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Ciguatera fish poisoning: a review

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Abstract

This review contains information on the ciguatera intoxication syndrome and the provoking ciguatoxins (CTXs) and gambiertoxin-4b (GTX-4B), of which CTX-1 is a major component at the end of food chain (the carnivore fish). Data on chemical structures and detection methods of ciguatoxin (CTX), sources for CTX, marine organisms associated with CTX, toxicity of CTX for animals and man, possible preventive measures for ciguatera intoxication, case reports of outbreaks of ciguatera intoxication and regulations and monitoring of CTX are included. Finally some recommendations are given for a better control of the putative CTX problem in the future.

Contents

Samenvatting

Ciguatera vergiftiging is een complex syndroom bij de mens, dat vooral voorkomt in het Pacifisch en Caribisch gebied, en veroorzaakt wordt door het eten van roofvissen die ciguatoxinen via hun voeding hebben opgehoopt. Ciguatera toxinen (CTX) worden geproduceerd door bentische algen als *Gambierdiscus toxicus*. Via voedselketens kunnen ciguatoxinen ophopen in vissen, levend in het koraalriffengebied. De ciguatoxinen analogen (CTX-1, CTX-2, CTX-3 en gambiertoxin-4b (GTX-4B)) zijn de belangrijkste toxinen die ciguatera veroorzaken. CTX-1 is het meest toxisch en wordt bovendien het meest gevonden. GTX-4B wordt geoxideerd tot CTX gedurende de overdracht in de voedselketen (alg-vis-roofvis). CTX zijn lipofiel en hittebestendig, en manifesteren zich in verschillende vormen. Een groep toxinen die qua structuur en farmacologische activiteit nauw verwant is met CTX uit het Pacifisch gebied (P-CTX) is verantwoordelijk voor ciguatera in het Caribisch gebied (C-CTX). Afhankelijk van de mate van contaminatie met CTX kan vis 0.1 tot 5 µg CTX-1/kg nat visvlees bevatten. In tegenstelling tot de meeste "shellfish" toxinen (Paralytic, Diarrhoeic en Amnesic Shellfish Poisoning toxinen) is er geen direct verband tussen het optreden van explosieve groei (bloei) van bentische algen en de contaminatie van vis met CTX. Dit komt vooral doordat de roofvis aan het eind van de voedselketen staat, en de toxinen zich in deze keten geleidelijk aan ophopen. *G. toxicus* hecht zich aan dode algen op het koraaloppervlak en aan op de bodem liggende dode algen. De vissen zijn zelf ongevoelig voor de toxinen. Rapportages van ciguatera vergiftigingen zijn beperkt tot de consumptie van grote roofvissen, die in de Pacifisch en Caribisch gebied leven, zoals barracuda, red snapper, jack, grouper, and surgeonfish. De CTX concentratie is het hoogst in de ingewanden, vooral in de lever, milt, nieren en het laagst in de graten. De concentratie van CTX is ook aanzienlijk lager in het vlees van de vis. Als eenmaal de toxische concentratie is bereikt in de roofvis, kan deze relatief lang (enkele maanden) aanwezig blijven.

Voor de bepaling van CTX worden zowel *in vivo* bioassays, biochemische en chemische methoden toegepast. Analysemethoden moeten in ieder geval CTX-1 kunnen detecteren. CTX kunnen worden geëxtraheerd met organische oplosmiddelen van intermediaire polariteit. Bij de bioassays wordt een extract van CTX intraperitoneaal of oraal toegediend aan muis, kip, mangoest, rat, garnaal, mug of diptera larven. De toxische effecten, in de meeste gevallen sterfte (LD_{50}) worden vergeleken met die, waargenomen na toediening van een standaard reeks (calibratie) van bv CTX-1. De meeste bioassays zijn weinig specifiek.
Verscheidene immunochemische methoden zijn ontwikkeld, zoals een radio-immunoassay en enzym immunoassays. Deze methoden lijken veelbelovend. De chemische bepalingsmethoden berusten vooral op een combinatie van chromatografische scheiding en detectie met behulp van nuclear magnetic resonance (NMR) en massaspectrometrie (MS). De ontwikkeling van deze methoden is in volle gang. De combinatie van NMR en MS lijkt gevoeliger en selectiever dan de meest gebruikte methode: de muisbioassay. Deze chemische methoden vereisen echter extractie op een grotere schaal en zijn tamelijk tijdrovend. Tot nu toe zijn de methoden, die gebruikt worden, ontwikkeld voor de detectie van P-CTX, en niet voor C-CTX. Met uitzondering van een enzym immunostick test is geen van de bestaande analysemethoden voor CTX gevalideerd in formele interlaboratorium-studies. Een tekort aan goede standaarden en referentiematerialen vertraagt de validatie van deze analysemethoden.

Het globale werkingsmechanisme van CTX moet nog verder worden opgehelderd. Er is weinig bekend over de lange-termijn-effecten na vergiftiging met CTX. CTX zijn competitieve remmers van de brevetoxinen en hebben een gemeenschappelijke bindingsplaats. Dit is een neuronaal voltage-afhankelijk natriumkanaal. Ondanks dat ciguatera in delen van de wereld frequent wordt waargenomen, is de vergiftiging zelden fataal, omdat de concentratie van het toxine in het visvlees laag is. Ciguatera vergiftiging werd gerapporteerd in veel landen zoals Australie, Bahamas, Canada, China, Haiti, Hawai, Madagaskar, Mexico, Tonga, USA, en zelfs incidenteel in Duitsland en Nederland. Medische behandeling van ciguatera-patiënten is gebaseerd op symptoombestrijding, zoals het geven van een infuus met d-mannitol, of een infuus om uitdroging en bloeddrukdaling te voorkomen. Er is echter geen tegengiftherapie beschikbaar. In de preventieve sfeer zijn het vermijden van de consumptie van grote roofvissen uit gebieden die “ciguatera prone” zijn en het uitvoeren van screeningstesten momenteel de enige mogelijkheden, om ciguatera-vergiftigingen te voorkomen. Het risico wordt ook verminderd als het viscerale deel van de vis niet wordt gegeten. Momenteel bestaat er wereldwijd nauwelijks regelgeving omtrent CTX.

Summary

Ciguatera food poisoning is a complex syndrome in humans principally encountered in the Pacific and Caribbean areas associated with the ingestion of a wide variety of coral reefs associated carnivorous fish that had accumulated ciguatoxin through their diet. Ciguatera toxins are produced by the benthic algae *Gambierdiscus toxicus* and are transmitted to the living fishes of coral reef by their food chain. The ciguatoxin analogues CTX-1, CTX-2, CTX-3 and gambier toxin-4b (GTX-4B) are the most important toxins, causing ciguatera. CTX-1 is the most potent toxin, and also the major toxin found in carnivorous fish. GTX-4B is oxidized to CTX during the transmission in the food chain (algae-herbivorous fish-predatory fish) in ocean waters. CTX are lipid-soluble and heat-resistant and exist in multiple forms. A family of toxins structurally and pharmacologically related to the Pacific family (P-CTX) is responsible for ciguatera in the Caribbean (C-CTX). Depending on the degree of contamination with CTX the fish contains 0.1 to 5 μg CTX-1/kg wet tissue. There is no direct link between occurrence of blooms of benthic algae and the contamination of fish with ciguatoxins, as is the case in shellfish poisoning (Paralytic, Diarrhoeic and Amnesic Shellfish Poisoning toxins). The carnivorous fish accumulates the CTX through its food chain. The *G. toxicus* adheres to dead coral surfaces and bottom-associated algae. The fish involved in the food chain are insensitive to the toxins. Case reports of ciguatera intoxication in humans are restricted to the consumption of the large predatory fish living in the Pacific and the Caribbean such as Barracuda, Red Snapper, Jack, Grouper and Surgeon fish. The CTX concentrates mostly in viscera, particularly in the liver, spleen and kidney and lowest in the bones. In the flesh of the fish the CTX concentration is considerably low. Once a toxic concentration is reached in the predatory fish, this can last for a long period, up to several months.

In vivo bioassay methods as well as biochemical or chemical methods are used for the determination of CTX. These analytical methods detect the CTX-1 in any case. The CTX can be extracted by using organic solvents of intermediate polarity. An extract of CTX from contaminated fish - in the bioassays - is administered intraperitoneally or orally to mouse, chicken, mongoose, rat, brine shrimp, mosquito and diptera larvae. The toxic effects - in most cases mortal - (LD_{50}) are compared to those seen with a range of concentrations of ciguatoxin standards (calibration curve), preferably CTX-1. All bioassay have in common that they lack specificity.
There are several immunochemical methods developed such as a radio immunoassay, a competitive enzyme immunoassay, a rapid enzyme immunoassay stick test, and enzyme-linked immunosorbent assay. These methods are promising. However, the lack of CTX standards and reference material has hampered their validation. To obtain antibodies against different ciguatoxins is challenging. The chemical detection methods are chromatographic detection and the combination of Nuclear Magnetic Resonance (NMR) and mass spectrometry (MS). The development of these methods is also in progress. The combination of NMR and MS appears to be more sensitive and selective than the mostly used mouse bioassay. This method requires a large-scale extraction and is rather time-consuming. In spite of the similarity between the C-CTXs and the P-CTXs, the methods have only been developed to determine the P-CTXs.

