Sellin, 1992; Lewis *et al.* 1991) this relationship is approximated by $\log \text{MU} = 2.3 \log (1 + T^{-1})$, where MU is the number of mouse units of ciguatoxin injected and T is time to death in hours (see also Table 7.2). One MU is the LD₅₀ dose for a 20 g mouse which is equivalent to 5 ng, 48 ng and 18 ng of CTX-1, CTX-2 and CTX-3, respectively. (Lewis and Sellin, 1992; Lewis *et al.* 1991). It is recommended that additional purification is undertaken to separate the various toxins, especially the maitotoxins (see Chapter 7.3.1) from ciguatoxins since maitotoxins induce effects in mice, often mistaken for effects of ciguatoxins despite the clear differences (see Table 7.2). Therefore modified extraction procedures have been reported that may improve separation of these two types of toxins (Yokayama *et al.*, 1988; Holmes *et al.*, 1991; Holmes and Lewis, 1994; Legrand *et al.*, 1992).

The mouse assay has been traditionally used but it is unsuitable as a market test. There are other disadvantages such as the variation in mouse weight, that must be limited involving a large breeding colony of the mice, and the death time relationship to dose is non-linear.

chicken assay

This assay provides a rapid means of assaying the toxicity of fish liver by administering small portions of liver directly into the crop of young chickens at 10 percent of their body weight. Administration of fish flesh is physically more difficult but can be accomplished (Vernoux *et al.*, 1985).

mongoose and cat assay

For the mongoose (Banner *et al.*, 1960) and cat assay (Lewis, 1987; Bagnis *et al.*, 1985) the same procedure is followed as with chicken, only flesh of fish is fed and also in large quantity (5 to 15 percent of the test animal weight was fed). The cat is less satisfactory as test model because it often regurgitates part of the test meal. Test animals are observed for 48 hours. Although the tests are simple in screening fish for toxicity, they are cumbersome and not quantitative (Bagnis *et al.*, 1987).

brine shrimp assay

The brine shrimp assay was the first non-vertebrate assay developed. However, false positive results were caused by the toxic effects on brine shrimp of the Tween 80 recommended to emulsify the extract and no toxic effect attributable to ciguatoxin could be detected (Granade *et al.*, 1976; Hungerford, 1993).

mosquito assay

A bioassay using mosquitoes has also been developed. Only a few laboratories perform this assay, perhaps because of difficulties in obtaining and housing mosquitoes and a lack of familiarity in handling and recognising signs characteristic of intoxication by ciguatoxins. This procedure involves intrathoracic injection of the mosquitoes of serially diluted fish extract, and the toxicity is expressed in mosquito LD₅₀. It is a rapid assay, depending on a simple extraction requiring a small amount of fish. However, the assay is non-specific and non-quantitative (Bagnis *et al.*, 1985, 1987).

diptera larvae assay

The diptera larvae assay could replace the mouse bioassay in the absence of alternative *in vitro* tests. However, the assay is not validated yet (Labrousse *et al.*, 1992). In this assay the diptera larva (*Parasarcophaga argyrostoma*) is used to detect ciguatoxin in fish flesh. These larvae are selected for their simple breeding and easy handling, their ability to consume spontaneously large quantities of fresh meat, and their very high sensitivity to ciguatoxin. For the growth test the larvae were fed about 5 g of the test sample. Larvae grown overnight on meat can easily be seen with the naked eye. After 24 hours, the larvae are weighted. Weight loss or a smaller increase of weight compared to healthy samples indicates the degree of toxicity of the sample. The limit of detection for ciguatoxin expressed as CTX-1 was determined either by weighing the larvae or examination with the naked eye, and fluctuated around 0.15 ng/g flesh. Samples containing more than 1 ng of CTX/g flesh (moray eel) killed the larvae in three hours, samples with lower concentrations inhibited larval growth. The reading with the naked eye seems to be satisfactory down to 0.2 ng of CTX/g, while that by weighing, a more objective method, was

acceptable down to 0.10 to 0.15 ng CTX/g. The test is very sensitive, simple and inexpensive, but it would be useful to establish a standard growth curve. Another element to improve the test is the response time. The response is acceptable for toxic fish, but more time is needed for low-toxicity samples (comparable to the response in the mouse bioassay) (Labrousse and Matile, 1996).

in vitro assays

sodium channel binding assays for ciguatoxins

Ciguatoxins bind to sodium channels causing them to open at normal cell resting membrane potentials. This results in an influx of sodium ions, cell depolarization and the appearance of spontaneous action potentials in excitable cells. This sodium influx can be enhanced by the addition of sodium channel activator toxins through an allosteric mechanism. The reported cell based assay for the ciguatoxins (Manger *et al.*, 1993, 1994, 1995) takes advantage of this phenomenon to produce an assay that is highly sensitive to ciguatoxins and other sodium channel activator toxins. This assay is 10 000 times more sensitive than the mouse assay for ciguatoxins.

An assay for ciguateric fish based on the ability of ciguatoxins to selectively inhibit the binding of $^3\text{[H]}$ -brevetoxin to sodium channels in rat brain synaptosomes was reported by Legrand and Lotte (1994).

Both in vitro sodium channel assays mentioned are more sensitive than the mouse bioassay and have considerable potential to replace this assay for the detection of ciguatoxins in crude fish extracts. However, in their current format, these assays are unlikely to be cost-effective for routine screening of individual fish.

alternative bioassays

Several assays have been developed such as the guinea pig ileum assay (Dickey *et al.*, 1982), the guinea pig atrium assay (Lewis, 1988; Lewis and Endean, 1986), the isolated frog nerve fibre assay (Benoit *et al.*, 1986), and assays with human and mouse hemolytic blood cells (Escalona De Motta *et al.*, 1986), and the bioassay that measures the mouse body temperature depression following intraperitoneal injections of toxic fish extracts (Gamboa *et al.*, 1990; Sawyer *et al.*, 1984). With the guinea pig atrium assay, the tissue extract is used to bath the atrium after removal from the guinea pig. Observations are made then for the characteristic inotropic effects indicative of ciguatoxin (DeFusco *et al.*, 1993).

7.2.3 Biochemical assays

immunoassays

An ideal assay for the detection of marine toxins should be simple, highly sensitive and specific. Therefore the evaluation of marine toxin detection assays has moved in the direction of immunologic analysis (Hokama and Smith, 1990). Immunochemical methods such as a radioimmunoassay (RIA) (Hokama *et al.*, 1977), a competitive enzyme immunoassay (EIA) (Hokama *et al.*, 1983, 1984, 1986), and a rapid enzyme immunoassay stick test (Hokama, 1985; Hokama *et al.*, 1985, 1987) have been developed. Problems with these immunochemical methods are their cross-reactivity with other polyether compounds and the limited antibody supply.

The presence of another family of ciguatoxins in the Caribbean region has important implications for the detection of ciguateric fish. Antibody detection methods, which are being developed based on antibodies raised against P-CTX-1 or P-CTX-1 fragments, may not be suitable for detecting Caribbean ciguatoxins (Vernoux and Lewis, 1997).

radioimmunoassay

In 1977, a radioimmunoassay (RIA) was developed for the detection of ciguatoxin directly in contaminated fish (Hokama *et al.*, 1977). In this assay, CTX conjugated to human serum albumin was injected into sheep and rabbits, thereby producing antibodies. The sheep antibody to CTX was used in the RIA after being purified and coupled to ^{125}I as a label. In practice, some false positives were reported. This method could not be used for analyses of large numbers of fishes.

enzyme-linked immunosorbent assay (ELISA)

The practicality of detection improved when Hokama *et al.* (1983) developed an enzyme immunoassay (EIA) for the detection of CTX. The procedure incorporated a sheep anti-ciguatoxin horseradish peroxidase conjugate and colorimetric determination of absorbance following the enzymatic reaction. The assay was shown to be similar in efficacy to the earlier RIA developed, but less expensive and more practical. However, it was still tedious and therefore abandoned as detection method.

stick tests

The speed of detection improved when Hokama (1985) further simplified the enzymatic procedure by

incorporating correction-fluid coated skewered bamboo sticks as test tools which meant that fish tissue need only be poked with the bamboo stick and the stick with the adherent tissue fluid mixed with reagents. This method proved to be successful in separating toxic from non-toxic fish. However, six tests per fish appeared necessary for accurate determination of ciguateric fish that were tested close to the borderline level.

The final goal, a rapid visual colour test, was achieved by coating a bamboo stick that had been inserted into fish flesh with sheep anti-ciguatoxin coupled to horseradish peroxidase. After a ten minute incubation the colour of the stick is evaluated visually, ranging from colourless (non-toxic) to intense bluish purple (highly toxic) (Hokama *et al.*, 1987).

Later, a rapid (within 15 minutes) stick-enzyme immunoassay using horseradish peroxidase-labelled sheep anti-ciguatera toxin antibody has been developed by Hawaii Chemtect International (Ciguatetect®) for detecting ciguatera toxins and toxins associated with diarrhoeic shellfish poisoning. The Ciguatetect® test can only be used as a general screening method to select samples for further analysis because the lack of CTX standards has hampered the determination of relative cross reactivity with various derivatives. The rate of false positive responses has not yet been determined (Park, 1995). The Ciguatetect® test was planned to be studied in a formal AOAC International Collaborative Study. To date, the study has not yet been carried out because the antibodies used were not monoclonal, which questioned the long-term availability and quality necessary for this type of methodology development and validation. The study coordinators are developing new hybridoma cell lines for the production of anti-ciguatoxin monoclonal antibodies (Quilliam, 1998a, 1999).

immunoassays based on monoclonal antibodies

Early studies all employed a polyclonal antibody raised to ciguatoxin in sheep. A disadvantage of such an approach is that for long-term antibody production a continuous supply of antigen is required for booster injections. Monoclonal antibodies on the other hand can provide a continuous supply of a selected antibody. Hokama *et al.* (1985, 1989a) and Hokama (1990) reported production of monoclonal antibodies to a related polyether toxin, as well as to ciguatoxin (likely CTX-1).

Speed, practicality and specificity were all combined when the technology of monoclonal antibodies was incorporated into the stick test procedure (Hokama *et al.*, 1989b). With this assay, CTX was conjugated to human serum albumin with carbonimide, and BALB/c mice were injected with the conjugate. The non-immunoglobulin synthesising mouse myeloma cells used for fusion were those designated PBX63-Ag8·65B as used in other studies (Hokama *et al.*, 1989a). The stick enzyme immunoassay than remains essentially the same as the original design (Hokama *et al.*, 1987), except that the horseradish peroxidase was now conjugated to the anti-CTX monoclonal antibody (MAB-CTX). This method has been used extensively for surveys and for clinical confirmation.

solid-phase immunobead assay

In 1990, a solid-phase immunobead assay (SPIA), with coloured polystyrene particles coated with MAB-CTX began to be used for direct detection of CTX adsorbed on bamboo paddles coated with organic correction fluid (Hokama, 1990; Hokama *et al.*, 1993). The membrane immunobead assay (MIA) presented by Hokama *et al.* (1998) is based on the immunological principles used to develop the SPIA. It uses a monoclonal antibody prepared against purified moray eel (MAB-CTX) coated onto coloured polystyrene beads. The polyether toxins extracted from a piece of fish tissue bind to the hydrophobic polyvinylidene membrane on a plastic support (membrane stick) and can be detected with the MAB-CTX coated onto the coloured polystyrene beads. The intensity of the colour on the membrane portion of the membrane stick is related to the concentration of CTX in the methanolic extracts. Overall, the MIA showed a reasonable limit of detection for CTX (approx. 0.032 ng CTX/g tissue). During development of the MIA, several factors critical to obtaining accurate and repeatable results were noted: i) the membrane portion of the membrane stick must not be touched, because touching may cause false-positive reactions; ii) the membrane stick must be soaked in the methanol/fish sample suspension for at least 20 minutes for optimal results; iii) the stick and the test tube must be completely dry before the latex immunobead suspension is added to the test tube; and iv) the membrane stick should not be soaked in the latex immunobead suspension for more than 10 minutes. The method of Hokama *et al.* (1998) was subjected to a semi-quantitative collaborative study of AOAC International in 1999 (Hokama and Ebesu, 2000). The study collaborators received dried fish samples, non-spiked or spiked with standard extract containing CTX. The study is still in the evaluation process with AOAC's Methods Committee on Natural Toxins, but a first assessment of the results has shown a sensitivity (defined as percent of truly (known) positive samples that are found by the method to be positive) and a specificity (defined as percent of truly (known) negative samples that are found by the method to be negative) of 91 percent and 87 percent respectively.

