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REPORT OF THE EXPERT MEETING ON CIGUATERA POISONING

ROME, 19–23 NOVEMBER 2018

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
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ABBREVIATIONS AND ACRONYMS

ADME	absorption, distribution, metabolism and excretion
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
C-CTX	Caribbean ciguatoxin
CBA	cell bioassay
CDC	Centers for Disease Control and Prevention
CP	ciguatera poisoning
CIFOCOss	FAO/WHO Chronic Individual Food Consumption database
CTX	ciguatoxin
EFSA	European Food Safety Authority
ELISA	enzyme-linked immunosorbent assay
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration (the United States of America)
FISH	fluorescence in situ hybridization
GABA	gamma amino butyric acid
GEADE	Global Estimate of Acute Dietary Exposure
HRMS	high-resolution mass spectrometry
HTS	high-throughput sequencing
I-CTX	Indian Ocean ciguatoxin
IAEA	International Atomic Energy Agency
ICR	Institute of Cancer Research
IESTI,	International Estimate of Short-term Intake
InsP3	inositol triphosphate
ip	intraperitoneal
iv	intravenous
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC-MS	liquid chromatography coupled with mass spectrometry
LD	lethal dose
LOAEL	lowest observed adverse effect level
LOD	limit of detection
LOQ	limit of quantitation

MBA	mouse bioassay
MTT	3-[4,5-dimethylthiazole-2-yl]-2,5 diphenyltetrazolium bromide
MTX	maitotoxin
MU	mouse unit
N2A (N2a)	mouse neuroblastoma assay
NMDA	N-methyl-D-aspartic acid
NOAA	National Oceanic and Atmospheric Administration
NOAEL	non-observable adverse effect level
P-CTX	Ciguatoxins (CTX) initially isolated from biota from the pacific region
PbTx	brevetoxin
PCR	polymerase chain reaction
PICTs	Pacific island countries and territories
PKS	polyketide synthase
PSS	Poison Severity Score
qPCR	quantitative polymerase chain reaction
RASFF	Rapid Alert System for Food and Feed
RBA	receptor binding assay
RFLP	restriction fragment length polymorphism
SEM	scanning electronic microscopy
SP	substance P
SPATT	solid phase adsorption toxin tracking
STX	saxitoxin
TEF	toxicity equivalency factor
TRPA1	transient receptor potential ankyrin 1
TTX	tetrodotoxin
UHPLC	ultra-high-pressure liquid chromatography
Vd	volume of distribution
VGSC	voltage-gated sodium channel
VGPC	Voltage-gated potassium channel
WHO	World Health Organization

DECLARATIONS OF INTEREST

All participants in the Joint FAO/WHO Expert Meeting on Ciguatera Poisoning completed a declaration of interest form in advance of the meeting. In relation to the subject of this Expert Meeting, the following declarations were made: (i) Ann Abraham, James M. Hungerford and Clémence Mahana declared having paid employment; (ii) Mireille Chinain, Ana Gago-Martínez, *David Timothy Harwood, Philipp Hess, Iddya Karunasagar and Clémence Mahana declared having received or anticipating research support; (iii) Marie-Yasmine Dechraoui Bottein, Jorge Diogene, Ana Gago-Martinez, David Timothy Harwood and Clémence Mahana reported participating in an expert committee or scientific advisory group; (iv) Peter Cressey and Jorge Diogene have provided expert opinion or testimony as part of a regulatory, legislative, judicial, or other governmental process; and (v) David Timothy Harwood indicated knowledge of a technology that could be impacted by the outcome of the meeting.*

Following the FAO Guidance Document for Declaration of Interests, the declarations noted above were assessed as to the extent to which each interest could be reasonably expected to affect and exercise influence on the experts' judgement. The declared interests of Ann Abraham, Mireille Chinain, Peter Cressey, Marie-Yasmine Dechraoui Bottein, Jorge Diogene, Ana Gago, David Timothy Harwood, Philipp Hess, James M. Hungerford, Iddya Karunasagar and Clémence Mahana were considered unlikely to impair their objectivity or have a significant influence on the impartiality, neutrality and integrity of the work. Meeting participation by these individuals was neither reasonably expected to create unfair competitive advantages, nor were the meeting outcomes reasonably foreseen to affect the individuals' declared interests. Neither FAO nor WHO received any public comments in response to the online posting of the names and brief biographies of the individuals considered for participation in the Expert Meeting. The interests of all participants were disclosed to all attendees at the beginning of the Expert Meeting.

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EXECUTIVE SUMMARY

Ciguatera poisoning (CP) is reported in historical documents of the sixteenth century. The first report of the organism *Gambierdiscus* (originally referred to as *Goniodoma* sp.) dates from October 1948, in Cabo Verde. Today, the term ciguatera identifies poisoning caused by the ingestion of certain reef fish and shellfish from tropical and subtropical regions, especially the South Pacific Ocean, Indian Ocean and the Caribbean Sea. Through the food chain, these fish and shellfish have accumulated certain lipid-soluble toxins (ciguatoxins [CTXs]) that are produced by dinoflagellates of the genera *Gambierdiscus* and *Fukuyoa.* Ciguatera is a worldwide problem that is expanding due, among other reasons, to climate change. In general, CP can be regarded as the most significant non-bacterial poisoning associated with fish consumption worldwide. A typical sign of the poisoning is cold allodynia, and there are more than 175 gastrointestinal, cardiovascular and neurological symptoms. It is unclear whether the toxins cause harm to those herbivorous or carnivorous fish that take them through the food chain.

In 2016, at the Thirty-Second Session of the Codex Committee on Fisheries and Fishery Products, the Pacific Nations raised CP as an issue that is increasingly affecting the tropical and subtropical regions of the Pacific Ocean, Indian Ocean and Caribbean Sea between the latitudes of 35°N and 35°S. Indeed, it was noted that, due to climate change, the frequency of storms and hurricanes is increasing, as is the sea surface temperature, which affects the distribution and proliferation of CTXs and makes the occurrence of CP less predictable. The issue of CP was raised at the Eleventh Session of the Codex Committee on Contaminants in Food. The Committee agreed to request scientific advice from FAO/WHO to enable the development of appropriate risk management options, in particular: full evaluation of known CTXs (toxicological assessment and exposure assessment), including geographic distribution and rate of illness, congeners, and methods of detection; and guidance for the development of risk management options.

There are now 16 described *Gambierdiscus* species: *G. australes*, *G. balechii*, *G. belizeanus*, *G. caribaeus*, *G. cheloniae*, *G. carpenteri*, *G. carolinianus*, *G. excentricus*, *G. pacificus*, *G. polynesiensis*, *G. scabrosus*, *G. toxicus*, *G. silvae*, *G. lapillus*, *G. honu* and *G. jejuensis*. Recently, two globular species *Gambierdiscus* have been reclassified as *Fukuyoa* (*F. yasumotoi* and *F. ruetzleri*), and a new species described (*F. paulensis*). Both *F. ruetzleri* and *F. paulensis* produce toxins. Optimum growth takes place between 26.5 °C and 31.1 °C, with thermal limits from 15–21 °C to 31–34 °C, and salinities from 24.7 g/litre to 35 g/litre with light irradiances below 231 µmol photons per square metre per second. A variety of techniques can be considered for the identification of species at any given site, including optical microscopy as

a screening tool, and scanning electronic microscopy (SEM) and/or molecular techniques (sequencing, PCR, RFLP, FISH probes, etc.) as confirmation tools.