With the exception of the enzyme immuno-stick test there is no collaborative study done yet to validate the methods used for determination of CTX. The lack of good quality reference material causes a delay of the validation of these methods.

The mode of action of ciguatera intoxication still needs further elucidation. Especially little is known about the long-term consequences of the intoxication. Ciguatoxins are competitive inhibitors of the brevetoxins and have a common binding site. This is a neuronal voltage-dependent sodium channel. Despite frequent occurrences of ciguatera poisoning in many parts of the world, this poisoning is rarely fatal, because of the low concentration of the toxin in fish flesh. Ciguatera intoxication has been reported in many countries such as Australia, Bahamas, Canada, China, Haiti, Hawaii, Madagaskar, Mexico, Tonga, US, and even in Germany and the Netherlands. Medical treatment for ciguatera patients is based on elimination or reduction of the symptoms, such as infusion with d-mannitol or infusion to prevent dehydration and hypotension. However, a real antidotal therapy is not known yet. Apart from the avoidance of consumption of large predatory fish from ciguatera prone areas, the use of screening tests are the only tools presently available to prevent intoxication with ciguatoxins. It also helps not to consume the visceral part of these fish.
At present there are hardly worldwide regulations for ciguatoxins.

1. Ciguatera

1.1 Introduction

Ciguatera food poisoning is a complex syndrome in humans principally encountered in the Pacific and Caribbean areas associated with the ingestion of a wide variety of coral reef-associated fish that can accumulate through their diet ciguatoxin, the main causative toxin. (Angibaud and Rambaud, 1998). Although the poisoning has been known for over 200 years, it has been during the last two decades that it has become a public health problem. In the past the ciguatera food poisoning in humans had been highly localised 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. (Sanner et al. 1997). No indicator such as the highly visible surface phenomenon, the so-called red tide as seen by paralytic shellfish poisoning, has ever been associated with ciguatera. It is this lack of warning signal that has contributed to the dread of ciguatera poisoning. (Scheuer, 1994).

Ciguatera is characterised by a wide array of neurological and gastrointestinal symptoms. (Hallegraeff et al.1995). Sometimes the illness is fatal due to cardiorespiratory failure, which may be caused by severe dehydration. The reported mortality rate varies, in Australia and New Zealand it is known to be under 0.1 per cent (Swift and Swift, 1993).

Although other toxins have been mentioned that might be active in ciguatera poisoning, including maitotoxin, scaritoxin, palytoxin and okadaic acid, only maitotoxin has, in addition to ciguatoxin, been isolated from the dinoflagellate *Gambierdiscus toxicus*, the tropical benthic species from which ciguatoxin originates (Moore and Scheuer, 1971; Kodama et al. 1989). It is assumed that *G. toxicus* produces so-called gambiertoxins, less polar toxin precursors which are oxidatively metabolised into the more polar ciguatoxin by the fish itself (Holmes et al. 1991).

1.2 History

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 a univalve mollusk, *Turbo pica*, reputed to cause indigestion. The term ‘cigua’ somehow was transferred to an intoxication caused by the ingestion of coral reef fishes. (Juranovic and Park, 1991; Scheuer, 1994).

Although the name ciguatera is only 200 years old, European explorers to the New World already described it in the 16th century. The reports of similar fish poisonings were documented by Peter Martyr (1457-1526) in the West Indies and by the Spanish explorer de Quiros (1606), while sailing off the coast of New Hebrides (South Pacific). Also Captain Cook suffered from this poisoning after eating a toxic fish caught in the same waters of New Hebrides in 1774. (Banner, 1976; Scheuer, 1994 and references therein).

2. Chemical features

2.1 Chemical properties and structures

There are several toxins mentioned in relation to the ciguatera phenomenon, including ciguatoxin (CTX), maitotoxin (MTX) and scaritoxin. The ciguatoxin analogues (CTX-1, CTX-2, CTX-3, and GTX-4B (gambiertoxin)) are the principal toxins associated with ciguatera, with CTX-1 being the most potent toxin, and also being the major toxin found in carnivorous fish (Murata et al. 1990; Lewis et al. 1991). The molecular formula is $C_{60}H_{86}O_{19}$ with an estimated molecular weight of 1111.5843 ± 0.0053 (Murata et al. 1990). Apparently, GTX-4B produced by *G.toxicus* is oxidised to CTX during transmission through the food chain. (Yasumoto and Satake, 1996). The mouse lethality of GTX-4B was enhanced 11 fold by the oxidation to CTX (Murata et al. 1989). Ciguatoxins are lipid-soluble toxins. (Murata et al. 1989, 1990; Lewis et al. 1991, 1993), (See figure 1).

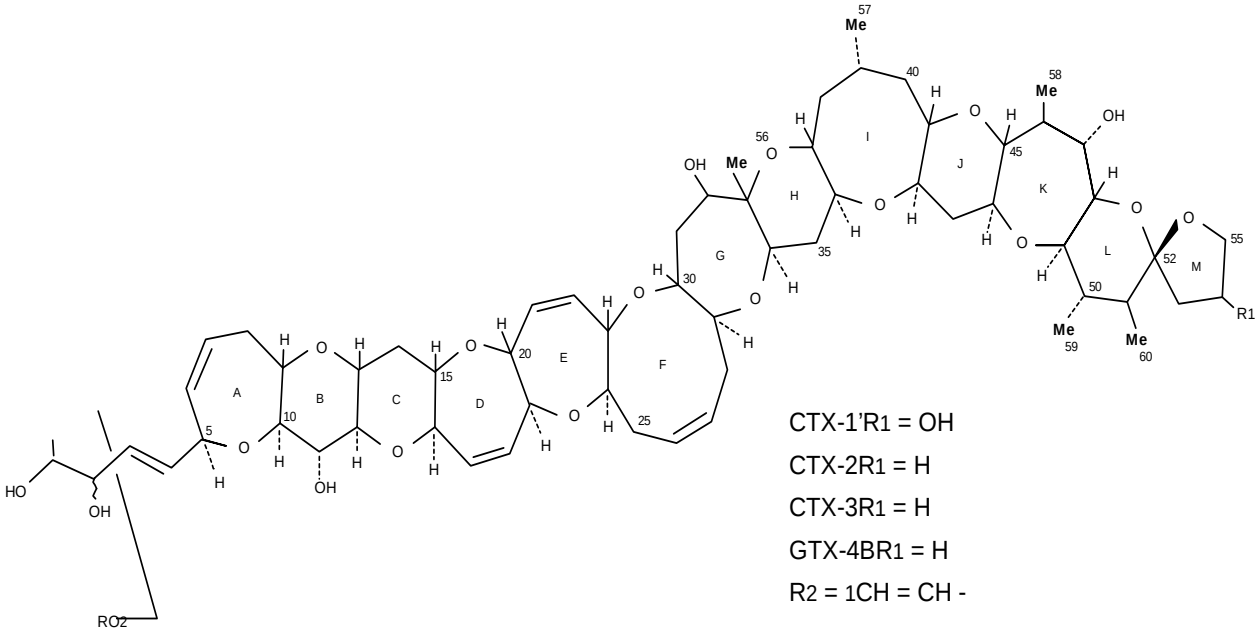

Figure 1. Structures of the major ciguatoxins (CTX) and gambier toxin-4b (GTX-4B) (from Lewis *et al* 1991)

Ciguatoxins have a brevetoxin-like polyether structure comprising 13 contiguous ether rings with a primary alcohol at 1 terminal of the molecule (Murata et al. 1989). It was shown that the main toxic compound extracted from the dinoflagellate *Gambierdiscus toxicus*, named gambiertoxin 4b (GTX-4B), possesses a ladder-shaped skeleton identical to that of CTX extracted from the moray eel, but with fewer oxidised terminal groups (Murata et al. 1990). Ciguatoxins are relatively inert molecules, which remain toxic after cooking, salting, drying, smoking, or marinating (Lange, 1994 and references therein). The toxins cannot be identified by visual appearance (Barton et al. 1995; Ting et al. 1998).

3. Etiology of ciguatera intoxication

3.1 Causative organisms

Until the middle 1950's the etiology of ciguatera poisoning was not clear. Between 1960 and 1977, numerous investigators sought to identify the basic organism(s) in the food chain responsible for ciguatera poisoning. Adachi and Fukyo (1979) proved the responsible organism to be *Gambierdiscus toxicus*.

Satake et al. (1993) provided the first chemical evidence for the production of a CTX analog by *G. toxicus* in laboratory cultures. Three strains of the dinoflagellate were collected in French Polynesia and Japan. The result clears up the question as to whether gambiertoxin-4B and the other toxins that were found in the *G. toxicus* samples collected at the Gambier Islands were derived from the dinoflagellate itself , or other contaminant benthic algae. The authors are convinced that ciguatera starts in the food chain from *G. toxicus*.