7.2.4 Chemical assays

chromatographic detection

Ciguatoxins do not possess a useful chromophore for selective spectroscopic detection but contain a

relatively reactive primary hydroxyl group through which (after appropriate clean-up) a label could be attached prior to detection. High performance liquid chromatography (LC) coupled to fluorescence detection provides a highly sensitive method that has the potential to detect natural levels of ciguatoxins in crude extracts from fish flesh. Dickey *et al.* (1992b) and Yasumoto *et al.* (1993) have reported encouraging results by labelling ciguatoxin with novel coumarin-based fluorescent reagents or the fluorescent 1-anthroylnitrile, respectively, prior to LC separation and fluorescence detection. LC coupled to selective-ion monitoring ionspray mass spectrometry (MS) is an alternative to fluorescence detection of ciguatoxin in LC eluants. This approach has shown considerable potential for the detection of labelled diarrhoeic shellfish toxins (Pleasant *et al.*, 1992b). Preliminary studies with CTX-1 indicate that such an approach could form the basis of a confirmatory analytical assay for ciguatoxins in fish (Lewis *et al.*, 1994).

nuclear magnetic resonance (NMR)/mass spectrometry (MS)

NMR and/or MS techniques have been used to characterize ciguatoxins present in fish viscera (Murata *et al.*, 1990; Lewis *et al.*, 1991) and flesh (Lewis and Sellin, 1992) and to characterize gambiertoxins in wild and cultured *G. toxicus* extracts (Murata *et al.*, 1990; Satake *et al.*, 1993).

Present analytical methods used to characterize ciguatoxins (NMR and MS) require large-scale extraction of ciguatoxins present in low concentrations in highly toxic fish and in most instances the characterization of ciguatoxins present at levels below 0.1 nmol/kg has not been possible. Lewis and Jones (1997) described gradient reverse-phase liquid chromatography/mass spectrometry (LC/MS) methods to identify the ciguatoxins accumulated by fish. The analysis was performed on 5 mg samples of partially purified highly toxic moray eels from the Pacific Ocean. P-CTX-1, the major toxin in the flesh and viscera of carnivorous ciguateric fish of the Pacific, was used as the reference ciguatoxin in this study. The method appears to be more sensitive and selective than the mouse bioassay, identifying 11 new P-CTX congeners in an enriched fraction from the viscera of moray eels. The potency and origin of these congeners remain to be established.

mass spectrometry

A state-of-the-art LC-ESI-MS/MS (ESI= ElectroSpray Ionisation) application paper with very practical notes on the detection and determination of ciguatoxins was reported by Lewis *et al.* (1999). Levels equivalent to 40 ng/kg P-CTX-1, and 100 ng/kg C-CTX-1, in fish flesh could be detected. Several real-life samples were analysed.

capillary zone electrophoresis

A method applying capillary zone electrophoresis (CZE) with UV detection was developed to detect maitotoxin (MTX) (see Chapter 7.3.1), a toxin associated with ciguatera fish poisoning (Bouaïcha *et al.*, 1997b). The authors demonstrated the applicability of CZE in the rapid and high-resolution separation of MTX in a solution of a commercial standard (which was not pure). They reported that an amount as low as 50 pg was visible in the electropherogram, by UV absorption at 195 nm. They concluded that CZE is a promising alternative compared to existing techniques such as LC/MS, to the determination of MTX in food, although solid-phase extraction would be a necessary technique for the extraction of the toxin from fish, as it is normally present in ng/kg amounts in ciguateric fish.

7.3 Source organism(s), habitat and distribution

7.3.1 Source organism(s)

Gambierdiscus toxicus is the source of two types of marine toxins, i.e. the water-soluble maitotoxins (MTXs) and the fat-soluble ciguatoxins. MTXs are produced by all strains of *G. toxicus* examined to date, with each strain apparently producing only one type of MTX. MTXs are principally found in the gut of herbivorous fishes and have no proven role in CFP. On the other hand, ciguatoxins are produced only by certain strains of *G. toxicus*, are found in the liver, muscles, skin and bones of large carnivorous fishes, and are regarded as the principal cause of CFP in humans (Chinain *et al.*, 1999; Lehane and Lewis, 2000).

The dinoflagellate *Gambierdiscus toxicus* was identified in the late 1970s near the Gambier Islands. This dinoflagellate lives in epiphytic association with bushy red, brown and green seaweeds and also occurs free in sediments and coral rubble (Hallegraeff *et al.*, 1995). The dead coral and marine algae thriving in tropical and subtropical reef systems are eaten by herbivorous fish; these fish accumulate and concentrate the toxins produced by the dinoflagellate. The herbivorous fish are eaten by larger carnivorous fish. During the passing through the food chain there is an oxidative biotransformation of the less oxidized gambiertoxins to the more oxidized and more toxic ciguatoxins (Durborow, 1999; Lehane and Lewis, 2000). In the stomach of herbivorous fish, incomplete biotransformation of gambiertoxins to ciguatoxins could be seen. After accumulation in herbivores the toxins are transferred to carnivorous fish. Carnivorous fish have been shown to contain ciguatoxins and no gambiertoxins, indicating that any remaining gambiertoxins present in the herbivorous prey is completely biotransformed in the carnivorous fish (Burgess and Shaw, 2001). In the Puerto Rico area, the benthic dinoflagellate *Ostreopsis lenticularis*

was shown to be a vector of CFP (Tosteson *et al.*, 1998). In the literature, other dinoflagellates were also mentioned, which may play a role in the production of toxins associated with ciguatera poisoning such as *Prorocentrum concavum*, *P. mexicanum*, *P. rhathytum*, *Gymnodinium sangienseum* and *Gonyaulax polyedra* (Aseada, 2001).

The Caribbean (C-CTXs) and Pacific toxins (P-CTXs) possess closely related structures but are chromatographically distinguishable from each other, indicating that the ciguatoxins from the Caribbean Sea are members of another family of ciguatoxins. The presence of different families of toxins may underlie the differences in ciguatera symptoms found between the Pacific and Caribbean region. It is likely that the Caribbean ciguatoxins arise from a small number of precursor toxins, similar to ciguatera in the Pacific where one gambiertoxin (GTX-4A) can give rise to at least four ciguatoxins which accumulate in fish. Probably different strains of *G. toxicus* are able to produce different arrays of polyether toxins and a Caribbean strain of *G. toxicus* is suggested to be a source of C-CTX-1 and -2 (De Fouw *et al.*, 2001).

7.3.2 Predisposing conditions for growth

G. toxicus is slowly growing and distributed circumtropically between 32° N and 32° S. It appears to be most prolific in the shallower waters away from terrestrial influences, with most ciguateric endemic areas being characterized by oceanic salinity waters (De Fouw *et al.*, 2001). Low salinity and high light intensities adversely affected *G. toxicus* growth. Research on *G. toxicus* populations in the Florida Keys showed that *G. toxicus* preferred depths of 1 to 4 m, grew best at 11 percent of full sunlight and that maximum abundance occurred at a water temperature of about 30° C (Lehane and Lewis, 2000). *G. toxicus* is commonly found growing epiphytically on macroalgae colonizing damaged coral reefs, such as *Turbinaria ornata*, *Amphiroa* spp., *Halimeda opuntia* and *Jania* spp. (De Fouw *et al.*, 2001).

Environmental studies suggested that the development of *G. toxicus* increased with insolation (exposure to sunlight), with the presence of silicates and oxides from land lateral soils, and with algal detritus which results in the development of peculiar algal turfs *Turbinaria*, *Jania* and *Amphiroa* species. Population densities of *G. toxicus* are patchy and can increase or decrease rapidly. Such growth patterns presumably underlie the spatial and temporal variability of ciguatera outbreaks. However, little is known of the precise environmental conditions that result in increased gambiertoxin production in nature (De Fouw *et al.*, 2001). In the Puerto Rico area maximum toxicity of the benthic dinoflagellate *Ostreopsis lenticularis* was seen in October to December preceded by several months (August to September) of exposure to sustained elevated sea surface temperatures lasting to an average of 20 days. *Spyraenea barracuda* caught in this area in October to December showed maximum toxicity following 24 days of exposure to elevated sea surface temperatures during the preceding months (August to October). Several factors may account for the correlation between increased sea surface temperatures and ciguatoxicity in fish. Changes of two or three degrees in ambient temperatures would be expected to produce marked responses in respiration and metabolic rates, circulating hormones and predatory activity in a variety of fishes (Tosteson *et al.*, 1998).

From February 1993 to December 1997, *Gambierdiscus* spp. population densities were monitored weekly in the French Polynesian Papara area in relation to temperature and salinity. A total of 58 blooms were recorded of which 65 percent occurred in 1995 and 1996 alone. Seasonality in cell densities was found from February 1993 to May 1995. During this period *Gambierdiscus* spp. populations tended to reach maximum abundance at the beginning and the end of the hot season. In contrast, salinity did not appear to be a determining factor in the seasonal abundance of this dinoflagellate. The noticeable increase in both peak densities and frequency of blooms further noticed in 1995 and 1996 was preceded by unusually high water temperatures in January to April 1994, concomitant with a severe coral-bleaching episode. Toxicity screening revealed that toxin production was maximum from October 1994 through December 1996 and no correlation was found between toxicity of the blooms and their biomass, nor the seasonal pattern of temperatures (Chinain *et al.*, 1999).

Lehane (2000) stated that the presence of *G. toxicus* is unpredictable and its abundance does not necessarily reflect the potential to produce gambiertoxins. Some research indicates that certain bacteria are found symbiotically associated with dinoflagellates and play a role in the elaboration of toxins by the symbiont dinoflagellates. It was suggested that bacteria might produce nutrients that were assimilated by dinoflagellates and were necessary for producing toxins. Another suggestion was the synthesis by bacteria of toxins which are then phagocytosed by dinoflagellates (Lehane, 2000).

Over the last decades, evidence has been accumulating that reef disturbance by military and tourist developments increase the risk of ciguatera by increasing benthic substrate for dinoflagellate growth (Hallegraeff *et al.*, 1995). Although there seems no seasonal variation in the occurrence of ciguatera intoxication, according to some authors the frequency of ciguatoxic barracuda caught, varied seasonally, with peak values (60 to 70 percent toxic fish) in the late winter-early spring (January to May) and autumn (August to November). Minimal frequencies (0 to 10 percent toxic fish) were observed in summer (June and July) and December. The seasonal variations in barracuda ciguatoxicity may reflect variability in the toxicity of their immediate prey, as well as the capacity of their detoxification system (the detoxification mechanism is inhibited by hormones produced in the reproductive cycle, and at reduced temperatures) (De Fouw *et al.*, 2001).

7.3.3 Habitat

G. toxicus is distributed circumtropically between 32° N and 32° S and consequently ciguatera is mostly confined to discrete regions in the Pacific Ocean, western Indian Ocean and the Caribbean Sea (Lewis, 2001).

7.4 Occurrence and accumulation in seafood

7.4.1 Uptake and elimination of CFP toxins in aquatic organisms

The uptake and distribution of ciguatoxins was determined in Caribbean fish caught from 1980 to 1983 on the island of St. Barthelemy (French Caribbean). Extracted lipids from several parts of these fish were analysed by mouse bioassays. The fish species belonged to the families of *Muraenidae*, *Serranidae*, *Scombridae*, *Carangidae*, and *Sphyraedinae*. The ciguatoxin concentration was highest in the viscera, particularly in the liver, spleen, and kidney, and lowest in the bones. The ratios of the toxin concentrations in the liver or viscera to that in the flesh were high and varied with the species suggesting that the toxin is distributed in different ways in different fish. The fact that highly vascularized organs such as liver, spleen, and kidney retained the highest quantity of ciguatoxin per unit weight suggests that blood is involved in the distribution of ciguatoxin to other tissues (De Fouw *et al.*, 2001; Pottier *et al.*, 2001).