Gambierdiscus cells are distributed in a very patchy manner; coefficients of variation among adjacent samples range from 50 percent to > 150 percent. The frequency distributions of average cell densities are similar in both the Atlantic and Pacific. Ten percent of the abundance estimates are between 1 000 cells/g wet weight algae and 10 000 cells/g wet weight algae, with 5 percent exceeding 100 000 cells/g wet weight algae.

More than 425 species of fish have been linked to ciguatera events. Coral reef fishes contribute to the expansion of CP intoxications. Reef fish known to potentially accumulate these toxins are: barracuda (Sphyraenidae), amberjack (*Seriola*), grouper (Serranidae), snapper (Lutjanidae), po'ou (*Labridae* spp.), jack (*Carangidae* spp.), trevally (*Caranx* spp.), wrasse (*Labridae* spp.), surgeon fish (*Acanthuridae* spp.), moray eel (*Muraenidae* spp.), roi (*Cephalopholis* spp.), and parrotfish (*Scaridae* spp.). A large variety of marine invertebrates including urchins, gastropods, bivalves and echinoderms have also been reported to contain CTXs, but their implication in CP is far less important than fish. Due to world trade, and consumption of imported fish there are poisoning reports in many geographic areas, such as Canada, Germany (e.g. Hamburg), the Paris area (France) and California, New York, Rhode Island and Vermont (the United States of America), and also CP has been reported after returning to their countries by patients having consumed ciguateric fish in endemic areas. Global warming is facilitating the expansion of *Gambierdiscus*, but there are bodies of water that are warm enough to depress their growth. Several reports indicate the presence of *Gambierdiscus* in new areas (Brazil, Morocco or Thailand), but there is no solid link yet to this being caused by climate change. Another subject not yet clarified is how the change in pH and carbon dioxide (CO2) levels and in sea surface temperatures may affect toxin production, as the growth of microalgae is influenced by these parameters. The approach followed by many countries is to impose fish size restrictions as a ciguatera risk management action, but the toxicity of some species is associated to seasonal variations and for most species, there is no proven correlation between the toxicity of fish and their size/weight.

Ciguatoxins (CTXs) are a class of large polyether ladder-like lipid-soluble compounds that are thermostable and resistant to mild pH changes; they contain 13–14 fused rings. Representative backbone structures of CTXs identified to date are represented by CTX4A, CTX3C and C-CTX1. The diastereoisomers 52-*epi*-54-deoxyCTX1B and 54-deoxyCTX1B (also known as CTX2 and CTX3, respectively) represent less oxidized forms of CTX1B. The only difference between CTX1B, 52-epi-54-deoxyCTX1B and 54-deoxyCTX1B involves modification at one end of the CTX. The backbone structure of CTX3C toxins differs from CTX4A group on the E-ring (i.e. an eight-membered ring in CTX3C and a seven-membered ring in CTX4A) and by the absence of an aliphatic side chain on the A-ring. Several toxic Caribbean CTX analogues have been identified and isolated from fish. The major Caribbean toxin is C-CTX1 and its 56-epimer C-CTX2. C-CTX2 was found as a minor analogue in fish. The C-CTX backbone shares characteristics with CTX4A and CTX3C but does not possess the aliphatic side chain on the A-ring and

it contains an additional ring on the right wing of the molecule. Additional Caribbean CTXs have been reported in several studies but have yet to be structurally elucidated. An Indian Ocean group has also been described, and although masses have been reported in the literature, no molecular structures have been determined to date. The most toxic analogue described to date is CTX1B, which is stable at 100 °C, 1 N NaOH, and in sunlight for 1 h, but loses toxicity in 1 N HCl after 10 min. Ciguatoxins are odourless, tasteless, heat stable and present at very low (typically < ppb) levels in contaminated seafood, making them difficult to detect without advanced detection methods. The toxic potency of CTXs has been shown to increase as they become more oxidized. Analogues isolated from the Pacific are currently thought to be the most potent and have been well characterized. Some CTXs are metabolites generated through the process of enzyme-mediated biotransformation in invertebrates and fish. Experimental CTX1B oral and intraperitoneal dosing studies in mice have confirmed the rapid absorption capacity of CTXs, and their ability to cross the blood–brain barrier. There is a similar compartmentalization of toxins across tissues into liver, spleen, brain, muscle, gonads, fat and bone. A remarkable feature of *Gambierdiscus* is its unique biochemical machinery, responsible for the production of multiple structurally complex polyether toxins, including CTXs, gambierol, gambierone, gambieroxide, gambieric acids and maitotoxins. Three families of CTXs have commonly been classified according to their geographical location, i.e. Pacific CTXs, Caribbean CTXs (C-CTXs) and Indian Ocean CTXs (I-CTXs). However, it is now possible and appropriate to classify CTXs based on the known chemical structures. The structural characteristics of CTXs actually allow further classification into two separate groups based on their chemical structure, i.e. CTX3C vs CTX4A backbones

and their derivatives. The Caribbean CTXs represent a third group. To date, only two Caribbean CTXs have been structurally elucidated (C-CTX1 and C-CTX2). A classification into five groups has been suggested, and this report uses this classification: CTX3C, CTX4A, C-CTX, I-CTX (the existence of this group is still speculative as the structure elucidation is pending), and other *Gambierdiscus* metabolites.

None of the methods described has been reported to have undergone single- or multi-laboratory validation. While some laboratories have produced reference materials and quantified standards on a small scale, recent data have most consistently reported CTX3C equivalents, owing to commercial availability. Several assays for the screening of fish samples for CTXs have been described, based on *in vitro* assays (N2A-MTT assay, immunoassays, receptor binding assay [RBA]) and *in vivo* (mouse) bioassays. These *in vitro* assays all have high throughput capacity due to the 96-well plate formats used, which allow parallel measurements at the respective endpoints of each assay. However, reliable quantification of the bioactivities and/or toxicity of CTXs in seafood extracts requires a certified reference and, where possible, a matrix-matched reference. Liquid chromatography coupled with mass spectrometry (LC-MS) is a suitable approach for the identification and confirmation of CTXs and related compounds in a range of matrices.

Information on fish consumption, particularly in countries with high rates of CP, is fragmentary. The estimated doses eliciting CP are in the range 48.4–429 pg/kg bw CTX1B equivalents. The minimum eliciting dose of 48.4 pg/kg bw CTX1B equivalents provides an estimate of a lowest observed adverse effect level (LOAEL) for CP in humans. Both the United States Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) have proposed a fish CTX concentration of 0.01 µg CTX1B/kg fish flesh as being unlikely to elicit symptoms of CP. This concentration is just below the lowest concentrations seen in fish samples associated with CP cases (0.02 µg CTX1B equivalents/kg fish flesh). As documented by mouse ip LD_{50}, variability in the potencies exists between some different analogues of CTXs. On the basis of LD_{50} estimates in mice, oral CTX1B (also known as CTX1) (0.22 µg/kg bw) was similar in potency to ip CTX1B (0.25 µg/kg bw). CTX1B and CTX4C have a cumulative effect on the cardiac tissue at a dose of 0.1 µg/kg bw for 15 days. A medium term low dose CTX1B exposure impairs spatial learning and reference memory in rats. Repeated exposures of rats (every 3 days for 8 weeks) to a low dose of CTX1B (0.065 µg/kg bw) after an initial high dose (0.26 µg/kg bw) leads to the development of anxiety-like behaviour learning and memory deficits, and decision-making impairment. Neurotoxic effects in rats are similar to symptoms reported in humans. After about 18 weeks, mice treated with 0.1 µg/kg bw show hypertrophy and histological changes in the heart. No effects are observed in mice treated with 0.05 µg/kg bw. A non-observable adverse effect level (NOAEL) for heart toxicity is 0.05 µg/kg bw (after 40 weeks, once a week). None of these studies were considered suitable to establish a health-based guidance value (acute or chronic). Human data allowed identification of a LOAEL of 50 pg CTX1B/kg bw after actute exposure.