This large dinoflagellate is strongly flattened. Although usually sessile the cells can swim when disturbed. The length is 24-60 μm, the transversal diameter: 42-140 μm. (Jackson et al. 1993). In a study of Chinain et al. (1997) the intraspecific variation among 19 isolates of the dinoflagellate *G. toxicus* that were collected from the Australian Islands (4), Gambiers Islands (1), New Caledonia (2), Society Islands (5), Tuamotu Islands (6), and French West Indies (1), was investigated by isoenzyme analysis. The 19 isolates exhibited identical morphological features, although cell sizes varied significantly. It appeared that the dinoflagellate species is comprised of numerous biochemically distinct strains (thus high genetic variability) and no clear relationship was found between the electrophoretic profiles of these isolates and their capacity to produce ciguatoxic compounds. Eight of the isolates were capable of producing ciguatoxin-like compounds.

G. toxicus is a widely distributed, slow growing dinoflagellate which is common but not restricted to coral reef waters. It appears to be most prolific in the shallower waters (3-15 m) away from terrestrial influences, with most ciguateric endemic areas being characterised by oceanic salinity waters. (Lewis and Holmes, 1993).

The dinoflagellates attach themselves to dead coral and marine algae thriving in tropical and subtropical reef systems (Barton et al. 1995). Bomber (1985) and Bomber et al. (1989) found a maximum abundance for *G. toxicus* of 2.28 x 10^3 cells/g wet weight on *Heterosiphonia gibbesii*, a rhodophyte. However, abundances on other groups of algae were less than 10^3 cells/g. According to Scheuer (1994) *Turbinaria ornata*, a brown alga, was the preferred substrate by far, while a calcareous red alga, *Jania* sp., was most heavily settled at another site, where no *Turbinaria* sp. was growing. The basis of this apparent chemotaxis is still unknown. Also the importance of algal host to the growth and toxicity of wild *G. toxicus* is unclear. It is possible that host algae merely provide support and/or shelter. Although most studies of *G. toxicus* have focussed on its density on macroalgal substrates, considerable numbers of *G. toxicus* also occur on turf algae.

Population densities of *G. toxicus* are patchy and can increase or decrease rapidly (Gillespie et al. 1985). 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. (Lewis and Holmes, 1993). Natural extracts from the algae combined with certain bacteria are believed to cause the development of *G. toxicus*, indicating that interactions between certain bacteria and the algae could trigger the production of gambiertoxin (Juranovic and Park, 1991). 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 result in the development of peculiar algal turfs *Turbinaria, Jania* and *Amphiroa* species (Caire et al. 1985; Taylor, 1985; Ballantine et al. 1985; Thomassin et al. 1992). Isolation and mass culture of various *G. toxicus* clones have revealed a constant *in vitro* production of the water-soluble maitotoxin fractions, while a variety of clones were grown in a non-ciguatoxic state (Holmes et al. 1991). The production of ciguatoxins by *G. toxicus* appears to be limited to certain strains and therefore, ciguatera outbreaks could probably occur after blooms of genetically defined ciguatoxic strains. (Glaziou and Legrand, 1994). At present such a correlation between the occurrence of a bloom of *G. toxicus* and the outbreak of ciguatera has not been observed, however.

3.2 Toxins produced

Scheuer et al. (1976) were the first to isolate ciguatoxin. It has been observed that moray eel which comes near the top in the coral ecosystem, metabolises more polar congeners of CTX, while the dinoflagellate produces less polar congeners. CTX-1, the most oxygenated member of this class of toxins is found to be absent in the dinoflagellates. The data suggest that less polar toxins produced by the dinoflagellate *G. toxicus* are the precursors of the more polar toxins found in the fish. (Bhakuni, 1995) Firm evidence that cultures of *G. toxicus* produce ciguatoxin was provided by Baden et al. (1985) by using radioimmunoassays and electrophysiological experiments to characterise the toxin. It is possible that cultured *G. toxicus* produces only trace amounts of ciguatoxin and that levels of production comparable to those found in natural populations are dependent on yet undefined environmental parameters. (Frelin et al. 1990). Ciguatoxins exist in multiple forms, produced both by the benthic dinoflagellate *Gambierdiscus toxicus*, which is frequently a causative organism, and as a result of biotransformation by passage through the food web. (Peng et al. 1995).

Ciguatera results predominantly from CTX-1 which is present at > 0.1 µg/kg (approx. 10^{-10} mole/kg) in the flesh of carnivorous fish. Consequently CTX-1 should be the principal target of any assay for ciguateric fish. However, significant levels of other ciguatoxins, particularly CTX-2 and CTX–3, may also accumulate in fish and such toxins could interfere with the response of an assay. (Lewis, 1994). CTX (1,2 and 3) likely arise through biotransformation and acid-catalysed spiro-isomerisation of gambiertoxin produced by *G. toxicus* and it is unlikely that other toxic benthic dinoflagellates are involved. (Lewis and Holmes, 1993).
G. toxicus appears to be the source of two of potent types of toxins: the maitotoxins and ciguatoxins. The CTXs, which are only produced by certain strains of *G. toxicus* are regarded as the toxins responsible for human illness. MTXs, which are produced by all examined strains of *G. toxicus* have no proven role in ciguatera poisoning. (Chinain et al. 1997).

A new ciguatoxin congener, ciguatoxin-4A (CTX-4A) was isolated from cultures of *Gambierdiscus toxicus* and chromatographic and spectral experiments indicated that CTX-4A is 52-*epi*CTX-4B. Various species of parrotfish have previously been reported to contain a toxin less polar than CTX-1, a toxin named scaritoxin (Chungue et al. 1977a). Judging from the reported chromatographic properties, scaritoxin seems to correspond to a mixture of CTX-4A and CTX-4B.

3.3 Contamination with ciguatera

3.3.1 Food chain

The first step consists of the production of toxins by benthic dinoflagellate, *G. toxicus*, which adheres to dead coral surfaces and bottom-associated algae.
The second step involves small herbivorous fish species that ingest the organism and its toxins. Subsequently the ciguatoxins become concentrated in large predatory fish, with increasing concentrations at each succession (these longer-living carnivorous species of fish at higher end of the food chain may remain toxic for several years). Finally, human individuals develop the disease after ingestion of a toxic fish (Barton et al. 1995).

3.3.2 Species involved

3.3.2.1 Fish

Ciguatoxin does not appear to harm fish, the fish merely transport the toxin up the food chain.

Puerto Rico

A systematic determination of the frequency of ciguatoxic barracuda caught along the Southwestern coast of Puerto Rico was undertaken by Tosteson et al. (1988). Head, viscera and flesh tissue components of 219 barracuda (528 tissue samples) were processed and screened for their toxicity during the period from March 1985 through May 1987. Twenty nine percent of these fish yielded toxic preparations in at least one of their tissue components.

Continental United States

The grouper, red snapper, jack, and barracuda are the most common reported fish species associated with ciguatera poisoning in the continental United States (Noone, 1996).

Florida

In most cases the great barracuda is involved in ciguatera poisonings in Florida between 1954 and 1992. Apart from the barracuda, the commonly reported species are snapper, hogfish, jack, and grouper.(Lewis et al. 1994b)

Hawaii

In Hawaii, jack, black snapper, and surgeonfish are most frequently involved with ciguatera toxin (Noone, 1996).

Mascareignes archipel

Thirty-four fish species have been identified to be involved in ciguatera poisoning in this region. Large predators such as grouper (*Serranidae* 53%, *Carangidae* 10%, *Lethrinidae*

15%) are mostly involved in ciguatera. Most toxic fish were caught by fishing offshore on coral banks located north of Mauritius. (Quod and Turquet, 1996).

3.3.2.2 Other species

Although the vast majority of ciguatera fish poisoning is seen after ingestion of carnivorous fish, other marine species are suspect in human ciguatera intoxication since ciguatera fish poisoning associated with cindarian (shellfish) ingestion was reported (Zlotnick et al. 1995).

Lewis et al. (1994b) suggested that also invertebrates (small shrimps and crabs) may be a vector in the transfer of gambiertoxins to carnivorous fish. The suggestion was made based on a study they performed with the often ciguateric blotched javelin fish (*Pomadasys 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.

3.3.3 Toxin distribution

In order to investigate the distribution of ciguatoxin in individual Caribbean fish, fishes were caught from 1980 to 1983 on the island of St. Barthelemy (French Caribbean), and extracted lipids from several parts of these fish were 'analysed' by mouse bioassays. The fish species caught 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, lowest in the bones. The ratios of the toxin concentrations of the liver or viscera to that of the flesh were high and varied with the species suggesting that the toxin is stored in different ways in different fish. The fact that highly vascularised organs such as liver, spleen, and kidney retain the highest quantity of ciguatoxin per unit weight suggests that blood is involved in the distribution of ciguatoxin to other tissues. (Vernoux et al. 1985).