Ciguatoxin becomes more concentrated as it moves up the food chain and its level is up to 50 to 100 times more concentrated in the viscera, liver and gonads of affected fish than in other tissues. It is not known why the fish are asymptomatic after toxin ingestion and how affected fish can remain toxic for years (De Fouw *et al.*, 2001).

Toxins in tissues from the herbivorous surgeonfish (*Ctenochaetus striatus*) collected in the Great Barrier Reef were characterized by mouse bioassay and chromatography. The biodebris (on turf algae) on which the fish feeds, were collected and the toxins present were compared with those found in *C. striatus*. It appeared that levels of gambiertoxins entering the fish were typically higher than levels found later in the liver. Consequently, the gambiertoxins and biotransformed products (ciguatoxins) do not appear to be accumulated in a simple, additive manner, suggesting that depuration of ciguatoxins and/or gambiertoxins may be significant in *C. striatus* (De Fouw *et al.*, 2001).

7.4.2 Fish containing ciguatoxins

Many species and many families of reef fishes are involved in ciguatera globally. These include the herbivorous *Acanthuridae* and corallivorous *Scaridae* (parrot fish), which are considered key vectors in the transfer of ciguatoxins to carnivorous fish. Many more species of carnivorous fish cause ciguatera. These include *Muraenidae* (moray eels) and *Lutjanidae* (snappers such as red bass) which are notorious in the Pacific, *Serranidae* (groupers) including coral trout from the Great Barrier Reef, *Epinephelidae*, *Lethrinidae*, *Scombridae* (mackerel), *Carrangidae* (jacks) and *Sphyraenidae* (barracudas). The latter two families are a particular problem in the Caribbean (Crump *et al.*, 1999b; Lewis, 2001). More than 400 species of bony fish have been reported in the literature to have caused ciguatera poisoning. The larger carnivores such as moray eels, snappers, groupers, carrangs, Spanish mackerels, emperors, certain inshore tunas and barracuda are the most toxic (IPCS, 1984).

Along the southwest coast of Puerto Rico, the caught barracuda is involved in ciguatera poisoning. Head, viscera and flesh tissue components of 219 barracudas (528 tissue samples) were screened for their toxicity during the period March 1985 through May 1987. Twenty nine percent of these fish yielded toxic preparations in at least one of their tissue components (De Fouw *et al.*, 2001).

In the continental United States, the grouper, red snapper, jack, and barracuda are the most commonly reported fish species associated with ciguatera poisoning (De Fouw *et al.*, 2001). In Florida, in the majority of cases, the great barracuda has been involved in ciguatera poisonings between 1954 and 1992. Apart from the barracuda, other commonly reported species are snapper, hogfish, jack, and grouper (De Fouw *et al.*, 2001).

In Hawaii, jack, black snapper and surgeonfish are most frequently involved with ciguatera toxin (De Fouw *et al.*, 2001). In the Mascareignes archipelago, 34 fish species have been identified to be involved in ciguatera poisoning. Large predators such as grouper (*Serranidae* 53 percent, *Carangidae* 10 percent, *Lethrinidae* 15 percent) are mostly involved in CFP. Most toxic fish were caught by fishing offshore on coral banks located north of Mauritius (De Fouw *et al.*, 2001).

An incomplete list of fish species associated with ciguatera is presented in Table 7.1. A complete list would be nearly impossible because in some areas hundreds of fish species may be involved in CFP.

CTX-1, CTX-2 and CTX-3 are the major ciguatoxins (determined by LC/MS and mouse bioassay) present in the flesh of ciguateric fish (*Scomberomorus commersoni*, *Plectropomus* spp. and *Pomadasys maculatus*) caught at Australian coasts. Two minor toxins, which may be further oxidized analogues of CTX-1 and CTX-2, were also identified (De Fouw *et al.*, 2001).

Table 7.1 Examples of fish associated with ciguatera

Species	Distribution
Lined surgeonfish (<i>Acanthurus linearis</i>)	Indo-Pacific
Bonefish (<i>Albula vulpes</i>)	Worldwide in warm seas
Gray triggerfish (<i>Balistes carolinensis</i>)	Atlantic, Gulf of Mexico
Gaucereye porgy (<i>Calamus calamus</i>)	Western Atlantic
Horse-eye jack (<i>Caranx latus</i>)	Atlantic
Whitetip shark (<i>Carcharinus longimanus</i>)	Worldwide
Humphead wrasse (<i>Cheilinus undulatus</i>)	Indo-Pacific
Heavybeak parrotfish (<i>Chlorurus gibbus</i>)	Indo-Pacific
Red grouper (<i>Epinephelus morio</i>)	Western-Atlantic
Giant moray (<i>Gymnothorax javanicus</i>)	Indo-Pacific
Hogfish (<i>Lachnolaimus maximus</i>)	Western Atlantic
Northern red snapper (<i>Lutjanus campechanus</i>) Tarpon (<i>Megalops atlanticus</i>)	Western Atlantic, Gulf of Mexico Eastern Atlantic
Narrowhead gray mullet (<i>Mugil capurri</i>)	East Central Atlantic
Yellowtail snapper (<i>Ocyurus chrysurus</i>)	Western Atlantic
Spotted coral grouper (<i>Plectropomus maculatus</i>)	Western Pacific
Blue parrotfish (<i>Sparus coeruleus</i>)	Western Atlantic
Spanish mackerel (<i>Scomberomorus maculatus</i>)	Western Atlantic
Lesser amberjack (<i>Seriola fasciata</i>)	Western Atlantic
Great barracuda (<i>Sphyrnaea barracuda</i>)	Indo-Pacific, Western Atlantic
Chinamanfish (<i>Symphorus nematophorus</i>)	Western Pacific
Swordfish (<i>Xiphias gladius</i>)	Atlantic, Indo-Pacific, Mediterranean

Source: Farstad and Chow, 2001

7.4.3 Other aquatic organisms containing ciguatoxins

Although the vast majority of ciguatera fish poisoning is seen after ingestion of carnivorous fish, other marine species are suspect in human ciguatera intoxication. Notably ciguatoxin was found in the viscera of a turban shell (*Turbo argyrostoma*, a marine snail). This snail has occasionally caused ciguatera-like intoxication in humans (IPCS, 1984).

Invertebrates (small shrimps and crabs) may also be a vector in the transfer of gambiertoxins to carnivorous fish. This suggestion was made based on a study with the often ciguateric blotched javelin fish (*Pomadasy maculatus*) which was found to feed predominantly on small shrimps and crabs in Platypus Bay, Queensland. Only shrimps contained detectable levels of ciguatoxin-like toxins (detected by mouse bioassay). It remains to be established if shrimps are capable of biotransformation of the gambiertoxins to ciguatoxins or if this capacity is exclusive for fish (De Fouw *et al.*, 2001).

In Platypus Bay, inside Fraser Island, Queensland (Australia), *Alpheidae* shrimps appeared to be an important vector transferring ciguatoxins to the small carnivore *Pomadasy maculatus*. *P. maculatus* probably passes these toxins to the large mackerel (*Scomberomorus commersoni*) which is notorious in this region. Given the diversity of prey preferences among the families of carnivores, it seems likely that additional herbivore vectors of ciguatoxins will be identified in the future (Lewis, 2001)

7.5 Toxicity of CFP toxins

7.5.1 Mechanism of action

The mechanism of action of ciguatoxins is related to its direct effect on excitable membranes. Such membranes are critical to the function of nerve and muscle, mainly in their ability to generate and propagate action potentials. Ciguatoxins are characterized by their affinity binding to voltage sensitive sodium channels, causing them to open at normal cell resting membrane potentials. This results in an influx of Na⁺ ions, cell depolarization and the appearance of spontaneous action potentials in excitable cells. As a consequence of the increased Na⁺ permeability, the plasma membrane is unable to maintain the internal environment of cells and volume control. This results in alteration of bioenergetic mechanisms, cell and mitochondrial swelling and bleb formation on cell surfaces. Ciguatoxin acts at the same receptor site (site 5) of the Na⁺ channel as brevetoxin, but the affinity of CTX-1 for voltage-dependent Na⁺ channels was around 30 times higher than that of brevetoxin, while CTX-4B had about the same affinity as brevetoxin. CTX-1 and CTX-4B were shown to competitively inhibit the binding of brevetoxin to the voltage-dependent Na⁺ channel of rat membranes. Ciguatoxin exerted a significant slowing of nerve conduction velocity and prolongation of the absolute refractory and supernormal periods indicating an abnormally prolonged Na⁺ channel opening in nerve membranes (Lehane and Lewis, 2000 and De Fouw *et al.*, 2001).

Cardiovascular effects of ciguatoxins were thought to result from a positive inotropic effect on the

myocardium. When ciguatoxin affects voltage-dependent Na^+ channels causing Na^+ to move intracellularly, normal cellular mechanisms begin to extrude sodium and take up calcium. Calcium is the intracellular trigger for muscle contraction. Although much of the increased calcium is buffered by the sarcoplasmic reticulum, it is likely that locally increased calcium concentrations increase the force of cardiac muscle contraction as is observed at ciguatoxin poisoning.

A similar mechanism of ciguatoxin-induced intracellular transport of calcium occurs in intestinal epithelial cells. The increased concentration of intracellular calcium caused by ciguatoxin acts as a second messenger in the cell, as it disrupts important ion-exchange systems. This results in fluid secretion, which presents itself as diarrhoea (Lehane and Lewis, 2000).

7.5.2 Other toxins mentioned to play a role in ciguatera

Maitotoxins are also produced by *G. toxicus* and are, via the intraperitoneal route, more toxic than ciguatoxin. However, maitotoxins are approximately 100 times less potent by the oral route compared with the intraperitoneal route, whereas the ciguatoxins are equipotent (De Fouw *et al.*, 2001).

While ciguatoxins act on Na^+ channels in nerves and muscles, maitotoxin stimulates the movement of Ca^{2+} ions across biomembranes and is a potent activator of changes in the intracellular Ca^{2+} concentrations of cells from a wide variety of organisms. As a consequence of an influx of Ca^{2+} , maitotoxins can produce several effects: hormone and neurotransmitter secretion; phosphoinositides breakdown and activation of voltage gated Ca^{2+} channels due to membrane depolarization. No specific blocker has been identified for this maitotoxin-induced channel. However, the primary target of MTXs remains still undefined. It is strongly suggested that these toxins have no ionophoretic activity. Among natural products, maitotoxins have the largest molecular weight (3422 Da) compared with any natural product known, besides biopolymers like proteins or polysaccharides. Molecular mechanic studies suggested that rather than being a flat accumulation of linked rings, the molecule might represent a molecular 'wire' (Escobar *et al.*, 1998). Maitotoxins also accumulate in the viscera of herbivorous fish, but obviously are not accumulated at sufficiently high doses in carnivorous fish to cause problems at human consumption. If maitotoxins were involved in CFP, qualitative differences in symptomatology might be expected, given that the pharmacology of maitotoxins is quite different from that of ciguatoxins (Lewis, 2001).

Various species of parrot fish have previously been reported to contain a toxin less polar than CTX-1, named scaritoxin. Judging from the reported chromatographic properties, scaritoxin seems to correspond to a mixture of CTX-4A and CTX-4B. Poisoning with scaritoxin is not well described. The name is derived from the poisonous fish *Scarus gibus*. Poisonings have two phases of symptoms, the first set of symptoms resembling typical ciguatera poisoning, the other, developing five to ten days after onset with failure of equilibration and marked locomotor ataxia (De Fouw *et al.*, 2001).