The main target of CTXs is the voltage-gated sodium channel (VGSC, Na_v), causing hyperexcitability of the nerve membrane, eliciting spontaneous and repetitive action potentials by interacting with receptor-site 5 of the alpha subunit pore of the VGSC. Ciguatoxins show affinity for all the VGSC isoforms (Na_v 1.1–1.9), with differences in potency between the different toxin types. CTX binding to the VGSC causes a shift in voltage dependence of Na^+ conductance to more negative membrane potentials, allowing an increase in Na^+ influx and spontaneous action potential firing. The overall effect is an increase in excitability. This action, especially in peripheral nerves where binding of CTXs to Na_v is long-lasting, explains most of the effects of the group.

Data on CTX toxicokinetics in humans are very limited. Many ciguatera cases express central nervous system symptoms, suggesting that CTXs can enter the brain. Ciguatoxins have been measured in blood several hours after ingestion. However, toxins may not persist long in blood as they are undetectable in serum, plasma or urine 90 hours after poisoning. Ciguatoxins have been detected in human liver in an autopsy of a lethal case six days after fish consumption. Case reports describing symptoms among infants of ciguatera-affected mothers suggest that women may eliminate toxins via breast milk, and that toxins may be resorbed through breast milk. Transplacental toxin transfer is possible. The consumption of ciguatoxic fish is followed by the onset within 48 hours of specific, incident neurological symptoms: cold allodynia (which may be considered as nearly pathognomonic), paraesthesia, dysaesthesia, pruritus, myalgia, arthralgia and/or dizziness.

Unspecific symptoms such as severe fatigue and any kind of pain (e.g. myalgia, arthralgia and dentalgia) are very common. More than 175 different symptoms have been reported to date. Chronic ciguatera symptoms are those that persist beyond three months after the initial poisoning, and concern at least 20 percent of ciguatera-affected persons. Ciguatera poisoning may have neurological, psychiatric and/or general symptoms that can persist for months or years after the initial poisoning. There is no specific treatment. The fatality rate has been estimated as < 0.5 percent, but in some contexts may exceed 10 percent. Death due to CTX exposure often follows cardiovascular and/or complications of the central nervous system. It might be preventable by avoiding consumption of fish heads, liver and viscera, or possibly through better clinical management practices. If, as suspected, only 10–20 percent of actual intoxications are formally reported to the authorities, the problem of CP poisoning is much larger than official figures show.

Although there are many gaps in the available information about CP, there are some needs that require urgent attention regarding both risk management and research. The main needs for risk management are for the definition of clear protocols to avoid the risk of consuming toxic fish, mainly by local people and tourists, but also consumers purchasing imported fish from certain areas. This includes a well-defined information and outreach programme, and a clear identification of the geographic distribution of fish and causative organisms. The main research needs refer to detection methods, both screening and analytical, and the need to have a stable supply programme of analytical standards.

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CHAPTER 1
INTRODUCTION

1.1 BACKGROUND

The first historical event involving a ciguatera poisoning was reported in 1521, it affected several captains of the Spanish army in the Gulf of Guinea and led to the death of Juan Sebastian Elcano and others (Urdaneta, 1580; Baeza, 2009). Captain Cook's crew was also poisoned in 1786, as described by the ship's surgeon (Pearn, 1994). In 1787, Parra described the neurological symptoms after ingestion of a local gastropod (*Livona* sp.) in the Antilles, calling the poisoning "siguatera" [cited in Lee, 1980]). The first report about the toxin-producing organism *Gambierdiscus* (originally referred as *Goniodoma* sp.) dates from October 1948, in Cabo Verde (Sousa e Silva, 1956). In 1955, Martyr described (Gudger, 1930; Holmes, Brust and Lewis, 2014) Ciguatera poisoning (CP) as the term used for an poisoning originally named in Cuba after the ingestion of *Turbo pica*, a marine snail called cigua by local people (Guzmán-Pérez and Park, 2000; Gudger, 1930).

Today, the term ciguatera identifies an poisoning syndrome caused by the ingestion of certain reef fish and shellfish from tropical and subtropical regions, especially South Pacific, Indian Ocean and the Caribbean. These fish and shellfish have accumulated certain toxins (ciguatoxins [CTXs]) through the food chain. These lipid-soluble toxins are produced by dinoflagellates of the genus *Gambierdiscus* and *Fukuyoa* (Vlamis and Katikou, 2014). Ciguatera is a worldwide problem, in some countries it is considered a globally neglected tropical disease that is expanding due, among other reasons, to climate change (Gingold, Strickland and Hess, 2014; Rhodes *et al*., 2014a; Hallegraef, 2015). Mild cases of palytoxin poisoning have been wrongfully identified as ciguatera (Lewis and Holmes, 1993), but in general CP can be regarded as the most significant non-bacterial poisoning associated with fish consumption worldwide (Lewis, Molgó and Adams, 2000). A typical sign of the poisoning is cold allodynia, and there are more than 175 gastrointestinal, cardiovascular and neurological symptoms (Gatti, Oelher and Legrand, 2008). It is unclear whether the toxins cause harm to those herbivorous or carnivorous fish that take them through the food chain, but there is evidence that they may also affect fish nerves (Flowers, Capra and Cameron, 1992).

More than 425 species of fish have been linked to ciguatera events (Perez-Arellano *et al*., 2005). Coral reef fishes are a premium sea product with global distribution, which contributes to the expansion of CP intoxications. Reef fish commonly involved in ciguatera are: barracuda (Sphyraenidae), amberjack (*Seriola*), grouper (Serranidae), snapper (*Lutjanidae*), po'ou (*Labridae* spp.), jack (*Carangidae* spp.), trevally (*Caranx* spp.), wrasse (*Labridae* spp.), surgeon fish (*Acanthuridae* spp.), moray eel

(*Muraenidae* spp.), roi (*Cephalopholis* spp.), and parrotfish (*Scaridae* spp.) (FDA, 2011). *Gambierdiscus* are different from other open-water dinoflagellates in that they flourish in calm and protected locations, such as coral reefs or atolls, where water movement is low. For this reason, destruction or alteration of the reefs has also been associated with an increase in CP events, as the spread of *Gambierdiscus* allows a wider dissemination to the food chain (Rongo and van Woesik, 2013; Bagnis, 1994; Dickey, 2008).

1.2 OBJECTIVES

In 2015, FAO organized an interagency meeting to discuss CP as an increasing food safety threat. At the meeting, a plan of action was defined and the need for international-level guidance was identified.