The toxin becomes more concentrated as it moves up the food chain and it is up to 50 to 100 times more concentrated in the viscera, liver and gonads of affected fish. It is not known why the fish are asymptomatic after toxin ingestion or how affected fish can remain toxic for years (Swift and Swift, 1993 and references therein).

Toxins in tissues from the herbivorous surgeonfish (*Ctenochaetus striatus*) collected in the Great Barrier Reef were characterised by mouse bioassay and chromatography. The biodetritus (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*. Such depuration by herbivores could, at least in part, contribute to the rapid decline in the ciguatoxin levels in a population of moray eels and the rapid decline in ciguatera incidence in some Pacific Island countries. (Lewis et al. 1994b)

3.3.4 Transmission of intoxication

Person to person transmission of ciguatera is possible via breastmilk to breastfed infants (Blythe and De Sylva, 1990; Thoman, 1989, Senecal and Osterloh, 1991), and ciguatoxins apparently have the ability to pass across the placenta (Pearn et al. 1982).

Also sexual transmission of ciguatera from female to male (penile pain after intercourse, Geller et al. 1991) and vice versa (pelvic and abdominal pain after intercourse, {Lange et al. 1989; Pearn et al. 1989}) has been described. The case reports described by Ting et al. (1998) support the suggested possibility that ciguatera may be transmitted by ciguatoxic semen during sexual intercourse. 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. Apart from 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 dysaesthesiae, pruritus, arthralgia, nausea and lethargy within 24 hours of having unprotected sexual intercourse with him.

4. Determination of ciguatoxin

Ciguatoxins are odourless, tasteless, and generally undetectable by any simple test; therefore, bioassays have traditionally been used to monitor suspect fish. Many native tests for toxicity of fish have been examined, including the discoloration 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 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 focussed on the development of chemical methods, such as TLC and HPLC 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 programs. (Park, 1994).

4.1 Chemical detection

Depending on the degree of contamination with ciguatoxins, ciguateric fish contains 0.1 to 0.7 nmol/kg CTX-1 (Lewis and Sellin, 1992) or up to 10-fold higher levels of the less potent ciguatoxins (CTX-2 and CTX-3) and gambiertoxins. The ciguatoxins can be extracted with organic solvents of intermediate polarity, but even after several clean-up procedures, the ciguatoxins in the remaining lipid mixture are still present at relatively low levels from 3 to 500 nmol/kg.

4.1.1 Chromatographic detection

Ciguatoxins do not possess a useful chromophore for selective spectroscopic detection but contain a relatively reactive primary hydroxyl through which (after appropriate clean-up) a label could be attached prior to detection. High performance liquid chromatography (HPLC) 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. (1992) 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 HPLC separation and fluorescence detection. HPLC coupled to selective-ion monitoring ionspray mass spectrometry (MS) is an alternative to fluorescence detection of ciguatoxin in HPLC eluants. This approach has shown considerable potential for the detection of labelled diarrhoeic shellfish toxins (Pleasance et al. 1992). 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. 1994a).

4.1.2 Nuclear magnetic resonance (NMR) and mass spectrometry (MS)

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

Present analytical methods used to characterise ciguatoxins (NMR and MS) require large-scale extraction of ciguatoxins present in low concentrations in highly toxic fish and in most instances the characterisation of ciguatoxins present at levels below 0.1 nmol/kg has not been possible. Lewis and Jones (1997) describe gradient reverse-phase high-performance liquid chromatography/mass spectrometry (HPLC/MS) methods to identify the ciguatoxins accumulated by fish. The analysis was performed on 5 μg samples of partially purified highly toxic moray eels from the Pacific Ocean. P-CTX-1 (P stands for Pacific), 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.

4.2 Bioassays

4.2.1 *In vivo* bioassays

4.2.1.1 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. (1984). 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 ^{o}C and observed over 7 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 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 1). One MU is the LD_{50} dose for a 20 g mouse which is equivalent to 5 ng , 48ng and 18ng of CTX-1,CTX-2 en 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 from ciguatoxins since maitotoxins induce effects in mice often mistaken for effects of ciguatoxins, despite the clear differences (see table 1). 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.

4.2.1.2 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% of their body weight. Administration of fish flesh is physically more difficult but can be accomplished. (Vernoux et al. 1985).

4.2.1.3 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 (5-15% 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).

4.2.1.4 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)

4.2.1.5 Mosquito assay

Also a bioassay using mosquitoes has been developed. Only few laboratories perform this assay, perhaps because of difficulties 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 LD_{50}. 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)

4.2.1.6 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 larvae (*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 laid on about 5 g of the test sample. Larva 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 > 1 ng of CTX/g flesh (moray eel) killed the larvae in 3 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).

Conclusion

All the mentioned bioassays have in common the limited chemical specificity for individual toxins (Juranovic and Park, 1991), although for a broad screening this property can be advantageous detecting a poisoning. The bioassays are semi-quantitative, sensitive and ciguatoxin induces characteristic signs of toxicology but the use of some animal species can be problematic (costly, difficult on ethical grounds).

4.2.2 *In vitro* bioassays

4.2.2.1 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 depolarisation 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, 1994a, 1994b) takes advantage of this phenomena 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. Legrand and Lotte (1994) have reported an assay for ciguateric fish based on the ability of ciguatoxins to selectively inhibit the binding of ^{3}[H]-brevetoxin to sodium channels in rat brain synaptosomes.

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.

4.2.2.2 Alternative bioassays in vitro

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 haemolytic blood cells (Escalona De Mota et al. 1986), and the bioassay that measures the mouse body temperature depression following ip 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).

4.3 Immunoassays

The problems with animal assays are that they involve large numbers of test animals which increase assay cost, and use relatively large amounts of fish tissues. In addition, their results are interpreted subjectively, and, most importantly, they lack specificity. An ideal assay for the detection of marine toxins should be simple, highly sensitive and specific. For these reasons, the evaluation of marine toxin detection assays has moved in the direction of immunological 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 have been developed. Problems with these immunochemical methods are their cross-reactivity with other polyether compounds and the limited antibody supply.

4.3.1 Radioimmunoassay

In 1977, a radioimmunoassay (RIA) was developed for the detection of ciguatoxin directly in contaminated fish (Hokama et al. 1977). In the 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.

4.3.2 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.

4.3.3 Stick tests

The speed of detection improved when Hokama et al. (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 (Ciguatect•) for detecting ciguatera toxins and toxins associated with diarrhoeic shellfish poisoning. The Ciguatect• 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 crossreactivity with various derivatives. The rate of false positive responses has not yet been determined. (Park, 1995). The Ciguatect• test was planned to be studied in a formal AOAC International Collaborative Study. Thus far the study has not been carried out yet, 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 in the process of making new hybridoma cell lines for the production of anti- ciguatoxin monoclonal antibodies (Quilliam, 1998).

4.3.4 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, okadaic acid, as well as to ciguatoxin (likely CTX-1).

Speed, practicality and specificity were all combined when Hokama incorporated the technology of monoclonal antibodies to the stick test procedure (Hokama et al. 1989b). With this assay, CTX was conjugated to human serum albumin with carboniimide, 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 remain 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.

4.3.5 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. 0.032ng CTX/g tissue). During development of the MIA, several factors critical to obtaining accurate and repeatable results were noted: 1) the membrane portion of the membrane stick must not be touched, because touching may cause false-positive reactions. 2) the membrane stick must be soaked in the methanol/fish sample suspension for at least 20 minutes for optimal results. 3) the stick and the test tube must be completely dry before the latex immunobead suspension is added to the test tube. 4) 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% and 87% respectively.

4.3.6 C-Ciguatoxin versus P-Ciguatoxin

The structures of toxins contributing to ciguatera (e.g. CTX-1, -2, -3) are known only for fish of the Pacific Ocean (Murata et al. 1990; Lewis et al. 1991, 1993). In an attempt to characterise the ciguatoxins in the Caribbean, the toxins involved in ciguatera fish poisoning in the Caribbean Sea were isolated from *Caranx latus*, a pelagic fish often implicated in ciguatera in the Caribbean region, and purified by mouse bioassay directed fractionation. Two major Caribbean ciguatoxins (C-CTX-1 and –2) and 3 ciguatoxin-like toxins were isolated upon reverse-phase high-performance liquid chromatography. The major Caribbean ciguatoxin (C-CTX-1) accounted for 65% of the toxicity, a minor toxin (C-CTX-2) for 13%. The identified 3 ciguatoxin-like toxins were a sleep-inducing fraction (< 1% of total toxicity), a minor toxin (€ 1% of total toxicity) and a hydrofobic fast-acting toxin (€ 19% of total toxicity). Ionspray MS indicated that C-CTX-1 and C-CTX-2 have the same parent ion. The fact that both toxins have the same mass suggests that they are diasteriomers. Turbo-assisted HPLC-MS identified C-CTX-1, C-CTX-2 and three C-CTX-1-related compounds but no Pacific ciguatoxins were detected. (Vernoux and Lewis, 1997)

Although NMR evidence indicated that C-CTX-1 and P-CTX-1 are closely related structures (Crouch et al. 1995), each of the Caribbean toxins was chromatographically distinguishable from the ciguatoxins found in the Pacific (Murata et al. 1990, Lewis and Holmes, 1993), indicating that the ciguatoxins from the Caribbean Sea are members of a new family of ciguatoxins. The presence of different families of toxins probably underlies the differences in ciguatera symptoms found between the Pacific and Caribbean region. It is likely that the Caribbean ciguatoxins arise from a smaller 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 (Lewis and Holmes, 1993). Given the fact that Caribbean and Pacific ciguatoxins are structurally related and that different strains of *G. toxicus* are able to produce different arrays of polyether toxins (Lewis and Holmes, 1993), Vernoux and Lewis (1997) suggest that a Caribbean strain of *G. toxicus* is a source of C-CTX-1 and –2.