7.5.3 Pharmacokinetics

Ciguatoxins are fat soluble and absorption from the gut is rapid and substantial, although an early onset of vomiting and diarrhoea may exist in expelling some of the toxins before they are absorbed. Since cleaning ciguateric fish can cause tingling of the hands and eating them can cause altered sensation in the oral cavity and dysphagia, it would appear that ciguatoxins can penetrate the skin and mucous membranes. The related brevetoxins also have this property. Ciguatoxins are carried in the blood bound to human serum albumin and moderate (unspecified) levels of ciguatoxin in serum of a patient were reported 22 weeks after consuming ciguatoxic fish. Ciguatoxins are also transmitted in breast milk and are able to cross the placenta and affect the foetus (Lehane and Lewis, 2000).

Sexual transmission of ciguatera from female to male (penile pain after intercourse) and vice versa (pelvic and abdominal pain after intercourse) has been described (De Fouw *et al.*, 2001).

Dysuria, or painful urination, suggest that ciguatoxins are excreted at least in part and possibly unchanged in urine. However, such excretion could be neither rapid nor complete given the serum levels 22 weeks after poisoning. As ciguatoxins accumulate in the body, they may reactivate clinical symptoms from time to time. If stored in adipose tissue, ciguatoxins are probably not a problem unless the tissue is rapidly broken down for example at rapid weight loss (Lehane and Lewis, 2000). Because of their similar structure, ciguatoxins are supposed to behave in a similar pharmacokinetic manner to brevetoxins. This means that the biliary/faecal route is the major route of elimination for ciguatoxins as was demonstrated for brevetoxins (Lehane and Lewis, 2000).

7.5.4 Toxicity to experimental animals

acute toxicity

To determine the origin of watery secretion and type of diarrhoea seen at ciguatoxin poisoning a study with male mice was carried out. Semi-pure ciguatoxin (85.7 percent) was extracted from the viscera of the moray eel. The CTX amounts are expressed by MU (mouse unit). MU was defined here as the

amount of CTX to kill a mouse (15 g) in 24 hours, and corresponds to 7 ng of pure CTX. This definition deviates from the definition given below and in Table 7.2. To estimate the potency of CTX causing diarrhoea, it was compared with diarrhoea caused by the cholera toxin. CTX was administered by gastric tube and intraperitoneal route at different doses. Diarrhoea and morphological influences on digestive tracts caused by CTX were observed microscopically. The results of the study revealed that:

- Diarrhoea occurred by intraperitoneal treatment but not by *per os* treatment. It is likely that CTX given per oral route was absorbed and metabolised in a slightly different manner from that of intraperitoneal route, and therefore did not cause diarrhoea.
- There was an effective dose range to cause diarrhoea of 0.14 to 1 MU.
- Diarrhoea probably resulted from hypersecretion of mucus in the colon and accelerated excretion at the rectum, so only the lower portion of the intestine was affected.
- Diarrhoea stopped within one hour, the mucus secretion was stimulated even after 24 hours accompanied by an abnormal increase in the number of goblet cells.
- The type of diarrhoea was similar to that seen at cholera toxicosis. The potency of CTX to cause diarrhoea was suggested to be about 1 300 to 8 500 times stronger than that of cholera toxin (De Fouw *et al.*, 2001).

In mice, symptoms are well defined and hypothermia is a characteristic response. However, whether ciguatoxin has direct effects on the central nervous system and what its targets in the brain may be are not known. The action of intraperitoneally administered ciguatoxin (0.5 MU) (1 MU = LD₅₀ dose for a 20 g mouse) isolated from the *G. toxicus* MQ2 Caribbean strain, in ICR female mice was investigated in order to identify discrete central nervous system targets for ciguatoxin. As a marker for neuroexcitability *c-fos* was used. The effect of CTX on *c-fos* mRNA was investigated to establish a time course of action on the brain and its effect on the *c-fos* translation product was examined to identify specific neuronal pathways activated by this toxin. A pronounced decrease in body temperature was seen between 10 and 20 minutes after administration. Ciguatoxin causes a rapid induction of *c-fos* mRNA in the brain that corresponds with the decrease in body temperature. The primary targets of CTX appeared to be the hypothalamus and brain stem. The results indicate that CTX has neuroexcitatory actions on brain stem regions receiving vagal afferents and ascending pathways associated with visceral and thermoregulatory responses (De Fouw *et al.*, 2001).

Table 7.2 (1) Effects of ciguatoxins (CTXs), gambiertoxins (GTXs) and maitotoxins (MTXs) administered intraperitoneally (i.p.) to (18-)20 g mice

Toxin	ip. LD ₅₀ (µg/kg bw)	MU ⁽²⁾ (ng)	Signs of intoxication	Min./max. time to death ⁽³⁾
P-CTX-1	0.25	5	hypothermia below 33 C, piloerection, diarrhoea, lachrymation, hypersalivation, dyspnoea, wobbly upright gait, gasping, terminal convulsions with tail arching, death from respiratory failure	37 min./»24 h
CTX-1B	0.33			mean survival time 10-20 h ⁶
P-CTX-2	2.3	9	as for P-CTX-1, plus progressive hind limb paralysis	53 min./»100 h
CTX-2A2	1.9			mean survival time 10-20 h ⁶
CTX-2A1	3.5			mean survival time 3.5-4.5 h ⁶
P-CTX-3	0.9	18	as for P-CTX-1, plus progressive hind limb paralysis	60 min./»26 h
CTX-3C	2.5			mean survival

				time 10-20 h ⁶
GTX-3C	1.3	26		
CTX-4B	10			mean survival time 3.5-4.5 h ⁶
GTX-4B	4.0	80	as for P-CTX-1, plus hind limb paralysis	
MTX-1 (4)	0.05	1	hypothermia, piloerection, dyspnoea, progressive paralysis from hind extending to fore limbs, mild gasping, mild convulsions preceding death > 30 seconds	72 min./»72 h
MTX-2 (4)	0.08	1.6	as for MTX-1	41 min./»72 h
MTX-3 (4)	»0.1	»2	as for MTX-1	72 min./»72 h
C-CTX-1(5)	3.6			
C-CTX-2(7)	1			

(1) Hallegraef *et al.* (1995).

(2) Mouse unit is 1/50 x LD₅₀ (~1 MU is the LD₅₀ dose for a 20 g mouse) (Lewis and Sellin, 1992)

(3) Minimum time to death estimated; maximum time to death estimated from effects of doses near the LD₅₀ dose (De Fouw *et al.*, 2001).

(4) From *Gambierdiscus toxicus* but are unlikely to accumulate in flesh of fish to levels toxic for humans via the oral route. MTXs can induce slight watery anal secretion, but do not cause diarrhoea.

(5) Fouw *et al.* (2001).

(6) Dechraoui *et al.* (1999).

(7) Lehane and Lewis (2000).

From Table 7.2, it appears that maitotoxins are more lethal to mice after i.p. injection than ciguatoxins. However the maitotoxins are about 100-fold less toxic by the oral route than by i.p. route (Lehane and Lewis, 2000).

Male ICR mice were given ciguatoxin or ciguatoxin-4c (at a dose level of 0.7 mg/kg body weight) by the oral or intraperitoneal route. Ciguatoxin-4c was not specified. Histopathological and ultrastructural changes of various organs and the modifying effects of several antagonists on the membrane permeability of sodium were examined. The heart, medulla of adrenal glands, autonomic nerves and the penis appeared to be the target organs. There were no differences in clinical signs or histopathological changes in mice receiving ciguatoxin or ciguatoxin-4c. Ultrastructural changes in the heart after the administration were characteristic. Marked edema between myofibrils and other organelles was prominent. It is of interest that antagonists to cholinergic and adrenergic autonomic nerves used in this experiment had no effect on cardiac injuries. Therefore, the effect of ciguatoxin on cardiac muscle may be based on its direct activity on cardiac muscles. Despite the severe diarrhoea, there were no morphological changes in the mucosal layer of the small intestine but the autonomic nerve system in muscle layers of the small intestine was sensitive to the toxins. Pre-treatment with atropine prevented the diarrhoea caused by the toxins and therefore it was suggested that the diarrhoea is probably induced by a direct action of these toxins on the autonomic system in the small intestine. No changes were seen in the cortical layer of the adrenal glands but degeneration of the medulla of the adrenal glands was prominent. Erect penises of treated mice were observed even after death. The precise mechanism is unknown, but direct or indirect effects of the toxins on penile cavernous bodies via autonomic endings as well as the formation of thrombi in the cavernous bodies may play a role (De Fouw *et al.*, 2001).

The morphologic response of the mouse heart was examined after repeated (15 days) low dose (0.1 mg/kg body weight) exposures to ciguatoxin or ciguatoxin-4c after oral and intraperitoneal administration. Furthermore the sequential changes of the heart injuries up to 14 months after either repeated low doses or after a single high dose (0.7 mg/kg body weight) was investigated for both exposure routes and for both toxins. A single dose of 0.1 mg/kg body weight caused no discernible morphological changes in hearts of mice, in contrast to repeated administration which resulted in severe morphological changes such as marked swelling of the myocardial and the endothelial lining cells of blood capillaries. The effects seen after repeated exposure are similar to those observed after the administration of one single high dose. The prominent swelling of the endothelial lining cells is likely to cause serious alteration of the permeability, which may result in plasma migration from the degenerated endothelial lining cells into the interstitial space. Within one month after the administration, myocytes and capillaries appeared to be normal. The effusion in the interstitial spaces resulted in bundles of dense collagen, which persisted for 14 months. The results indicate that ciguatoxin and ciguatoxin-4c have a cumulative effect on the cardiac tissue. This means that if there are repeated exposures to low doses of ciguateric fish, even the ingestion of fish slightly contaminated by ciguatoxin may play a role in the development of the heart disease (De Fouw *et al.*, 2001).

repeated administration

No data

reproduction/teratogenicity

No data

mutagenicity

No data

in vitro studies

Experiments were carried out on nodes of Ranvier of myelinated nerve fibres isolated from the sciatic nerve of adult frogs. CTX-1b, the major toxin involved in ciguatera fish poisoning, was extracted and highly purified from moray-eel liver and viscera. The authors did not explain why they defined the ciguatoxin as CTX-1b. CTX-1b produced swelling of the nodes of Ranvier. The swelling was prevented by the Na⁺ channel blocker tetrodotoxin, indicating that the swelling originated in Na⁺ entry through voltage - dependent Na⁺ channels. D-mannitol caused shrinkage of nodes of Ranvier previously swollen by CTX-1b. CTX-1b induced spontaneous action potentials and caused a persistent activation of a fraction of Na⁺ current, D-mannitol suppressed these spontaneous action potentials (De Fouw *et al.*, 2001).

The results of a study with ciguatoxin on guinea pig atria and papillary muscles suggested that the toxic effects of ciguatoxin stem from its direct action of opening myocardial Na⁺ channels. Extrasystoles developed in atria and papillary muscles within 45 minutes of addition of ciguatoxin (> 0.15 MU/ml) and appeared to result mainly from its effect on neural Na⁺ channels causing an increased release of noradrenaline from the nerves associated with the myocardium. The papillary muscles were less sensitive to the toxic effects of ciguatoxin than those of the atrium. This corresponded to a 10-fold difference in their sensitivity to positive inotropic doses of ciguatoxin (De Fouw *et al.*, 2001).

7.5.5 Toxicity to humans

clinical symptoms

After consumption of ciguatoxin contaminated fish, the onset of the first symptoms can be as short as 30 minutes for severe intoxications, while in milder cases onset may be delayed for up to 24 hours to occasionally 48 hours. The first symptoms can be either gastrointestinal or neurological in nature (e.g. circumoral tingling). Gastrointestinal symptoms usually last only a few days, while some neurological symptoms can take several days to develop. Ciguatera symptoms typically last for several weeks to several months. In a small percentage of cases (less than 5 percent), certain symptoms may persist for a number of years.