At the Thirty-second Session of the Codex Committee on Fisheries and Fishery Products (2016), the Pacific Nations raised CP as an issue that increasingly affects the tropical and subtropical regions of the Pacific Ocean, Indian Ocean, and Caribbean Sea, between the latitudes 35°N and 35°S. Indeed, it was noted that, due to climate change, the frequency of storms and hurricanes is increasing, as is the sea surface temperature, which affects the distribution and proliferation of CTXs and makes the occurrence of CP less predictable.

In addition to climate change, globalization of trade might also contribute to the spread of CTXs. As such, further guidance might be needed for those countries that have not previously considered CTXs in their risk management programmes.

The matter of CP was raised at the Eleventh Session of the Codex Committee on Contaminants in Food. The Committee agreed to request scientific advice from FAO/WHO to enable the development of appropriate risk management options. In particular, the requested scientific advice of FAO/WHO entails:

> full evaluation of known CTXs (toxicological assessment and exposure assessment), including geographic distribution and rate of illness; congeners; and methods of detection;

> based on this, guidance for the development of risk management options.

1.3 MEETING APPROACH

The Expert Meeting was jointly organized by FAO and WHO. Both organizations posted a public call for data and a call for experts on their respective websites, and distributed the calls widely to all Codex contact points and elsewhere.

Applications to the call for experts were screened for relevant experiences, and the most suitable experts were identified considering the need for a gender-balanced representation of all regions. Selected experts were submitted to a process of evaluation of potential conflict of interest as per applicable FAO and WHO policies. No significant interests have been identified by the experts attending the meeting. All data received were made available to the experts and informed the discussions during the meeting and this report (main text and annex).

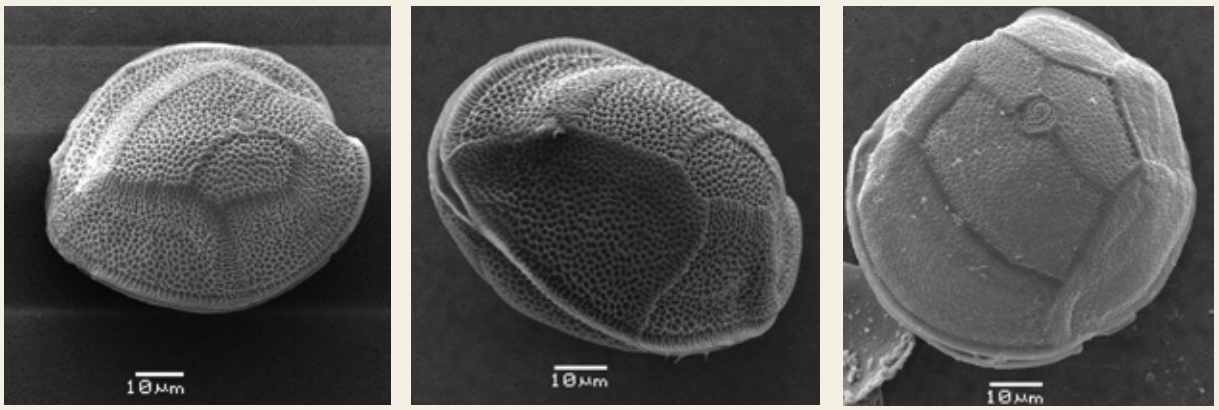
CHAPTER 2
OCCURRENCE OF CAUSATIVE ORGANISMS AND CTXs

2.1 OCCURRENCE OF GAMBIERDISCUS SPECIES

Ciguatera poisoning is a tropical disease, endemic between latitudes 35°N and 35°S, but the occurrence of ciguatoxic fish is global due to the international shipment of fish and other seafood products (Dickey and Plakas, 2010). The link between toxins produced by a benthic microorganism and CP was conjectured to be through grazing herbivorous fish to carnivorous fish and was proposed in 1958 (Randall, 1958). However, the causative organism, a dinoflagellate in the genus *Gambierdiscus*, was not described until 1977 by Yasumoto and colleagues (Adachi and Fukuyo, 1979) after having material collected previously by Bagnis and colleagues in the Gambier Islands, French Polynesia. Due to the lack of understanding of the ecology and taxonomy of *Gambierdiscus* at the time, the organism was named *Diplopsalis* (Dickey, 2008). *Gambierdiscus toxicus* (Adachi and Fukuyo, 1979; Bagnis, *et al.*, 1980; Vlamis and Katikou, 2014) was the only species in the genus for almost 20 years; hence, the early literature is rife with erroneous reports of *G. toxicus* (Figure 1). There are now 16 described *Gambierdiscus* species: *G. australes*, *G. balechii*, *G. belizeanus*, *G. caribaeus*, *G. cheloniae*, *G. carpenteri*, *G. carolinianus*, *G. excentricus*, *G. pacificus*, *G. polynesiensis*, *G. scabrosus*, *G. toxicus*, *G. silvae*, *G. lapillus*, *G. honu* and *G. jejuensis* (Jang, Jeong and Yoo, 2018; Kibler *et al.*, 2012; Fraga *et al.*, 2016; Pisapia *et al.*, 2017a; Smith *et al.*, 2016). Recently, two globular species of *Gambierdiscus* have been reclassified as *Fukuyoa* (*F. yasumotoi* and *F. ruetzleri*) and a new species described (*F. paulensis*) (Gomez *et al.*, 2015). Both *F. ruetzleri* and *F. paulensis* produce toxins (Laza-Martinez *et al.*, 2016; Rhodes and Smith, 2018; Leung *et al.*, 2018).

Gambierdiscus is an epiphytic benthic (bottom-dwelling) dinoflagellate, and toxic cells are the first step in contaminated marine food webs. There is poor understanding about the ecological or environmental factors that affect the production of toxins by *Gambierdiscus*. A study of eight species shows that maximum growth takes place between 26.5 °C and 31.1 °C, with thermal limits from 15–21 °C to 31.34 °C.

FIGURE 1 ***GAMBIERDISCUS: G. BELIZEANUS* (A), *G. AUSTRALES* (B), *G. CARIBAEUS* (C)**



Salinities range from 24.7 g/litre to 35 g/litre for maximum growth with sufficient light at irradiances generally below 231 µmol photons per square metre per second (Kibler *et al*., 2012). Each species has unique combinations of optimal temperature, salinity and light conditions (Kibler *et al*., 2012). *Gambierdiscus* grow slowly compared with many other dinoflagellates, and it is estimated that 1–5 months are necessary for significant increases in cell abundance to occur. At exponential growth, the division rate for *G. polynesiensis* is 0.13 ± 0.03 divisions/day (Chinain, *et al*., 2010a). The fastest rate of division was reported in a Hawaiian strain of *G. toxicus*, 0.55 div/day (Chinain *et al*., 2010a). There is no clear correlation between *Gambierdiscus* cell density and toxicity, depending on the species. Lag times of up to 17 months have been reported between a *Gambierdiscus* bloom and transfer of toxins in the food web leading to elevated CP incidences (Clausing *et al*., 2016). Correlation between water temperature and bloom occurrence in Hawaii, the United States of America, was reported after water temperature increased from 25.4 °C to 26.5 °C (Parsons, Settlemier and Bienfang, 2010), suggesting blooms may develop above a certain temperature threshold. However, this relationship weakens when temperatures exceed 31 °C. To understand how increasing temperatures may affect the distribution of *Gambierdiscus* and *Fukuyoa* species in the Caribbean, one study (Kibler *et al*., 2015) used temperature vs growth data in combination with projected water temperatures at six representative sites to predict how the growth rates of six *Gambierdiscus* species may change as oceans warm in the twenty-first century.