The presence of a new family of ciguatoxins in the Caribbean region has important implications for the detection of ciguateric fish. Antibody detection methods, which to date 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)

5. Toxicology

5.1 Ciguatera poisoning

5.1.1 Clinical symptoms

Data were obtained for 33 patients involved in 27 ciguatera outbreaks occurring between Jan 1 and April 10 in 1980 on St Thomas in the US Virgin Islands. All patients had some form of gastro-intestinal (GI) tract symptom, including diarrhoea (91%), vomiting (70%), or abdominal pain (39%). Other common symptoms described included malaise (70%), itching (58%), pain/weakness in lower extremities (58%), arthralgias (52%), circumoral paresthesia (36%), hot and cold reversal (36%), and parasthesia in extremities (33%). It appeared that the data were obtained for 33 patients involved in 27 ciguatera outbreaks occurring between Jan 1 and April 10 in 1980 on St Thomas in the US Virgin Islands. All patients had some form of gastro-intestinal (GI) tract symptom, including diarrhoea (91%), vomiting (70%), or abdominal pain (39%). Other common symptoms described included malaise (70%), itching (58%), pain/weakness in lower extremities (58%), arthralgias (52%), circumoral paresthesia (36%), hot and cold reversal (36%), and parasthesia in extremities (33%). It appeared that the GI tract symptoms tend to occur early and be of short duration (less than a day), while neurologic manifestations occur later in the course of the disease and persist for weeks to months. (Morris et al. 1982a) The above mentioned clinical symptoms observed in a short period of time for a limited number of ciguatera outbreaks are characteristic for the illness.

5.1.1.1 Gastrointestinal symptoms

The gastrointestinal symptoms develop within 12-24 hours after eating the poisonous fish and these symptoms are quite similar to any other food poisoning, but it is the neurological symptoms that are distinct for ciguatera food poisoning.

5.1.1.2 Neurological symptoms

Neurological symptoms may occur early or late, and these include tingling sensations, especially of the extremities, perceptions of loose teeth, and ataxia. The characteristic neurological symptom associated with ciguatoxin is paradoxal dysesthesia or the reversal of temperature sensation (cold objects feel hot to touch). (Swift and Swift, 1993 and references therein). The burning sensation of the mouth is typical of ciguatera and has been described previously as temperature reversal. However, other experiments have shown that gross temperature perception (external temperature receptors) remains intact in ciguatera (Cameron and Capra, 1993), while the burning sensation is generated in C-polymodal nociceptor fibres in skin and deep structures (Pearn et al. 1989). Neurological complaints are inherent to the ciguatera intoxication and may persist for weeks to months, sometimes even years (Barton et al. 1995).

5.1.1.3 Cardiovascular symptoms

Cardiovascular symptoms, such as hypotension and bradycardia, although serious, usually resolve within 5 days after onset (Swift and Swift, 1993 and references therein).Geller and Benowitz (1992) studied bradycardia and orthostatic hypotension related to ciguatera. They concluded that ciguatoxin does not have a direct chronotropic effect on the myocardium but that it does cause an increase in parasympathetic tone and impairs sympathetic reflexes. Bradycardia was attributed to the inhibition of cholinesterase.

5.1.1.4 Hallucinatory symptoms

Apart from the commonly reported gastro-intestinal, neurological, and cardiovascular symptoms, 16% of the patients in the Indian Ocean area experienced signs of hallucinatory poisoning: lack of co-ordination, loss of equilibrium, hallucinations, mental depression and nightmares. (Quod and Turquet, 1996)

5.1.2 Factors influencing clinical symptoms

5.1.2.1 Sensitisation

The phenomenon of sensitisation is observed, where persons who have previously had ciguatera may suffer a recurrence of typical ciguatera symptoms after eating fish that do not cause symptoms in other persons (Narayan, 1980). Such sensitisation can occur many months or even years after an attack of ciguatera.

Also Gollop and Pon (1992) noted that individuals suffering from ciguatera will often have symptoms after eating any seafood, and often nuts, nut oils and alcoholic beverages as well. Therefore it was recommended that a patient suffering from ciguatera be placed on a diet which involves avoidance of all seafood, nuts, nut oils, sesame oil and alcoholic beverages. (Bach and Terrell- Perica, 1988). Even in the absence of symptoms at first exposure, those with frequent intake of ciguatoxic fish are more likely to become ill. This may be a matter of accumulation of ciguatoxin in the host, or possibly an induction of an immunologic reaction.

5.1.2.2 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 man'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. (Kodama and Hokama, 1989).

5.1.2.3 Amount of fish consumed

Thirty French patients suffering from ciguatera fish poisoning due to ingestion of the same barracuda in Mexico were examined to identify prognostic factors All patients who presented a high severity in symptoms reported persistent manifestations between 1 and 7 months, and had eaten the largest amount of fish. Therefore it was concluded that ciguatera poisoning is dose-dependent in man. (De Haro et al. 1997).

5.1.2.4 Toxins involved

In the Caribbean region, gastrointestinal signs are more prominent and neurological symptoms less prominent than the incidence typically reported in the Pacific (Lewis et al. 1988), suggesting that the toxins involved may not all be identical to the toxins involved in the Pacific region. The instability of Caribbean but not Pacific ciguatoxins in basic conditions supports the suggestion that somewhat different toxins are indeed involved in the regions (Vernoux and Andaloussi, 1986)

5.1.2.5 Ethnic variation

Though variation in symptomatology is possibly the result of inconsistent reporting, it has also been speculated that it relates to differing toxins in affected fish. Ciguatera poisoning is confusing as it may cause severe symptoms in one person and mild or no symptoms in another even though both at similar portions from the same fish. Bagnis et al. (1979) reported that the symptoms correlate 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 Orientals had more diarrhoea and abdominal pain. (Swift and Swift, 1993).

5.1.2.6 Age or weight

An association between illness and age exists: in most studies, the largest age group is that between 30 and 49 years old. Children could be less susceptible to toxic fish (Morris et al. 1982b), or eating fish with low levels of toxin over several years could eventually result in sensitisation to the toxin. Although it is sometimes stated that children are particularly susceptible for ciguatoxin, one should be aware of the fact that children so often ingest more of the fish, and in a particularly toxic fish meal a relatively small increase in ingested mass (in relation to the child's body weight) may result in a supra-threshold level of ingested toxin. (Pearn, 1994).

The correlation between increasing age and weight with duration and severity of symptoms may be explained by prior sub-clinical toxin exposure and is consistent with the observation that repeated ciguatoxin exposures are associated with more severe illness. The association between amount of toxic fish consumed and bradycardia is consistent with an increased dose of ciguatoxin. (Katz et al. 1993).

5.1.2.7 Genetic component

A significant genetic component is likely, although, even within affected families, there is frequently widespread variation in the severity of symptoms and objective signs which is not completely explained by a difference in amount of fish eaten. (Pearn, 1994).

5.1.2.8 Sex difference

Sex differences in response to the toxin are often hinted at, but no formal attempts at initial dose quantification, with long term follow up by sex, have been reported. (Pearn, 1994).

5.1.3 Other symptoms

Symptoms less commonly associated with ciguatera include puritis, fevers, dysuria, hypersalivation, vertigo, tremors, blurred vision, cranial nerve palsy, cerebellar involvement (Sakamoto et al. 1987), chills, metallic taste in the mouth (Glaziou and Legrand (1994), myalgia, paralysis of limbs and facial muscles, fatigue, depression, neurosis, insomnia, hysteria, muscle spasticity, shock, respiratory failure (Swift and Swift, 1993 and references therein) and coma (DeFusco et al. 1993).

5.1.4 Mortality

Although the ciguatera intoxication is seldom fatal, death may occur due to respiratory failure The mortality rate for ciguatoxin fish poisoning is estimated under 0.1% of the cases (Swift and Swift, 1993 and references therein).