A combination of a few to more than 30 gastrointestinal, neurological and/or generalized disturbances have been reported. Gastrointestinal symptoms involving vomiting, diarrhoea, nausea and abdominal pain (>~50% of cases) typically occur early in the course of the disease and often, but not always, accompany the neurological disturbances. Neurological disturbances invariably occur in ciguatera and include tingling of the lips, hands and feet, unusual temperature perception disturbances where cold objects give a dry-ice sensation, and a severe localized itch of the skin (>~70 percent of cases). These symptoms and a profound feeling of fatigue (90 percent of cases) can occur throughout the illness. Muscle (>80 percent), joint (>70 percent) and teeth aches (>30 percent) occur to varying extents, and mood disorders including depression and anxiety (50 percent) occur less frequently. Severe cases can

involve hypotension with bradycardia, respiratory difficulties and paralysis but deaths are uncommon (less than 1 percent according to Lehane, 2000). The low fatality rate (2 percent) appears to arise because fish rarely accumulate sufficient levels of ciguatoxin to be lethal at a single meal, perhaps because fish succumb to the lethal effects of higher ciguatoxin levels (Lewis, 2001).

Lehane and Lewis (2000) noted that most cases of CFP in the Pacific involved the consumption of fish containing 0.1-5 nmol P-CTX-1/kg, which is equivalent to about 0.1-5 µg/kg of fish flesh.

persistence and recurrence of symptoms

Neurological disturbances usually resolve within weeks of onset, although some symptoms may persist for months or even years. Symptoms such as pruritus, arthralgia and fatigue can also persist for months or years. Analysis of ciguatoxins in blood samples suggests that the toxin can be stored in adipose tissue and that symptoms may recur during periods of stress, such as exercise, weight loss, or excessive alcohol consumption. Sensitivity to alcohol may also persist for years after the first attack (Lehane, 2000).

factors influencing clinical symptoms

sensitization

The phenomenon of sensitization has been observed where persons who previously were intoxicated with ciguatoxin may suffer a recurrence of typical ciguatera symptoms after eating fish that do not cause symptoms in other persons. Such sensitization can occur many months or even years after an attack of CFP (De Fouw *et al.*, 2001).

It was also noted that individuals who had suffered from CFP, often have symptoms after eating any seafood and often nuts, nut oils and alcoholic beverages as well. Therefore patients suffering from CFP are recommended to avoid these food products. Eating fish with low levels of toxin over several years in the absence of symptoms could eventually result in sensitization to the toxin. This may be a matter of accumulation of ciguatoxin in the host or possibly an induction of an immunological reaction (De Fouw *et al.*, 2001).

fish species involved

Large variations are noted in the frequency and severity of the symptoms after ciguatera poisoning. Ciguatera case reports from the Hawaii State Department of Health were examined for patterns of symptomatology in relation to the types of fish consumed. While individuality and variability of human's response to particular toxin cannot be ruled out as the cause of the wide variations, the data presented would suggest that there are also differences in symptoms which are fish-specific or toxin-specific. It may be postulated that the carnivores feed on different herbivores or metabolise the toxins from the same prey to more or less active forms (De Fouw *et al.*, 2001).

ethnic variation

Though variation in symptomatology is possibly the result of inconsistent reporting, it has also been speculated that it relates to differences in toxins within the same contaminated fish. Some authors reported that the symptoms correlated with ethnic groups. It appeared that Melanesians more commonly had pruritis, ataxia, abdominal pain and weakness, that Europeans experienced more neck stiffness, lachrymation, arthralgia and reversal of temperature sensation, and that Asians had more diarrhoea and abdominal pain (De Fouw *et al.*, 2001).

geographic variation

In the Pacific Ocean, neurological symptoms predominate, while in the Caribbean Sea, gastrointestinal symptoms are a dominant feature of the disease. These differences in symptoms provide clear evidence that different ciguatoxins may underlie ciguatera in Pacific and Caribbean waters. A third class of ciguatoxins is likely to underlie the different pattern of symptoms observed in the Indian Ocean where ciguateric fish cause a cluster of symptoms reminiscent of hallucinatory poisoning including lack of coordination, loss of equilibrium, hallucinations, mental depression and nightmares, in addition to symptoms typical of ciguatera. Ciguateric fish in the Indian Ocean are also more frequently contaminated by lethal levels of toxin (Lewis, 2001).

Percentages given for symptoms in different regions are:

- Neurological symptoms: paresthesia is found in 36 percent of cases in US Virgin Islands, 70 to 76 percent in Australia and Miami, and in 87 to 89 percent of cases in French Polynesia, Fiji and the Caribbean area (De Fouw *et al.*, 2001).
- Gastrointestinal symptoms: diarrhoea appears to be common in 32 percent of cases in Fiji to 86 percent in other regions (De Fouw *et al.*, 2001).

- Cardiac manifestations: Bradycardia and hypotension are reported in French Polynesia (16 percent) and Fiji (9 percent) (De Fouw *et al.*, 2001).

The toxin responsible for ciguatera in the Gove region of Northern Australia is the same as the major toxin responsible for poisoning from carnivorous fishes in the Pacific Ocean but differs from the toxins involved in the Indian Ocean and the Caribbean Sea (De Fouw *et al.*, 2001).

sexual transmission of intoxication

Four men became ill after the ingestion of freshly caught trevally and coral trout a few hours before the characteristic symptoms of ciguatera poisoning developed. In addition to these symptoms, two men complained of intense penile pain and one of these patient's female partner, who had not eaten any fish, complained of circumoral dysesthesiae, pruritus, arthralgia, nausea and lethargy within 24 hours of having unprotected sexual intercourse with him (De Fouw *et al.*, 2001).

effects during pregnancy

Ciguatoxin is transferred across the placenta from mother to foetus. It does not affect foetal development but has been attributed to accelerated foetal movements. It can also pass from mother to infant via breast milk. Mothers who breast fed their babies had reported excessive pain of their nipples. The babies showed diarrhoea. Women who had chronic symptoms with ciguatera occasionally reported worsening of symptoms during their menses (Beadle, 1997).

A family of four in Queensland (Australia), two children, father and mother who was 11 weeks pregnant, was diagnosed with ciguatera poisoning after eating a coral trout. The poisoning was confirmed clinically and by mouse bioassay. The concentration of ciguatoxin in the trout eaten, being 1.3 ng/g, is considered relatively high. The father and mother, showing more severe intoxication, were intravenously treated with 20 percent mannitol (250 ml over 30 minutes). The mother recovered quickly after mannitol infusion, in the father a second mannitol infusion a week after the poisoning had beneficial effects. Twenty-eight weeks later, the mother gave birth to a 3.4 kg male. The newborn showed respiratory problems at birth and was treated for persistent pulmonary hypertension which was not attributed to ciguatoxin exposure *in utero*. No residual symptoms were seen after two months (De Fouw *et al.*, 2001).

A pregnant woman in San Francisco (USA) showed symptoms characteristic of ciguatera poisoning, four hours after she had eaten a large portion of a barracuda fish. Many of the symptoms lasted for several weeks. The woman, who was in her second trimester, experienced an increase of foetal movements one hour after the poisonous meal, which lasted for a few hours. The presence of ciguatoxin was confirmed in two bioassays (guinea pig atrium stimulation test and a mouse bioassay) and a stick enzyme immunoassay. The newborn at term was normal and follow-up visits revealed no abnormalities in the first 10 months (De Fouw *et al.*, 2001).

Two days before the expected birth of a child, a woman had eaten ciguateric coral trout. Within four hours she experienced the characteristic gastrointestinal and neurological symptoms of CFP. Tumultuous foetal movements were experienced and an intermittent peculiar foetal "shivering", which began simultaneously with her own systemic symptoms. The bizarre foetal movements continued strongly for 18 hours and gradually decreased over the next 24 hours. A 3.8 kg male was delivered by Caesarean section two days later. He exhibited left-sided facial palsy (possibly myotonia of the small muscles of the hands) and respiratory distress syndrome but recovered within six weeks (Lehane and Lewis, 2000).

treatment

A real antidote therapy is not known. If the patient presents symptoms of ciguatera intoxication soon after ingestion of the fish, gastric lavage followed by treatment with activated charcoal might help. The biggest breakthrough in the treatment of ciguatera came with the use of mannitol. It does not seem to affect the cardiovascular or gastrointestinal symptoms but does reduce the severity and duration of neurological symptoms. Ideally mannitol should be administered in the acute phase to be effective. Clinical research shows that mannitol is not effective if administered more than 48 hours after symptoms appear (De Fouw *et al.*, 2001).

Only one single blind controlled trial with mannitol (patients were unaware of the treatment received) has been reported. This trial showed that 250 ml of 20 percent mannitol given intravenously in one hour was slightly more effective than a combination of vitamins and calcium also given intravenously in one hour. Treatment with 20 percent mannitol solution in water intravenously at a dose of 1 g/kg bw at an initial rate of 500 ml/hour caused an improvement in the symptoms (De Fouw *et al.*, 2001).

The mechanism of mannitol treatment is not completely understood. One theory is that mannitol actually competes with sodium channels. A second theory is that mannitol's effectiveness is in its ability to act as an osmotic agent at the cellular level to reduce fluid excess in the cytoplasm of nerve cells or to prevent an influx of sodium through sodium channels to stabilise the cell membrane. A third theory suggests that mannitol may react directly with the toxin to neutralise it or displace it from its binding site on the cell (De Fouw *et al.*, 2001).

It has also been suggested that the presence of mannitol in the extra-cellular fluid sterically inhibits the movement of sodium ions through channels which have been blocked by the ciguatoxin molecule. Another suggestion is that mannitol may act as a scavenger for hydroxyl radicals in ciguatoxic systems (De Fouw *et al.*, 2001).

In the case of dehydration and hypotension, intravenous crystalloid infusion and vasoactive agents may be required. Atropine sulphate for bradycardia and dopamine infusion for severe hypotension may be life-saving. In cases of respiratory depression, mechanical ventilation may be necessary (De Fouw *et al.*, 2001).

Two patients in a hospital in Santiago, Chile who had CFP after eating a dusky grouper in the Dominican Republic were successfully treated with gabapentin (400 mg orally three times a day) (Perez *et al.*, 2001).

Amitriptyline may be useful for treating dysesthesia which may be chronic (Crump *et al.*, 1999b).

experimental data

Five CFP patients still experiencing intense paresthesia were selected to perform temperature studies. It appeared that temperature perception covering a range from very cold to hot was normal in these patients. The cut-off point of the peculiar symptoms described as reversal of temperature perception (such as tingling, burning, smarting and electric) was recorded around 24 to 26 °C and this temperature appears to correlate very closely to the cold threshold from C-polymodal nociceptors (23°C). This finding suggests that the paradoxical sensory discomfort experienced is, most likely, a result of an exaggerated and intense nerve depolarization occurring in small peripheral nerve tissue such as A-delta myelinated fibres and in particular the unmyelinated C-polymodal nociceptor fibres. These kind of cutaneous unmyelinated fibres respond to mechanical, heat, cold, and chemical stimuli in the painful intensity range. By the same mechanism, the intense sensation of itch experienced in a large percentage of ciguatera patients is characteristic of lower frequency discharges in some C-polymodal nociceptor fibres (De Fouw *et al.*, 2001).

7.5.6 Toxicity to aquatic organisms

fish

Individual tropical fish can carry sufficient ciguatoxin in their tissues to poison several humans, without showing obvious pathology. However, ciguatoxin has been shown to be lethal to freshwater fish and marine fish. Na⁺ channels of marine fish are susceptible to ciguatoxin, and ciguatoxin exerts similar effects on fish and mammalian Na⁺ channels. It can be concluded that:

- fish are susceptible to ciguatoxin but at doses higher than those required to cause death in mammals
- Na⁺ channels and/or Na⁺ gates of both ciguatoxin-carrier and ciguatoxic-non-carrier fish were sensitive to being opened by ciguatoxin; and
- sensitivity of fish nerves to ciguatoxin and the lack of overt pathology in toxic fish suggested that carrier fish have a partitioning or detoxification mechanism to keep the toxin away from target sites.