They predicted:

> northward progression of species currently restricted to lower latitude environments;

> increased abundance of species already present in from subtropical to temperate areas;

> reduced occurrence of some species in the Caribbean Sea as temperatures exceed their upper thermal growth limits.

Temperature increases are not the only changes that will accompany global warming. Sea-level rise, regional increases in precipitation, and nutrient input and habitat (substrate) alterations will also follow. Each of these factors will affect habitat suitability and, in turn, the distribution and abundance of *Gambierdiscus* and *Fukuyoa* species (Yong *et al*., 2018).

2.2 DISTRIBUTION OF CP CAUSATIVE ORGANISMS

Species in the genera *Gambierdiscus* and *Fukuyoa* have a pan-tropical distribution and the genus is reported throughout the Caribbean Sea (Figure 2), the North East Atlantic and the Mediterranean Sea (Figure 3), and Hawaii (the United States of America), French Polynesia, Australia, Japan, Southeast Asia, the Pacific Ocean (Figure 4). In 2012 and 2014, two new *Gambierdiscus* species were described from the Canary Islands (Spain) and both are toxic (see Section 2.6.1, Table 2). This genus has been found in the Mediterranean Sea, the Gulf of Mexico, and in the Atlantic Ocean off the coast of North Carolina (the United States of America) (34.7°N) (Villareal *et al*., 2007; Aligizaki and Nikolaidis, 2008; Litaker *et al*., 2009; Fraga and Rodriguez, 2014; Fraga *et al*., 2011; Fraga *et al*., 2016; Rodriguez *et al*., 2017). *Gambierdiscus* has also been observed on the coast of Angola (Isabel Rangel, personal communication), and CTXs have been detected in fish from Cameroon (Bienfang, Oben and DeFelice, 2008), but the entire coast of Africa remains mostly unexplored in this regard. Until 2009, establishing the distribution of various *Gambierdiscus* species was difficult because the taxonomy of the genus was poorly defined. Studies simply reported counts as *Gambierdiscus "toxicus"* or *Gambierdiscus* sp. (Tester *et al*., 2008; Litaker *et al*., 2009). A revision of the genus in 2009, using both morphological and genetic methods, made it possible to unambiguously identify *Gambierdiscus* species. As with the global distribution (Figure 5) of most micro-organisms, it is possible with additional sampling that species now known as endemic to either the Atlantic or Pacific may eventually be found in both regions.

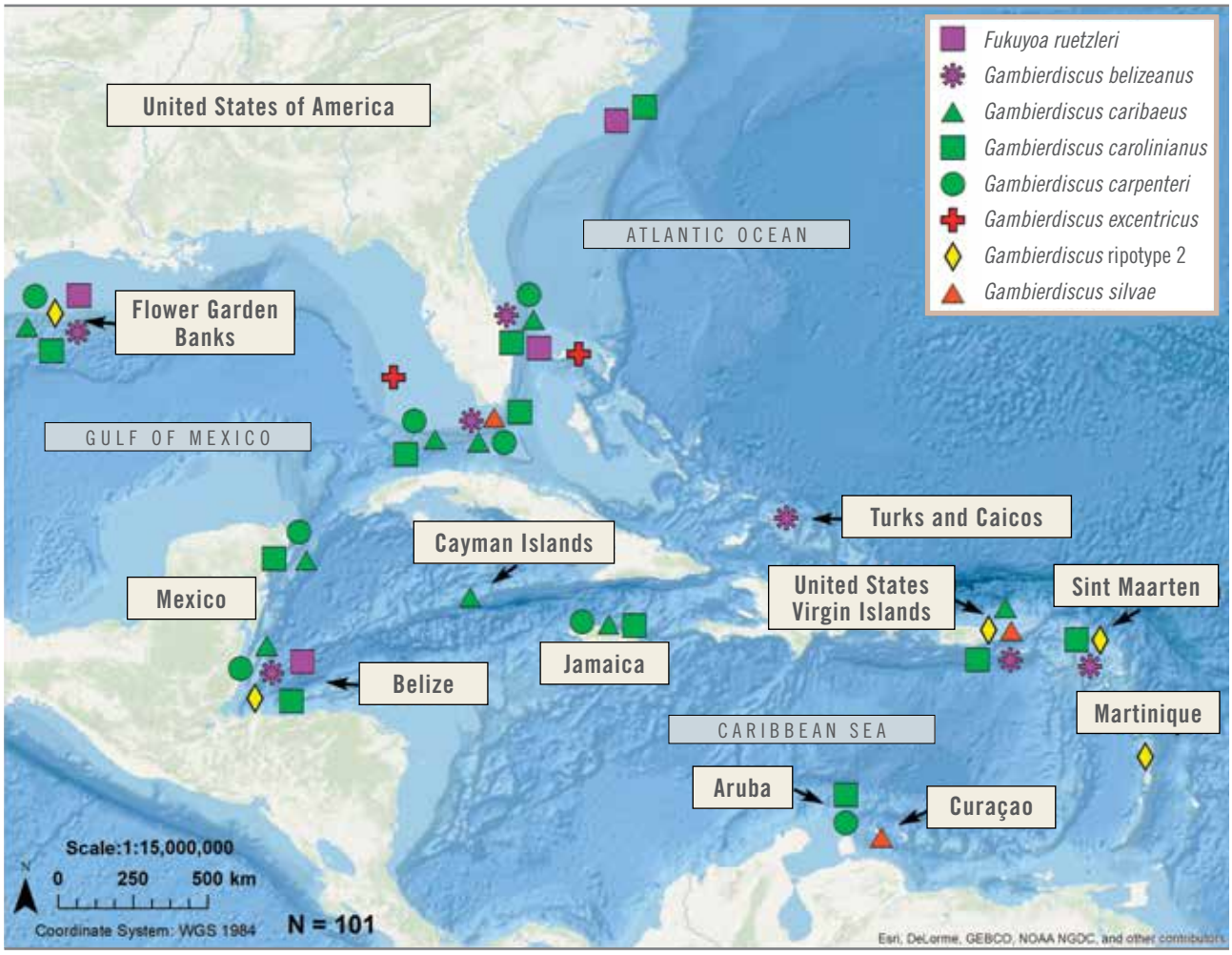
The different *Gambierdiscus* species have been identified on most tropical and subtropical coasts (Figures 2–5):

> *G. toxicus* has been reported in French Polynesia (Chinain, Faust and Pauillac, 1999), Mexican Caribbean (Hernández-Becerril and Amazán, 2004), New Caledonia, Réunion, Indian Ocean (Chinain, Faust and Pauillac, 1999), Viet Nam (Roeder *et al*., 2010), and Malaysian Borneo (Leaw, Lim and Tan, 2011).