It has been shown that there are differences in the response depending on whether the patient suffered with a first attack or suffered from a second (or further) attack (Glaziou and Martin, 1993). The majority of symptoms were significantly more common in those patients experiencing their second or later intoxication by ciguatera.

5.1.5 Geographic variation

Confusion exists about the relative incidence of different symptoms in different parts of the world. Some differences are undoubtedly due to sampling errors, differences in case descriptions and so on, but it is obvious that there are different toxins and different toxin subtypes in different areas. It seems clear that the human clinical syndrome of ciguatera is the result of ingestion of a cocktail of different ciguatoxins. There is some evidence that antibody profiles to toxins from fish taken from different parts of the world differ in their cross-reactivity. This gives further evidence that there are subtle differences in ciguatera syndromes in different parts in the world. (Pearn, 1994)

In the Caribbean, ciguatera is widespread in both the Greater and Lesser Antilles. The signs and symptoms associated with ciguatera display regional variation, but all include gastrointestinal and neurological disorders. In the Caribbean, ciguatera is dominated by gastrointestinal signs, while in the Pacific ciguatera is dominated by neurological signs (Lewis et al. 1988 and references therein).

Percentages given for symptoms in different regions are:

Neurological symptoms: paraesthesia is found in 36% of the cases in US Virgin Islands (Morris et al. 1982a), 70-76% in Australia and Miami (Lewis et al. 1988; Lawrence et al. 1980), and in 87-89% of the cases in French Polynesia, Fiji and the Caribbean area (Sorokin, 1975; Johnson and Jong, 1983).

Gastrointestinal symptoms: diarrhoea appears to be common, 32% of the cases in Fiji to 86% in other regions (as cited from Glaziou and Legrand, 1994).

Cardiac manifestations: Bradycardia and hypotension are reported in French Polynesia (16%) and Fiji (9%). (as cited from Glaziou and Legrand, 1994).

The toxin responsible for ciguatera in the Gove region (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. (Lucas et al. 1997)

5.1.6 Indirect clinical effects

Ciguatera may have indirect effects on health by predisposing victims to poor nutrition and other diseases, and via its social and economic effects (reducing trade of fishes and reducing tourism). (Ruff and Lewis, 1994).

5.2 Mode of action

5.2.1 Ciguatoxin

Relatively little is known about the chronic course of the ciguatera intoxication; for example, ciguatera is reported to continue as a neurological syndrome for months to years (Halstead, 1988; Lawrence et al. 1980, Bagnis et al. 1979), however this cannot be confirmed without

accurate diagnosis. Nor can appropriate comparisons be made between geographical areas for the same marine toxin disease without accurate diagnosis (as reported earlier there is an apparent clinical difference between ciguatera in the Caribbean and the Pacific) (Lange, 1987; Halstead, 1988; Bagnis et al. 1979 and Lawrence et al. 1980). Multiple forms of ciguatoxin with minor molecular differences and pathogenicity were described (Lewis et al. 1991). CTX-1 is the major toxin found in carnivorous fish were it typically contributes to approx. 90% of total lethality and poses a health risk at levels above 0.1 µg/kg fish (Murata et al. 1990; Lewis et al. 1991; Legrand et al. 1992; Lewis and Sellin, 1992).

The ciguatoxins involved in ciguatera intoxication are competitive inhibitors of the brevetoxins and have a common binding site on neuronal voltage dependent sodium channels. Even if their action remains partly understood a prolongation of axonal sodium channel activation, by contrast with other fish toxins is now described as the main mechanism by most authors. (Angibaud and Rambaud, 1998). So, the ciguatoxins are characterised 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 sodium ions, cell depolarisation and the appearance of spontaneous action potentials in excitable cells. Calcium is an intracellular trigger for muscular contraction (Lewis and Endean, 1986). A similar mechanism of ciguatera-induced intracellular transport of calcium occurs in intestinal epithelial cells. This increased concentration of calcium acts as a second messenger in the cell as it disrupts important ion exchange systems resulting in a fluid secretion which causes diarrhoea (Lewis and Endean, 1986; Hallegraeff et al. 1995). Ciguatoxin has increased binding affinity during channel activation (Lewis and Endean, 1986).

Cardiovascular effects associated with ciguatera are thought to result from a positive inotropic effect of the toxin on myocardium (Lewis, 1988). The contractile effects of ciguatoxin on the various types of muscles (skeletal, cardiac, and smooth) have been explained by a direct action on muscle Na^+ channels or by an indirect action on Na^+ channels of the nerves supplying the muscles, or by both. (Molgo et al. 1992).

5.2.2 Other toxins mentioned to play a role in ciguatera

With a LD_{50} of 0.17 µg/kg intraperitoneally in mice maitotoxin is more toxic than ciguatoxin (Bagnis et al. 1986). It is unlikely that maitotoxin affects sodium channels, maitotoxin (MTX) may act to mobilise intracellular calcium by means of a second messenger pathway (Sortino et al. 1991). The maitotoxins are approx. 100-fold less potent by the oral route compared with the intraperitoneal route, whereas the ciguatoxins are equipotent (Lewis et al. 1991). The different maitotoxins possibly involved are summarised in table 1.

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 (Chungue et al. 1977).

With a LD_{50} of 210 µg/kg intraperitonally in mice**,** okadaic acid is substantially less potent than ciguatoxin (Dickey et al. 1990). Because of similarities in origin, molecular properties, and presence in confirmed ciguatoxin fish, okadaic acid has been suggested as a factor in the etiology of ciguatera (Kimura et al. 1982).

Table 1 [1]. Effects of structurally defined dinoflagellate polyether toxins administered intraperitoneally (ip.) to 20 g mice.

Toxin	**ip. LD_{50}** (µg/kg b.w.)	**MU[2]** (ng)	**Signs of intoxication**	**Min./max. time to death [3]**
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 minutes/€ 24 hours
CTX-2	2.3	9	As for CTX-1, plus progressive hind limb paralysis	53 minutes/ €100 hours
CTX-3	0.9	18	As for CTX-1, plus progressive hind limb paralysis	60 minutes/ € 26 hours
GTX-4B	4.0	80	As for 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 minutes/€ 72 hours
MTX-2 [4]	0.08	1.6	As for MTX-1	41 minutes/€ 72 hours
MTX-3 [4]	€0.1	€2	As for MTX-1	72 minutes/€ 72 hours

[1] This table is copied from a larger table composed by Hallegraeff et al. (1995).
[2] Mouse unit, where 1 MU = LD_{50} 50 (ie. 1 MU is the LD_{50} dose for a 20 g mouse)
[3] Minimum time to death estimated as described by Molinengo (1979); maximum time to death estimated from effects of doses near the LD_{50} dose.
[4] From *Gambierdiscus toxicus* but are unlikely to accumulate to significant levels in the flesh of fish.

5.2.3 Experimental data

5.2.3.1 Human data

Cameron and Capra (1993) selected five patients from five separate outbreaks of ciguatera poisoning in the Brisbane area who were still experiencing intense paresthesia, to perform temperature studies in which their hands were immersed into baths of water of different temperatures. The first experiment was to determine whether or not a person could distinguish a normal progression of gross temperature changes (0 to 50 C). In a second experiment the ability to distinguish between two baths of different temperatures was assessed. The third experiment was designed to assess the severity of the paradoxical symptoms associated with cold temperatures and to determine if a distinct cut off temperature for these symptoms existed. All patients could accurately distinguish between baths of different temperatures and each patient was able to describe a normal gradation of gross

temperature perception, so the gross temperature perception covering a range from very cold to hot remains normal in ciguatera poisoning. The cut-off point of the peculiar symptoms described as reversal of temperature perception (such as tingling, burning, smarting and electric) was recorded around 24-26 C and this temperature appears to correlate very closely to the cold threshold from C-polymodal nociceptors (23 C). This finding suggest that the paradoxical sensory discomfort experienced is, most likely, a result of an exaggerated and intense nerve depolarisation occurring in small peripheral nerve tissue such as A-delta myelinated 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. C-polymodal nociceptors are not spontaneously active at normal temperature in the undamaged skin but show to have a threshold above 40 C and a cold threshold below 23 C (Torebjörk, 1974). According to the authors the symptoms described by the five patients with mild ciguatera poisoning are a result of a disturbance in the peripherical C-polymodal nociceptor and A-delta fibres. 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 (Bagnis et al. 1979; Lawrence et al. 1980).

5.2.3.2 Animal data

Benoit et al. (1996) carried out experiments 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. As shown by using a confocal laser scanning microscopy, 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.

A study with male mice (4-weeks old; 23-26 g) was carried out to determine the origin of watery secretion and to clarify the type of diarrhoea of ciguatoxin poisoning (Ito et al. 1996). Semi-pure ciguatoxin (85.7%) came from extracts 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 previously given definition on page (Lewis and Sellin,1992) To estimate the potency of CTX causing diarrhoea, it was compared with that of cholera toxin. CTX was administered by gastric tube, and intraperitoneal 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 it did not cause diarrhoea.
- there was an effective dose range to cause diarrhoea of 0.14 – 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 influenced.
- diarrhoea was finished within 1 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 choleratoxicosis. The potency of CTX to cause diarrhoea was suggested to be about 1300-8500 times stronger than that of cholera toxin.