It was suggested that the presence of a ciguatoxin-induced soluble protein-ciguatoxin association in the muscle of toxic species of narrow-barred Spanish mackerel may be the basis of a sequestration mechanism that diminishes the binding of ciguatoxin to the target sites of the Na⁺ channels of excitable membranes in fish (Lehane and Lewis, 2000).

The adverse effects of ciguatoxin on medaka (*Oryzias latipes*) embryos were quantified by microinjection into the egg yolk of the embryos. Embryos microinjected with 0.1-0.9 pg/egg showed tachycardia but no reduction in hatching success; however 22 percent of the fish which hatch at this dose range have lethal spinal defects. At higher levels (1.0-9.0 pg/egg) a direct decrease in success was seen together with a 93 percent incidence of lethal spinal defects. Embryos exposed to 10-20 pg/egg ciguatoxin have 0 percent hatching success. The results of this study indicated that maternal transfer of low levels of ciguatoxin may represent an unrecognized threat to the reproductive success of reef fish and a previously undetected ecological consequence of proliferation of ciguatoxin-producing algae in reef systems increasingly impacted by human perturbations (Edmunds *et al.*, 1999).

7.6 Prevention of CFP intoxication

7.6.1 Depuration

Ciguatoxin cannot be identified by odour, taste or appearance. It is also temperature stable so cooking or freezing will not destroy it. Ciguatoxin can also not be eliminated by salting, drying, smoking or

marinating. The contaminated fish can remain toxic for years, even on a nontoxic diet (Beadle, 1997). Apart from the avoidance of consumption of large predatory fish, the use of animal screening tests is the only tools presently available to prevent intoxication (De Fouw *et al.*, 2001).

7.6.2 Preventive measures

The major source of ciguatera cases has been the fish caught by sport fishing (79 percent). If people could be educated to avoid consuming heads, viscera and roe of reef fish, and avoid fish caught in the areas known for frequent occurrence of ciguatoxin intoxication, the incidences of ciguatera probably would decrease dramatically (De Fouw *et al.*, 2001).

Large predatory reef fish are most likely to be affected; the larger the fish, the greater the risk. Some authorities advocate avoiding fish that weigh more than 1.35 to 2.25 kg but this is only a relative precaution. However, there is no way of knowing the size of fish from which the steak or filet was cut. Organ meats, including the roe, appear to contain higher concentrations of toxins and should be avoided. Consuming small portions from several fish per meal instead of a large portion of any suspect fish will reduce the risk too (De Fouw *et al.*, 2001).

7.7 Cases and outbreaks of CFP

7.7.1 General

As many as 50 000 cases of CFP worldwide are reported annually; the condition is endemic in tropical and subtropical regions of the Pacific Basin, Indian Ocean and Caribbean. Isolated outbreaks occur sporadically but with increasing frequency in temperate areas such as Europe and North America. Increase in travel between temperate countries and endemic areas, and importation of susceptible fish has led to the encroachment of CFP into regions of the world where CFP has previously been rarely encountered (Ting and Brown, 2001). In the primary endemic areas including the Caribbean and South Pacific Islands the incidence is between 50 and 500 cases per 10 000 people (Perez *et al.*, 2001). In the developed world, CFP poses a public health threat due to delayed or missed diagnosis. Without treatment, distinctive neurologic symptoms persist, occasionally being mistaken for multiple sclerosis. Constitutional symptoms may be misdiagnosed as chronic fatigue syndrome (Ting and Brown, 2001). It was supposed that the incidence figures were likely to represent only 10 to 20 percent of actual cases, with the extent of under-reporting likely to vary between countries and over time (De Fouw *et al.*, 2001).

7.7.2 Europe

France

Two people showed signs of CFP after eating frozen fish (not specified) from China (Province of Taiwan) (IPCS, 1984).

After eating pieces of various fish, a 60 year old man developed CFP with diarrhoea, facial paresthesia, myalgia, cramps and weakness. Physical examination revealed a motor distal deficit of the four limbs, myokimia and ataxia. EMG testing was in favour of an axonal neuropathy. Neurological symptoms persisted for two months. This case illustrates a new pathophysiological mechanism of neuropathy: "axonal channelopathy" (Derouiche *et al.*, 2000).

A few days after eating a shellfish meal (trocas=*Tectus pyramis*), one patient suffered ataxia and stupor. The patient was confused with cerebellar signs and ocular disturbances (hypotropia). Blood results, cerebrospinal fluid and brain CT scan were unremarkable. The patient developed a septic shock and died four weeks after admission. No necropsy was performed. The clinical picture strongly suggested a seafood poisoning, namely ciguatera. However, no toxicological assay was performed. CFP has never been reported with trocas (Angibaud *et al.*, 2000). A confirmed case of CFP was reported in 2002 (EU-NRL, 2002).

Germany

A case of ciguatera poisoning in a 40 year old man in Germany following a travel to the Dominican Republic, has been described. The man showed the characteristic ciguatera symptoms after having eaten a meal of grouper. On return to Germany, he was admitted to the hospital. Due to the typical history and clinical findings, ciguatera toxin ingestion was diagnosed. All symptoms were finally resolved after 16 weeks (De Fouw *et al.*, 2001).

After cutting short their holidays in the Dominican republic, four people from a travel group presented, on return to Germany, complex neurological symptoms including paresthesia, nervousness, inverse temperature perception, muscle cramps, headache and dizziness. Dinner at the holiday location existing of "peak bass and lemon sauce" led to the diagnosis of ciguatera poisoning. The first symptoms in all members of the travel group (26 persons) were diarrhoea, sickness and sweating (Blume *et al.*, 1999).

A 45 year old woman showed signs of CFP on return to Germany after a journey to the Red Sea. She

appeared to have consumed a fish meal during her vacation. The usual treatment with mannitol etc. three weeks after the onset of the symptoms proved inefficient. However, during the 21 months of follow-up, a marked spontaneous clinical and electrophysiological reversal of symptoms occurred (Ruprecht *et al.*, 2001).

Italy

CFP has begun to appear in Italian travellers to the Caribbean islands (Bavastrelli *et al.*, 2000).

The Netherlands

Five patients with symptoms of ciguatera poisoning were seen in the outpatients department of Tropical Medicine in an Amsterdam hospital. The patients had eaten fish in Curaçao and Isla de Margarita (Venezuela). Ciguatera could only be diagnosed based on the clinical symptoms and the fact that a fish was eaten in the Caribbean area (De Fouw *et al.*, 2001).

7.7.3 Africa

Madagascar

A very severe outbreak of ciguatera poisoning, presumably caused by a shark, occurred in Manakara, a city on the east coast of Madagascar, on 28 November 1993. The mortality rate was 20 percent (98 out of 500 poisoned people died). When the medical team arrived five days after the tragedy, most of the serious cases had already died. One hundred and fifty patients were still in hospital (35 in a critical state, of whom 15 died within a few days). The symptomatology presented by the patients in critical state were not indicative for CFP as a consequence of their severity and included coma, body rigidity, myosis, mydriasis, convulsions, respiratory distress and pulmonary oedema, cardiovascular collapses, bradycardia, gengivorrhagia and dehydration. The symptoms in the moderately poisoned persons (115 cases) were typical for CFP. Unfortunately, no remains of the shark were available for chemical investigations (Boisier *et al.*, 1995).

Epidemiological data concerning the same outbreak in Manakara in November 1993 as described above, were reported. The attack rate was about 100 percent. Records of 188 hospitalized patients were reviewed. The first clinical signs appeared within five to 10 hours after ingestion. The overall mortality was close to 30 percent, perhaps because of the inadequacy of local life-support technology. The patients suffered almost exclusively from neurological symptoms, the most prominent being a constant, severe ataxia. Rare cases also manifested digestive or cardiovascular signs. Gastrointestinal troubles, like diarrhoea and vomiting, were rare. Two liposoluble toxins were isolated from the liver and tentatively named carchotoxin-A and -B, respectively. They were distinct from ciguatoxin in their chromatographic properties. The mouse lethality of the shark liver was about 30 mouse units (MU) per g of liver (1 MU was defined as the amount of toxin required to kill a mouse weighing 16 g within 24 hours). This figure exceeded highest ciguatoxin level reported from moray eel liver (20 MU/g liver). Both toxins caused diarrhoea, laboured breathing, paralysis of limbs, and convulsions before death in mice, as does ciguatoxin. However, a distinction was noted between the shark toxins and ciguatoxin in dose-survival time response. Mice given the shark toxins died within 4 hours, or otherwise survived. In contrast, mice given ciguatoxin died even after 24 hours (De Fouw *et al.*, 2001).

7.7.4 North America

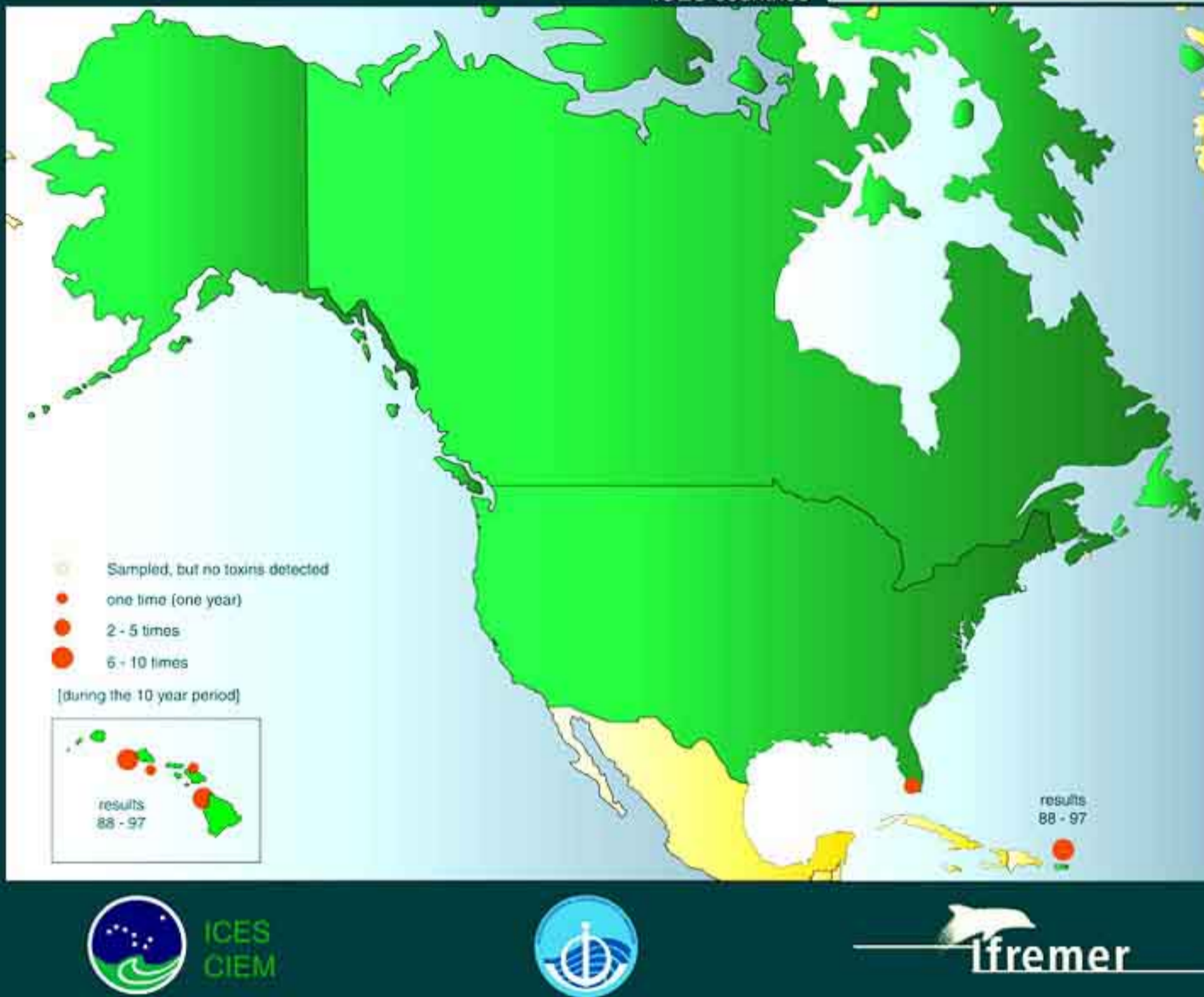
The presence of CFP toxins in North American ICES countries is illustrated in Figure 7.2.