- *G. belizeanus* has been reported in Kiribati (Xu *et al*., 2014), Saudi Arabia (Red Sea), Belize (Faust, 1995), Mexican Caribbean (Hernández-Becerril and Amazán, 2004), Malaysia, Florida (the United States of America) (Litaker *et al*., 2009), Pakistan (Munir, Siddiqui and Morton, 2011), Queensland (Australia) (Kohli, Farrell and Murray, 2015), and Saint Barthélemy – Caribbean (Litaker *et al*., 2010).
- *F. yasumotoi* has been reported in Kuwait and the Gulf of Aqaba (Jordan) (Saburova, Polikarpov and Al-yamani, 2013), New Zealand (Rhodes *et al*., 2014b), Singapore (Holmes, 1998), Japan (Nishimura *et al*., 2013), Mexican Caribbean (Hernández-Becerril and Amazán, 2004), Queensland (Australia) (Kohli, Farrell and Murray, 2015), and Nha Trang – Viet Nam (Kohli, Farrell and Murray, 2015).
- *G. australes* has been reported in French Polynesia (Chinain, Faust and Pauillac, 1999), Japan (Nishimura *et al*., 2013), Cook Islands (Rhodes *et al*., 2010), Hawaii (the United States of America) (Litaker *et al*., 2009; Pisapia *et al*., 2017a), Pakistan (Munir, Siddiqui and Morton, 2011), and the Kermadec Islands (New Zealand) and Zealandia regions of the Southwest Pacific (Rhodes *et al*., 2017a; Rhodes *et al*., 2017b).
- *G. silvae* (this species was formerly known as *G.* ribotype 1) has been reported in the Canary Islands (Spain) (Rodriguez *et al*., 2017; Pisapia *et al*., 2017a), and Belize – Caribbean (Litaker *et al*., 2010) and Japan (Nishimura *et al*., 2013).
- *G. pacificus* has been reported in French Polynesia (Chinain, Faust and Pauillac, 1999), the Marshall Islands and the Society Islands (French Polynesia) – Micronesia (Litaker *et al*., 2010), Kota Kinabalu and Sipadan Island, Malaysia (Kohli, Farrell and Murray, 2015), and Nha Trang – Viet Nam (Roeder *et al*., 2010).
- *G. polynesiensis* has been reported in French Polynesia (Chinain, Faust and Pauillac, 1999), Pakistan (Munir, Siddiqui and Morton, 2011), and Nha Trang – Viet Nam (Roeder *et al*., 2010).
- *G. caribaeus* has been reported in Florida (the United States of America), Belize – Caribbean, Tahiti (French Polynesia), Palau, Hawaii (the United States of America) (Litaker *et al*., 2009), Flower Gardens – Gulf of Mexico, Ocho Rios – Jamaica (Holland *et al*., 2013), the Bahamas, Grand Cayman Island, Tol-truk (Micronesia [Federated States of]) (Litaker *et al*., 2010), Hainan Island (China), Jeju Island (the Republic of Korea) (Jeong *et al*., 2012), and the Canary Islands (Spain) (Rodriguez *et al*., 2017).
- *G. carolinianus* has been reported in North Carolina (the United States of America), Atlantic Ocean (Litaker *et al*., 2009), Bermuda, Mexico (Litaker *et al*., 2010), Puerto Rico, Flower Gardens – Gulf of Mexico, Ocho Rios – Jamaica, Crete (Greece) (Holland *et al*., 2013), and the Canary Islands (Spain) (Rodriguez *et al*., 2017).

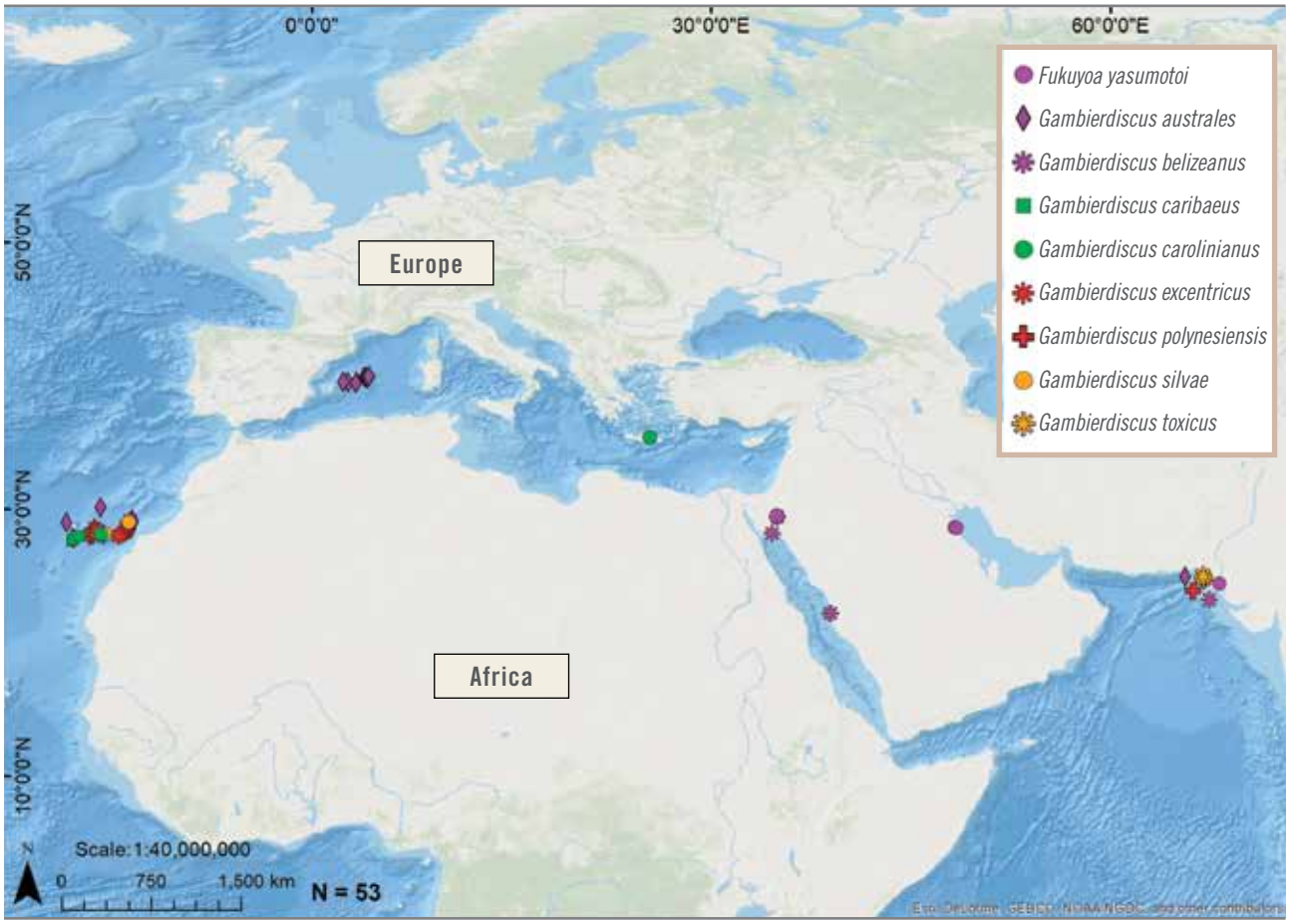
- *G. carpenteri* has been reported in Australia (Kohli *et al*., 2014b), Kiribati, Belize, Guam, Fiji (Litaker *et al*., 2009), Hawaii (the United States of America) (Litaker *et al*., 2010), Dry Tortugas – Florida (the United States of America), Flower Gardens – Gulf of Mexico, and Ocho Rios – Jamaica (Holland *et al*., 2013).
- *G. scabrosus* (formerly known as *Gambierdiscus* sp. type 1) has been reported in Japan (Nishimura *et al*., 2014), and Viet Nam (Pisapia *et al*., 2017a; Nishimura *et al*., 2014).
- *G. balechii* (formerly known as *Gambierdiscus* sp. type 6) has been reported in Kiribati (Dai *et al*., 2017), and Manado, Celebes Sea, Indonesia (Pisapia *et al*., 2017a; Fraga *et al*., 2016).
- *F. ruetzleri* has been reported in North Carolina (the United States of America), and Belize – Caribbean (Litaker *et al*., 2009).
- *G. honu* has been reported in Rarotonga (Cook Islands) and in North Meyer Island, and the Kermadec Islands and South West Pacific (Rhodes *et al*., 2017a).
- *G. cheloniae* has been reported in Rarotonga (Cook Islands) (Smith *et al*., 2016).
- *G. excentricus* has been reported in the Canary Islands (Spain) (Fraga *et al*., 2011; Rodriguez *et al*., 2017).
- *G. lapillus* has been reported in the Great Barrier Reef (Australia) (Kretzschmar *et al*., 2017).
- *G. jejuensis* (formerly known as *Gambierdiscus* sp. type 2) has been reported from Jeju Island (the Republic of Korea) (Jang, Jeong and Yoo, 2018).
- *Gambierdiscus* ribotype 2 has been reported in Belize – Caribbean, Martinique – Caribbean (Litaker *et al*., 2010), and Puerto Rico (Holland *et al*., 2013).
- *Gambierdiscus jejuensis* has been reported in the Republic of Korea (Jang, Jeong and Yoo, 2018), and Japan (Nishimura *et al*., 2013).
- *Gambierdiscus* sp. ribotype 3 has been reported in the Canary Islands (Spain) (Rodriguez *et al*., 2017) and Japan (Nishimura *et al*., 2013).
- *Gambierdiscus* has also been reported in many other areas, although the species were not identified, such as Cyprus, Rhodes (Greece), Saronic Gulf (Greece), French West Indies, Cuba, Veracuz, Costa Rica, Brazil, Angola, and Cameroon (Kohli, Farrell and Murray, 2015). Moreover, some species were thought to be endemic to certain zones, such as: *G. australes* and *G. pacificus* (Pacific area); *G. belizeanus*, *G. caribaeus*, *G. ruetzleri*, *G. carpenteri* and *G. carolinianus* (Atlantic and Pacific); and *G. yasumotoi* (Pacific) (Kohli, Farrell and Murray, 2015). Nevertheless, this might be due to lack of extensive searches, as *G. australes* has recently been found in the Canary Islands (Spain) (Rodriguez *et al*., 2017).