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. (Lewis, 1988).

In mice symptoms are well defined and hypothermia is a characteristic response. However, whether ciguatoxin has direct central nervous system effects and what its targets in the brain may be are not known. Peng et al. (1995) investigated the action of intraperitoneally administered ciguatoxin (0.5 MU as defined by Lewis and Sellin, 1992); isolated from the *G. toxicus* MQ2 Caribbean strain) in ICR female mice 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.

In order to study the histopathological and ultrastructural changes of various organs after the administration of ciguatoxin, male ICR mice were given ciguatoxin or ciguatoxin-4c (at a dose level of 0.7 μg/kg body weight) by oral route and via intraperitoneal route. The authors (Terao et al. 1991;1992) did not specify what ciguatoxin-4c was. Also 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 autonerves 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. (Terao et al. 1991).

The morphologic response of the mouse heart was examined after repeated (15 days) low dose (0.1 μg/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 μg/kg body weight) was investigated for both exposure routes and for both toxins. A single dose of 0.1 μg/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 of this study 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. (Terao et al. 1992).

6. Treatment

Medical treatment for ciguatera patients is based on elimination or reduction of the symptoms. 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. Several symptomatic therapies are used, 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. (Pearn , 1994). Mannitol given to the ciguatera patient is safe, and no synergism between the toxin and mannitol has been observed.

Only one single blind controlled trial (patients were unaware of the treatment received) has been reported by Bagnis et al. (1991). This trial showed that 250 ml of 20% mannitol given intravenously in 1 hour was slightly more effective than a combination of vitamins and calcium also given intravenously in 1 hour. Treatment with 20% mannitol solution in water intravenously at a dose of 1 g/kg body weight at an initial rate of 500 ml/hour caused an improvement in the symptoms. (Eastaugh, 1996).

The mechanism of mannitol treatment is not completely understood. One theory is that mannitol actually competes with sodium channels (Palafox et al. 1988; Lewis et al. 1992a). 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 (Pearn et al. 1989; Stewart, 1991). 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 (Swift and Swift, 1993 and references therein).

It has also been suggested that the presence of mannitol in the extracellular fluid sterically inhibits the movement of sodium ions through channels which have been blocked by the ciguatoxin molecule (Stewart, 1991). Another suggestion is that mannitol may act as a scavenger for hydroxyl radicals in ciguatoxic systems (Pearn et al. 1989).

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 (Bagnis et al. 1979).

7. Outbreaks of ciguatera poisoning

7.1 Occurrence

7.1.1 Factors influencing outbreaks

Over the last decades evidence is accumulating that reef disturbance by military and tourist developments increase the risk of ciguatera by increasing benthic substrate for dinoflagellate growth. As vectors for the dispersal of marine plankton (or their resistant spores) vessel ballast water and translocation of shellfish stocks were mentioned. Certain weather patterns and ecologic disturbances (tidal waves, earthquakes, hurricanes) are thought to be some of the natural causes of outbreaks. (Bagnis et al. 1985; Barton et al. 1995).

In addition to natural disturbances (such as cyclones), a human role in the occurrence of ciguatera is suggested including (1) collecting of marine animals, (2) fishing and the use of dynamite and poisons, (3) sewage and eutrophication, (4) petroleum hydrocarbons, heavy metals, and power plants, (5) dredging and filling, (6) construction activity, and (7) recreational activities (Salvat, 1987). Also the damage to coral reefs by the anchors and chains of cruise ships in the Caribbean islands has raised concern. (De Sylva, 1994)

Although there seems no seasonal variation in the occurrence of ciguatera intoxication according to De Sylva (1994), the frequency of ciguatoxic barracuda caught varied seasonally during the period of the study, with peak values (60-70% toxic fish) in the late winter-early spring (January-May) and fall (August-November). Minimal frequencies (0-10% 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 (Lech et al. 1982).

7.1.2 Geographical distribution

Around 400 million people live in areas where ciguatera fish poisoning is present.
Ciguatera is the most common of all fish poisonings, it is common in tropical and subtropical regions in a belt between laterals 35ºN and 35ºS. (Lange et al. 1992 and references therein). Although ciguatera is endemic in all nations of the Pacific and Caribbean region, isolated outbreaks are increasingly recognised in temporate countries (fish caught in endemic countries can be shipped elsewhere), including countries in Canada and even Europe. Since the notification of cases is voluntarily, little reliable data are available. (Ting et al. 1998)

<u>Pacific region</u>
Being a rare disease two centuries ago ciguatera now has reached epidemic proportions in French Polynesia. In the period 1960 to 1984 more than 24,000 patients were reported from this area. (Hallegraeff et al. 1995)
Ciguatera is also common in Australia (Gillespie et al. 1985), Puerto Rico, American Samoa, Guam, and the Virgin Islands. (Lange et al. 1992 and references therein).

<u>US</u>
Outbreaks have been reported from Hawaii and Florida, whereas other intoxications in different places in the US have been associated with consumption of imported fish (Smith et al. 1998)

Indian Ocean
In the Indian Ocean, ciguatera poisoning was first described in Mauritius, on the east coast of Madagascar. (Quod and Turquet, 1996).

7.1.3 Incidence

Despite frequent occurrences of ciguatera poisoning in many parts of the world, ciguatera poisoning is rarely fatal because of the low concentration of the toxin in fish flesh. Even by restricting to moray eel viscera, since the viscera contain more toxin than fish flesh, it took 75 kg of toxic viscera (approximately. 1,100 kg of eels) to produce 1.3 mg of HPLC-pure ciguatoxin (Tachibana et al. 1987). Depending on the geographic region, 5 to 600 cases per 10,000 people are reported annually (Lange, 1994 and references therein). The incidence figures are likely to represent only 10 to 20% of actual cases, with the extent of under-reporting likely to vary between countries and over time. Reasons for under-reporting of ciguatera include non-reporting of confirmed cases and mis-diagnosis, with mild cases often mistaken for common illnesses by victims. (Lewis, 1993)

Pacific region
The mean reported incidence rate of ciguatera for the South Pacific islands during a five-year period (1979 to 1983) was 97/100,000 (Lewis, 1986). The South Pacific Commission reported a mean annual incidence of 217 per 100,000 population in 1987 (cited in Glaziou and Legrand, 1994). During the years 1985-1990 the Pacific Islands Kiribati, Tokelau and Tuvalu reported 90 – 100 cases/10.000 population *per annum*. In French Polynesia, Vanuatu, Marshall Islands, and Cook Islands the reported cases varied from ca. 35 to 50/10.000 population *per annum*. Less than 20 cases/10.000 population *per annum* 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 Epidemiological and Health Information Service. (Lewis, 1993).

Australia
In Australia an annual incidence of 30 per 100,000 was estimated by Capra and Cameron (1985). The annual incidence in Queensland is reported to be about 1.6 cases per 100,000 population (Ruff and Lewis, 1994).

Indian region
Very little information is available on incidence in the islands in the Indian Ocean (Madagascar, Comores, the Seychelles, Mauritius and Rodrigues) but the annual incidence rate was estimated to be 0.78/10,000 residents. (Quod and Turquet, 1996)

US
From 1983 through 1992 in the US, 129 outbreaks of ciguatera poisoning involving 508 persons were reported, no ciguatera-related deaths were reported. Most outbreaks were reported from Hawaii (111) and Florida (10), the other outbreaks in different places in the US have been associated with consumption of imported fish. (Smith et al. 1998). Gollop and Pon (1992) reviewed the Hawaii State Department of Health epidemiological records for cases of ciguatera poisoning during a five year interval: from January 1984 through December 1988. A total of 150 outbreaks were reported during these years and involved 652 exposed individuals, resulting in 462 cases showing symptoms of ciguatera intoxication (overall attack rate 70.9%). The Kona coast of the island of Hawaii was responsible for most incidents.

Furthermore 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 (Anderson et al. 1983). In Miami, the estimated annual incidence rate is 50 per 100,000 population (Lawrence et al. 1980). A household survey in the US Virgin Islands showed an annual incidence rate of 730 per 100,000 population (Morris et al. 1982b)

Worldwide
It is estimated that worldwide over 25,000 people annually are affected by ciguatera fish poisoning (Lewis and Sellin, 1992).

7.2 Case reports

In this section only recent case reports are included.

Arafura region
An outbreak of ciguatera poisoning was reported arising from eating a single fish (coral cod) captured from the Arafura Sea (Northern Australia) that resulted in 20 poisoning events. When a 230 g sample of the fish was analysed by mouse bioassay and HPLC/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 determined. (Lucas et al. 1997).