Figure 7.2 Occurrence of CFP toxins in North American ICES countries from 1991 to 2000

Ciguatera Fish Poisoning 1991 - 2000

ICES countries

DISCLAIMER - WARNING
HAEDAT maps should be interpreted with caution regarding risk of intoxication by seafood products from the respective areas/regions/countries. The IOC and ICES are not liable for possible misuse of this information.



Source: <http://www.ifremer.fr/envlit/documentation/dossiers/ciem/aindex.htm>

Canada

Canadians have been affected by CFP through the consumption of tropical fish, mainly when travelling in the north Caribbean region or occasionally through imported fish. The second group of individuals who are exposed to ciguatoxins are those who buy tropical fish from local fish markets or who eat such fish in restaurants. During the years from 1983 to 1997, 22 cases of CFP were reported in Canada, mostly from imported fish (Todd, 1997). In 1998, a CFP incident was reported from hospitals in Montreal, Quebec, involving seven cases. The cases concerned members of three families each of whom had consumed barracuda. The patients revealed gastrointestinal and neurological symptoms. One of the cases had left over fish. At toxicological testing in the mouse assay mortality occurred. An ELISA assay was inconclusive (Anonymous, 2000b). Recently Health Canada was notified of thirty Canadians who developed ciguatera fish poisoning as a result of consuming cooked coral reef fish that had been brought back from Fiji (Anonymous, 2002b).

The United States of America

From 1983 through 1992, 129 outbreaks of ciguatera poisoning involving 508 persons were reported in the United States, however, no ciguatera-related deaths were reported. Most outbreaks were reported from Hawaii (111) and Florida (10). The other outbreaks in different parts of the country have been associated with consumption of imported fish (De Fouw *et al.*, 2001).

California

A 34 year old woman and a 40 year old man became ill within six hours of eating barracuda they caught in turbid water near Cancun, Mexico. Five weeks after the onset of the symptoms, the diagnosis CFP was made. Ten weeks after eating barracuda the patients were free of the symptoms (Farstad and Chow, 2001).

An outbreak of cases in Southern California was tracked to grouper harvested off the coast of the Baja peninsula during an El Niño year (Farstad and Chow, 2001).

Florida

In Miami, the estimated annual incidence rate is 50 per 100 000 population (De Fouw *et al.*, 2001). In 1972, 34 cases of CFP were reported during an outbreak after eating barracuda (Pottier *et al.*, 2001). In 1980, 129 people exhibited CFP symptoms after eating local grouper and snapper. No mortality was seen (IPCS, 1984). Twenty cases of CFP following consumption of amberjack were reported to the Florida Department of Health and Rehabilitative Services in August and September 1991. Forty percent of samples from amberjacks originating from a dealer in Key West and from restaurants and grocery stores in Florida and Alabama were positive in the mouse bioassay (Anonymous, 1993). The estimated rate of CFP in south Florida is 1 300 cases per year, among which 10 percent are caused by fish caught in Florida waters. Many of the ciguateric fish come from the Bahamas. An annual incidence of five CFP cases per 10 000 inhabitants in Dade County (Miami) is reported (Pottier *et al.*, 2001).

Maryland

In 1980, twelve people were reported to show CFP symptoms after eating grouper from Florida (IPCS, 1984).

New York

A 36 year old man was presented to the Emergency Department of a hospital in New York six hours after a late night flight from Aruba. The patient suffered from nausea and vomiting (five episodes), diaphoresis, abdominal pain and loose, watery stools (three episodes). The symptoms began about three hours after returning from vacation. The patient had eaten an unknown local fish stew just before departure home. After four hours in hospital the patient was sent home. Six hours later the patient returned to the hospital with continued gastrointestinal problems together with pruritus, a “numb” feeling around the mouth and mild difficulty in walking caused by myalgia. The patient had taken alcohol at home. Neurological evaluation showed sensory reversal dysesthesia and generalized paresthesia. The patient responded well to supportive therapy and was discharged home after two days (Aseada, 2001).

North Carolina

In 1987, 10 cases of CFP were reported during an outbreak after eating barracuda, dolphin fish and yellow fin tuna (Pottier *et al.*, 2001).

Rhode Island

A male patient in Rhode Island suffered from CFP after ingestion of a fish soup. The patient developed gastrointestinal and neurological symptoms, respiratory distress and cyanosis, progressing to stupor and coma. Coma is unusual but it has been reported. It might be that the patient had consumed a large amount of toxin. It is also possible that the alcohol consumption, the ingestion of non-seafood-related toxins or genetic susceptibility caused a more severe response to ciguatera toxin. A sample of the fish soup was tested and the stick immune assay resulted in “non-edible toxic”. The mouse bioassay resulted in death of the mouse within 48 hours, but the mouse response did not show all ciguatera-like symptoms. The guinea pig atrium assay was negative; both atria did not show the typical inotropic response to ciguatera toxin (De Fouw *et al.*, 2001).

Vermont

In 1986, two persons in Vermont showed CFP symptoms after eating barracuda originating from Florida’s coastal waters. Portions of a single barracuda frozen by one restaurant were positive for ciguatoxin by the enzyme immune assay (Anonymous, 1986).

Hawaii

Based on the epidemiological records for CFP cases of the Hawaii State Department of Health, over a five year period (January 1984 through December 1988) a total of 150 outbreaks were reported involving 652 exposed individuals, resulting in 462 cases showing symptoms of ciguatera intoxication (overall attack rate 70.9 percent). The Kona coast of the Island of Hawaii was responsible for most incidents (De Fouw *et al.*, 2001).

The South Point of the Island of Hawaii and the Napali coast of the island of Kauai were frequently implicated areas. An annual incidence rate in Hawaii of 8.7 per 100 000 from 1984 to 1989 was reported by Gollop and Pon (1992) as compared to 2.5 per 100 000 from 1975 to 1981 (De Fouw *et al.*, 2001).

A confirmed (in left-over fish by immunoassay EIA) ciguatera poisoning was reported in 1985 in which 15 persons of various ages became ill after eating an amberjack caught off the western shore of the island of Kauai (Hawaii). All individuals developed characteristic gastrointestinal and neurological symptoms within 1.5 to six days. Furthermore 10 of the 15 persons demonstrated cardiovascular symptoms, such as bradycardia and hypotension. Duration of the illness ranged from two to 132 days. Bradycardia was associated with increasing age and body weight as well as the amount of fish consumed. An increased duration of the illness (but not an increased severity) was correlated with both increasing age and weight, and was independent of amount and components of toxic fish consumed (De Fouw *et al.*, 2001).

7.7.5 Central and South America

Anguilla

The CFP incidence is reported to vary between two to five cases per 1 000 inhabitants per year (Pottier *et al.*, 2001).

The Bahamas

In March 1982, 14 members of a crew of an Italian freighter showed CFP poisoning after eating a local barracuda. No mortalities were reported (Anonymous, 1982).

After eating a contaminated barracuda caught from the Cay Sal Bank of the Bahamas on 12 October 1997, 17 crew members of a Norwegian cargo ship showed symptoms of ciguatera poisoning (nausea, vomiting, diarrhoea, and muscle weakness) two to 16 hours later. Three samples of left-over raw barracuda and red snapper, caught simultaneously with the consumed barracuda, were tested for ciguatoxin using an experimental membrane immunobead assay. The samples from both fish tested positive for ciguatoxin (Smith *et al.*, 1998)

Chile

A farm-raised salmon, possibly imported from Chile, was suspected of causing CFP in 1992. The affected woman became seriously ill 1.5 hours after eating the fish (Durborow, 1999).

Cuba

Ten cases per year are generally recorded officially except for in 1974 when 174 cases were reported (Pottier *et al.*, 2001). In 1978, 100 cases of CFP were reported after eating local moray eel and Spanish mackerel. No mortality occurred (IPCS, 1984).

In 1987, an outbreak involving 57 cases of CFP was reported (Pottier *et al.*, 2001). Three out of four people who ate barracuda on vacation in Cuba developed frequent watery diarrhoea and vomiting within five hours. The fourth patient developed similar but less severe symptoms within 12 hours. Gastrointestinal symptoms gradually subsided over 24 to 48 hours during which time weakness, generalized pruritus, and peri-oral and distal extremity paresthesias developed (Butera *et al.*, 2000).

Dominican Republic

In 1989, 81 CFP cases were reported. Six out of these 81 were isolated cases while the remaining 75 cases were seen in 13 outbreaks (Pottier *et al.*, 2001).

Guadeloupe

In Saintes Islands (southern Caribbean islands), a study over 20 years estimated an average incidence of three cases per 10 000 inhabitant per year. During the first six months of 1970, several outbreaks occurred in many localities. From 1980 to 1985, 255 cases were reported with five requiring resuscitation. Since 1992, a CFP incidence of 0.7 per 10 000 inhabitants per year was reported. However, this appears to be a gross underestimate because the reporting was done by only 32 out of 300 physicians in the archipelago. Medical supervision reported an estimate of 100 cases per year for Guadeloupe (Pottier *et al.*, 2001).

Haiti

In 1985, two cases of CFP were reported (Pottier *et al.*, 2001). In February 1995, six US soldiers in Haiti became ill after eating a locally caught fish, the so called greater amberjack (*Seriola dumerili*). The symptoms presented were characteristic for ciguatera with gastrointestinal and neurological symptoms. Three patients developed bradycardia and hypotension. All patients recovered fully in one to three months (gastrointestinal and cardiovascular symptoms abated within 72 hours). Analysis of a portion of the cooked fish showed indeed approximately 20 ng Caribbean ciguatoxin-1 (C-CTX-1)/g flesh. Additionally a less and a more polar minor toxin were detected (De Fouw *et al.*, 2001).

Jamaica

In 1978, 250 people showed CFP symptoms after eating local grouper and barracuda. No mortality occurred (IPCS, 1984). Reports on CFP are rare in Jamaica with most outbreaks involving five to 18 persons (Pottier *et al.*, 2001).

Martinique

Eighty intoxications were reported in 1982. In 1983, the annual incidence was estimated at 41 per 100 000 inhabitants per year (Pottier *et al.*, 2001).

Mexico

CFP is present at both coasts of Mexico. There are poisoning cases every spring and summer season, both on the Pacific as well as Caribbean coasts (Sierra-Beltrán *et al.*, 1998).

In 1974, 24 people on board of a ship were reported to show CFP symptoms after eating barracuda from the Gulf of Mexico. No mortality occurred (IPCS, 1984). In 1984, a total of 200 cases of ciguatera intoxication occurred in La Paz, Baja California Sur in Mexico after consuming contaminated snapper fish (*Lutjanus* sp.) (Ochoa *et al.*, 1998). In May 1993, the entire crew of a fishing boat became ill with symptoms resembling ciguatera after eating fish (*Serranidae* and *Labridae*) that were caught in the Alijos Rocks (west coast of the Baja California Peninsula) at depths fluctuating between 9 and 36 metres. After analysis of the suspected fish using the mouse bioassay, the presence of ciguatera-like toxins was confirmed (De Fouw *et al.*, 2001).

In July 1994, 10 cases of CFP occurred on the Isla de Mujeres after consuming barracuda. Between 20 minutes to 12 hours after eating the contaminated fish poisoning symptoms were reported. All suffered gastrointestinal disturbances as the main manifestation. Watery diarrhoea was the earliest complaint. Cold-to-hot temperature reversal dysesthesia occurred in all but there were differences in the occurrence and severity of other symptoms. No associations between the amount of toxic fish ingested with the latency period and the severity and duration of the symptoms were found (Arcila-Herrera *et al.*, 1998).

Twenty-five cases of ciguatera poisoning on the Pacific Coast of the USA as discovered by the Department of Health Services in San Diego (California) over a four-month period, were reported. All persons had eaten a fish called flag cabrilla captured at the coast of Baja California (Mexico). The persons suffered primarily from gastrointestinal symptoms (diarrhoea, vomiting, nausea) and neurological symptoms (extremity paresthesias, pruritis, paresis, dizziness, headache), one woman had bradycardia and hypotension (De Fouw *et al.*, 2001).

In the period from 1993 to 1996, in El Pardito, a small island complex in the Gulf of California, human CFP cases occurred after eating viscera of *Serranidae* and *Lutjanidae* fish (Sierra-Beltrán *et al.*, 1998). In 1997, an outbreak of CFP involving 30 French tourists was reported (Pottier *et al.*, 1997). Sierra-Beltrán *et al.* (1998) reported that the last outbreak in Mexico caused two deaths. Since the coasts of the country are frequently struck by hurricanes, it is possible that these conditions favour the spreading of the toxin producers *G. toxicus*, *O. ovata* or *P. mexicanum*.

Puerto Rico

CFP mostly involves the smaller islands. Between 1980 and 1982, 100 outbreaks involving 215 persons were recorded. An annual incidence of 90 per 10 000 inhabitants was estimated (Pottier *et al.*, 2001).

Saint Barthelemy

About 30 patients per year are treated by physicians. The patients are mainly tourists or fishermen who have eaten groupers, mackerels, jacks or snappers. However, with avoidance of local risk fish and an increased import of fish into Saint Barthelemy, the incidence of CFP will be reduced (Pottier *et al.*, 2001).

Saint Martin

The incidence of CFP is estimated to be two to five cases per 1 000 inhabitants per year (Pottier *et al.*, 2001).

Saint Vincent

In 1985, an outbreak of CFP with 105 patients after eating barracuda was reported (Pottier *et al.*, 2001).

Venezuela

Two hundred cases of CFP on a cruise ship resulted in several fatalities (Farstad and Chow, 2001).

Virgin Islands

A household survey in the United States Virgin Islands showed an annual incidence rate of 730 per 100 000 population (De Fouw *et al.*, 2001). In 1981, 14 outbreaks of CFP involving 65 patients were reported

after eating black fin snapper (Pottier *et al.*, 2001). In 1982 and 1983, 33 and 51 people, respectively showed CFP symptoms after eating local carrang and/or snapper. No mortality occurred (IPCS, 1984).

A CFP incidence of 940 cases per 60 000 persons on St. Thomas and St. John was estimated in 1980, while in 1982 estimates varying from 73 per 10 000 to 360 per 100 00 inhabitants per year were reported (Pottier *et al.*, 2001).

7.7.6 Middle East

Israel

Two families complained of a sensation of “electric currents”, tremors, muscle cramps, nightmares, hallucinations, agitation, anxiety and nausea of varying severity. The symptoms lasted for 12 to 30 hours and resolved completely. All patients had eaten rabbit fish (“aras”). The typical clinical manifestations along with the known feeding pattern of the rabbit fish suggested CFP (Raikhlin-Eisenkraft and Bentur, 2002).

7.7.7 Asia

China

In Hong Kong Special Administrative Region, 47 outbreaks of CFP involving 397 people were reported to the Department of Health from 1988 to 1992. Snapper had accounted for most (59.6 percent) of these outbreaks (Chan and Kwok, 2001).

A CFP incidence in Hong Kong Special Administrative Region occurred in humans a short time after consumption of a mangrove snapper caught in the South China Sea. All four persons became ill, showing the gastrointestinal and neurological features (nausea, abdominal pain, diarrhoea, paresthesia and numbness of extremities) typical for CFP. One patient showed also a life-threatening bradycardia and hypotension (De Fouw *et al.*, 2001).

Eight family members showed signs of CFP after consuming a grouper. One out of these eight patients was treated in the hospital with mannitol and improvement of the clinical symptoms occurred initially. After one week some of the symptoms (mainly neurological) recurred and stayed on for 45 days after consumption of the toxic fish (Chan and Kwok, 2001).

Fiji

In 1984, 925 cases of CFP were reported after eating local snapper, barracuda, grouper and emperor. One person died (IPCS, 1984).

French Polynesia - New Caledonia (South Pacific)

Although a rare disease two centuries ago, ciguatera now has reached epidemic proportions in French Polynesia. In the period from 1960 to 1984, more than 24 000 patients were reported from this area (Hallegraeff *et al.*, 1995).

Four adult tourists developed CFP after eating contaminated fish in Vanuatu (Ting and Brown, 2001). In 1979, 3 009 people were affected by CFP by eating local fish (surgeon fish, parrot fish, grouper, snapper, carrang, emperor and barracuda). Three people died (IPCS, 1984).

Indian Ocean

Very little information is available on incidence in the islands in the Indian Ocean (Comores, the Seychelles, Mauritius and Rodrigues) but the annual incidence rate was estimated to be 0.78 per 10 000 residents (De Fouw *et al.*, 2001).

La Reunion (Indian Ocean)

After eating snapper from Salya de Malha, 367 people were affected by CFP in 1978. No mortality occurred (IPCS, 1984).

South Pacific Islands

The mean reported incidence rate of CFP for the South Pacific islands during a five-year period (1979 to 1983) was 97 per 100 000. The South Pacific Commission reported a mean annual incidence of 217 per 100 000 population in 1987. During the years from 1985 to 1990 the Pacific Islands of Kiribati, Tokelau and Tuvalu reported 90 to 100 cases per 10 000 population per year. In French Polynesia, Vanuatu, Marshall Islands, and Cook Islands the reported cases varied from approximately 35 to 50 per 10 000 population per year. Less than 20 cases per 10 000 population per year were reported for Fiji, Northern Marianas, New Caledonia, Wallis and Futuna, American and Western Samoa, Niue, Guam, Nauru, Fed. St. of Micronesia, Palau, Tonga and Papua New Guinea. Data are from the South Pacific

7.7.8 Oceania

Australia

In Australia an annual incidence of 30 per 100 000 was estimated. The annual incidence in Queensland is reported to be about 1.6 cases per 100 000 population (De Fouw *et al.*, 2001). Each year, outbreaks of CFP occur from consumption of fish caught along the tropical coast of eastern Australia. In 1988, clinical details from a Queensland database of 617 cases from 225 outbreaks collected over 23 years were published. Major outbreaks occurred in Sydney in 1987 (63 people affected) and 1994 (43 people affected), after the consumption of Spanish mackerel from Queensland (Lehane, 2000 and Lehane and Lewis, 2000).

An outbreak of CFP was reported after eating a single fish (coral cod) captured from the Arafura Sea (Northern Australia) causing 20 poisoning events. When a 230 g sample of the fish was analysed by mouse bioassay and LC/MS the presence of Pacific ciguatoxin-1 (P-CTX-1) was found. This was the first time that the toxin contributing to ciguatera in the Arafura Sea has been identified (De Fouw *et al.*, 2001).

More recently from July 1997 to August 1998, there were three small outbreaks of CFP in the inner Sydney area caused by reef fish. In all three incidents, diagnosis was based on clinical grounds. The first outbreak (six cases) was caused by coral trout from Fiji; the second outbreak (10 cases) by coral trout from Queensland and the third outbreak (10 cases) by spotted cod from Queensland. The third outbreak included two exclusively breastfed infants who exhibited symptoms two days after onset of their mother's symptoms (Karakis *et al.*, 2000).

In September 1997, an outbreak of CFP in outer Melbourne was traced to a 16.2 kg Maori Wrasse fish imported in Victoria from Trunk Reef in Queensland. Thirty individuals who attended a banquet at an Asian restaurant consumed at least one of four different dishes prepared from the flesh and viscera of the fish. All 30 reported one or more symptoms, mainly gastrointestinal symptoms and/or in 18 cases neurological symptoms. Seventeen cases were seen in four different hospitals and nine were treated with parenteral mannitol therapy. Nine out of eighteen cases were still symptomatic 10 weeks after the episode (Ng and Gregory, 2000).

Two male patients were admitted to a hospital in Herston, Queensland in 1998 with CFP symptoms including cardiac toxicity. In one patient, the cardiac symptoms resolved over three days and the non-cardiac-symptoms over the subsequent 14 days. In the second patient, all symptoms normalised within six weeks (Miller *et al.*, 1999).

From 1990 to 2000, in total 132 CFP cases in 10 outbreaks were registered. Not included in this total is an average of 48 annual cases of CFP estimated in Queensland each year, which will increase the ten-year total to 612 cases (Sumner and Ross, 2002).

New Zealand

Three imported cases were notified in 1997 (Crump *et al.*, 1999a). A 42 year old man was presented at a hospital in Christchurch with CFP symptoms three weeks after returning from Fiji. In Fiji, he developed CFP symptoms within three hours of eating barbecued fish. The patient required a period of respiratory supportive therapy. Dysesthesia of the hands and feet persisted for weeks but resolved after five days on amitriptyline (Crump *et al.*, 1999a).

Tonga

A CFP case associated with cindarian (jellyfish and related invertebrates) ingestion was reported. Cindaria have not previously been associated with direct ciguatera intoxication in humans. A 12 year old Tongan girl had eaten jellyfish about two hours prior to the presentation of gastrointestinal and neurologic symptoms characteristic for CFP. All other persons who had eaten the jellyfish were without symptoms, which might suggest that the girl had prior ciguatoxin intoxication with sensitization and re-emergence of symptoms with new exposure. Serum samples of the girl were drawn and examined for ciguatera toxins. Following discharge, serum ciguatera toxin assay result was 3.5 (on a 1 to 5 scale), strongly positive, and comparable with values previously obtained from acute CFP victims. Attempts to obtain a portion of the ciguatoxic jellyfish served at meal, and to further specify the source or species of the jellyfish to evaluate ciguatoxin contamination were unsuccessful (De Fouw *et al.*, 2001).

7.8 Regulations and monitoring

Very few specific regulations exist for ciguatera toxins (Van Egmond, *et al.*, 1992). In some areas, public health measures have been taken that include bans on the sale of high risk fish from known toxic locations. Such bans have been used in American Samoa, Queensland, French Polynesia, Fiji, Hawaii and Miami. The bans were apparently with some success but with attendant economic loss (De Fouw *et al.*, 2001).

7.8.1 Europe

In the EU, Council Directive 91/493/EEC (EC, 1991b) is in force, laying down the health conditions for the production and the placing on the market of fishery products. This directive states: “The placing on the market of the following products shall be forbidden; fishery products containing biotoxins such as ciguatera toxins”, without further specific details about the analytical methodology.

In France, this directive is incorporated in French legislation and it is applicable for products imported from outside the EU. The regulation permits the import of certain marine fish species, for which a positive list exists (De Fouw *et al.*, 2001).

7.8.2 North America

The United States of America

Hawaii, Puerto Rico and Florida are the principal locations affected. There are neither standards, nor an official method. For this reason, there are no effective testing programmes for CFP, and the most widespread sanitary measure applied for its prevention is the prohibition of the sale of fish species known to be potentially toxic, or for which some CFP outbreaks have been reported (Fernández, 1998; Van Egmond *et al.*, 1992)

In Hawaii a limited programme has been instituted using an immunoassay. Fish testing positive are considered unsafe and removed from the market (Van Egmond *et al.*, 1992).

7.8.3 Oceania

Australia

In Platypus Bay, Queensland, a ban has been imposed on the capture of the ciguateric fish species Spanish mackerel (*Scomberomorus commersoni*) and barracuda (*Spyraena jello*) to reduce the adverse impacts of ciguatera. Reef carnivores such as the moray eel, chinaman, red bass and paddletail fish have long been considered regular ciguatera carriers and are now not sold by marketing authorities in Australia (De Fouw *et al.*, 2001).