Bahamas
When seventeen crew members of a Norwegian cargo ship had eaten a barracuda caught near the Cay Sal Bank of the Bahamas on 12 October 1997 they became ill with nausea, vomiting, diarrhoea, and muscle weakness 2 to 16 hours later, all showing symptoms of ciguatera poisoning. Three samples of leftover raw barracuda and red snapper that were caught simultaneously with the barracuda that was eaten, were tested for ciguatoxin using an experimental membrane immunobead assay. The samples from both fish tested positive for ciguatoxin. (Smith et al. (1998)

Canada
Bruneau et al. (1997) describe 5 cases of ciguatera poisoning in Quebec resulting from eating fish (barracuda) in a Montreal restaurant. All persons showed gastrointestinal symptoms (vomiting, nausea and diarrhoea) within hours after ingestion of the fish. They also showed pruritis, and one or more neurological symptoms within 12 to 24 hours such as temperature reversal (all), light-headedness (4), shivering (4), and paresthesia of hands and feet (3).

China
In the article of Chan and Wang (1993) cases of ciguatera poisoning in Hong Kong are reported for the first time. A short time after eating a mangrove snapper caught in the South China Sea, all four persons became ill, showing the gastrointestinal and neurological features (nausea, abdominal pain, diarrhoea, paraesthesia and numbness of extremities) typical for ciguatera poisoning. One patient showed also a life-threatening bradycardia and hypotension.

Germany
Sanner et al. (1997) describe a case of ciguatera poisoning in a 40 year old man in Germany following a travel to the Dominican Republic. The man showed the characteristic ciguatera symptoms after having eaten a meal of grouper. After his return in Germany he was admitted

to the hospital. Due to the typical history and clinical findings ciguatera toxin ingestion was diagnosed. All symptoms finally resolved after 16 weeks.

Haiti
In February 1995, six U.S. 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 1 to 3 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. (Poli et al. 1997)

Hawaii
The following report describes a confirmed ciguatera poisoning in 1985 (confirmed in leftover fish by immmunoassay EIA) 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 neurologic symptoms within 1.5 to 6 days. Furthermore 10 of the 15 persons demonstrated cardiovascular symptoms, such as bradycardia and hypotension. Duration of the illness ranged from 2 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 severity !) was correlated with both increasing age and weight, and was independent of amount and components of toxic fish consumed. (Katz et al. 1993).

Madagascar
Habermehl et al. (1994) described the first case of a very severe outbreak presumably caused by a shark. The outbreak occurred in Manakra, a city on the East coast of Madagascar on 23 November 1993 and had a mortality rate of 20% (98 out of 500 poisoned people died). When the medical team arrived 5 days after the tragedy most of the serious cases had already died. 150 Patients were still in hospital (35 in a critical state and 15 of them died within a few days). The symptomatology presented by the patients in critical state are not indicative because 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 moderate poisoned persons (115 cases) were typical for ciguatera poisoning. Unfortunately, no remains of the shark were available for chemical investigations.

Mexico
Lechuga-Devéze and Sierra-Beltrán (1995) describe the first confirmed case of ciguatera in Mexican waters. In May 1993, the entire crew of a fishing boat became ill with symptoms resembling ciguatera, after eating fish that were caught in the Alijos Rocks at depths fluctuating between 9 and 36 meters. After analysis of the suspected fish using the mouse bioassay, the presence of ciguatera-like toxins was confirmed. The surge of ciguatoxic activity at Alijos Rocks seems to have started in 1992.

25 Cases of ciguatera poisoning on the Pacific Coast of the US 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 parasthesias, pruritis, paresis, dizziness, headache), one woman had bradycardia and hypotension. (Barton et al. 1995).

Rhode Island
DeFusco et al. (1993) describe a male patient in Rhode Island suffering from ciguatera poisoning 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 'nonedible toxic'. The mouse bioassay resulted in death of the mouse within 48 hours, but the mouse response did not show all ciguatera-like responses. The guinea pig atrium assay was negative, both atria did not show the typical inotropic response to ciguatera toxin.

The Netherlands
5 patients with symptoms of ciguatera poisoning were seen in a hospital in Amsterdam (the Netherlands) in the policlinic outpatients' department of Tropical Medicin. The patients had eaten fish in Curaçao and Isla de Margarita (Venuzuela). Ciguatera could only be diagnosed based on the clinical symptoms and the fact that a fish was eaten in the Caribbean area. (Wetsteyn et al. 1995).

Tonga
Zlotnick et al. (1995) report a ciguatera fish poisoning associated with cindarian (jellyfish and related invertebrates) ingestion. Cindaria have not previously been associated with direct ciguatera intoxication in humans. A 12-year old Tongan girl had eaten jellyfish about 2 hours prior to the presentation of gastrointestinal and neurologic symptoms characteristic for ciguatera poisoning. All other persons who had eaten the jellyfish were without symptoms, which might suggest that she had prior ciguatoxin intoxication with sensitisation 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 1-5 scale), strongly positive, and comparable with values previously obtained from acute ciguatera fish poisoning 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.

7.3 Intoxication during pregnancy

Fenner et al. (1997) describe a family of four in Queensland (Australia), two children, father and mother who was 11 weeks pregnant, 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% 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. This is the only case known of severe ciguatera poisoning in the first trimester of pregnancy and it appears that the poisoning had no influence on the baby.

A pregnant woman in San Fransisco started to have symptoms characteristic of ciguatera poisoning, four hours after she had eaten a large portion of a barracuda fish. 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. (Senecal and Osterloh, 1991). In contrast to this case, Pearn et al. (1982) reported a newborn suffering from left-sided palsy and possible myotonia of the hands after a ciguatera intoxication of the mother. This may be explained by a difference in exposure dose and/or the different gestational timing (second versus third trimester).

8. Monitoring Programs/Regulations

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 (Dawson, 1977), Queensland (Lewis et al. 1988), French Polynesia (Lewis, 1986), Fiji (Sorokin,1975), Hawaii (Gollop and Pon, 1992), and Miami (Craig, 1980). The bans were apparently with some success, but with attendant economic loss.

Europe

In the EU, Council Directive 91/493/EEC 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 the French Legislation and it is applicable for products imported in France from outside the EU. The regulation permits the import of certain marine fish species, for which a positive list exists (Ledoux and Fremy, 1994).

USA

In Florida there are restrictions on the sale of certain fish species and sizes. In Hawaii a limited program has been instituted using an immunoassay; fish testing positive are considered unsafe and removed form the market (Van Egmond et al. 1992).

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. (Lewis et al. 1994b). Reef carnivores such as the moray eel, chinaman, red bass and paddletail fishes have long been considered regular ciguatera carriers and are now not sold by marketing authorities in Australia. (Ting et al. 1998)

9. Prevention

Apart from the avoidance of consumption of large predatory fish the use of animal screening tests are the only tools presently available to prevent intoxication. (Juranovic and Park, 1991). Presently there is no commercially available test shown to reliably detect the levels of ciguatoxin which contaminate ciguateric fish, however, the bioassays as screening test has considerable merit and warrants further research.

The major source of ciguatera cases has been the fish caught by sport fishing (79%). 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 ciguatoxins intoxication, the incidences of ciguatera would decrease dramatically (Gollop and Pon, 1992).

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 (Lewis, 1993).

10. Recommendations

A routine monitoring scheme particular in the area endemic for ciguatera should be established. Major advances in the analytical methodology are still required, however, before ciguatoxin can be detected as a part of a routine monitoring scheme to reduce the risk of ciguatera. Standards and reference materials for ciguatoxin are needed. Important recent advances in knowledge of the chemistry of the toxins involved in ciguateric fish and in screening methods have laid the foundations for such advances. (Hallegraeff et al. 1995).

Future research should include the following: (Lewis, 1993; Scheuer, 1994)

- determine the precise parameters, (ecological and/or genetic) that trigger a population explosion of dinoflagellates to render algae- and dendritus-feeding fish toxic to humans.
- develop improved treatment regimes, including simpler, orally effective, therapies.
- develop assays that distinguish toxic from non-toxic fish in a cost-effective and reliable manner.
- elucidate the molecular structure of ciguatoxin and its congeners completely (this seems still not be done).
- standardise definition of the mouse unit; the best approach would be to transpose mouse unit by means of a calibration curve into μg/kg fish tissue (liver or muscle flesh) expressed as CTX-1 equivalents.

Nomenclature should be standardised and internationally be agreed about.
Vernoux and Lewis (1997) propose a standard nomenclature for naming ciguatoxins, which is CTX together with a letter code prefix to indicate the region were it occurs and a numbering system to indicate the chronological order of reporting (e.g. P-CTX-1 is the major ciguatoxin from the Pacific Ocean).

Recommendations particularly applicable to the Netherlands

Ciguatera poisoning is sporadic in Europe, and the poisoning is rarely fatal. Therefore in Europe and particularly the Northern countries as the Netherlands which are not an endemic area for ciguatera (at present) it is considered adequate to have a regular analytical check on presence of ciguatoxins in imported large predatory fish from endemic areas. In addition education of medical staff people is needed. This would enable them to recognise a case of ciguatera poisoning and to take the most appropriate actions for the patients.

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