FIGURE 2 ***GAMBIERDISCUS*** **AND** ***FUKUYOA*** **OCCURRENCE IN THE CARIBBEAN AND ADJACENT SEAS**



Source: Tester *et al.*, 2020.

FIGURE 3 ***GAMBIERDISCUS* AND *FUKUYOA* OCCURRENCE IN MACARONESIA, EUROPE AND THE NEAR EAST**



Source: Tester *et al.*, 2020.

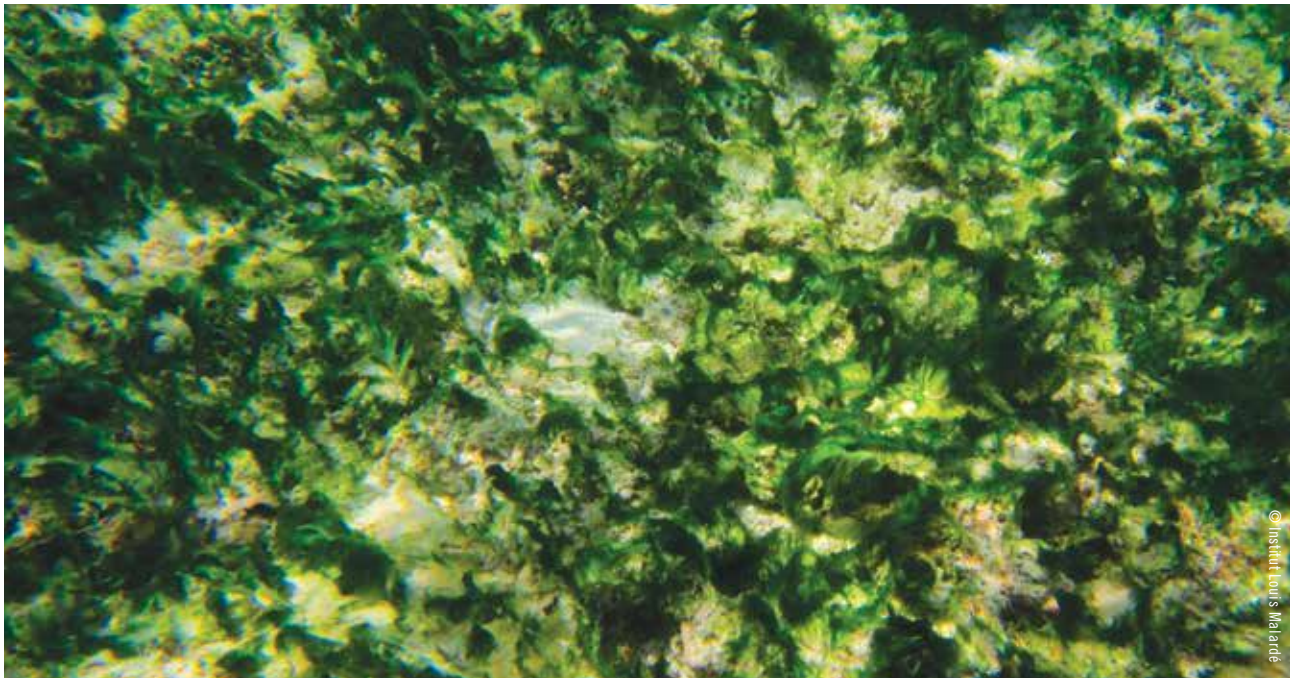
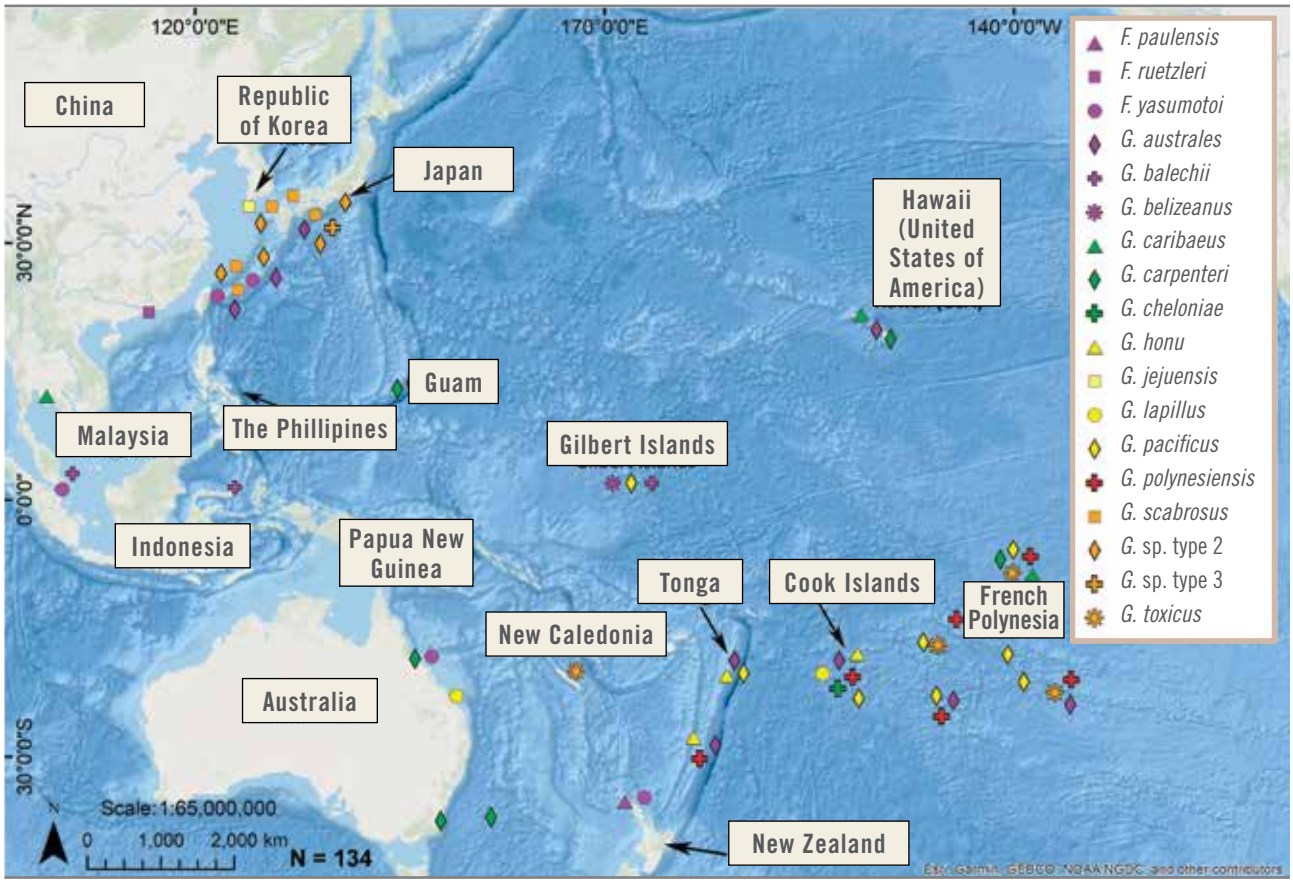
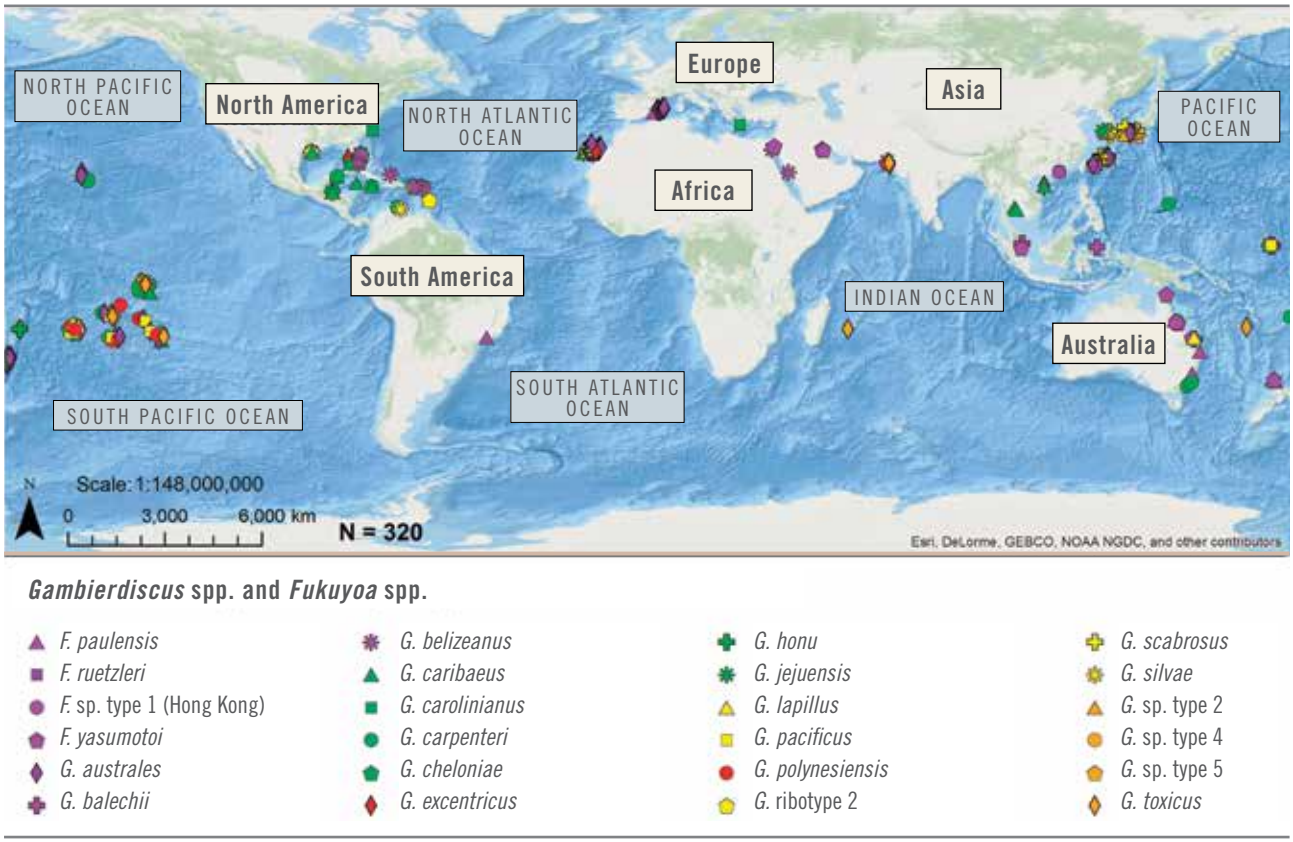


FIGURE 4 *GAMBIERDISCUS* AND *FUKUYOA* OCCURRENCE IN THE PACIFIC OCEAN, N=162



Sources: Tester *et al.*, 2020.

FIGURE 5 **GLOBAL *GAMBIERDISCUS* AND *FUKUYOA* OCCURRENCE FROM PUBLISHED RECORDS, 2009-2018**



Sources: Tester *et al.*, 2020.

2.3 METHODS FOR SPECIES IDENTIFICATION

A variety of techniques can be considered for the identification of species at any given site, including: optical microscopy as a screening tool, and then scanning electronic microscopy (SEM) and/or molecular techniques (sequencing, polymerase chain reaction [PCR], restriction fragment length polymorphism [RFLP], fluorescence *in situ* hybridization [FISH] probes, etc.) as confirmation tools. A combination of SEM and molecular techniques should be considered where new species are to be described.

2.3.1 POLYMERASE CHAIN REACTION (PCR)

Polymerase chain reaction (PCR) assays targeting unique sequences found within the SSU, ITS, and D1/D3 LUS ribosomal domains are available for most *Gambierdiscus* species to help detect described species or identify new ones. These assays are cost-effective, produce rapid, accurate measurements of relative cell concentrations, and can be adapted to survey large numbers of environmental samples. Semi-quantitative PCR assays are available for: *G. belizeanus*, *G. caribaeus*,

G. carpenteri, *G. carolinianus*, *G. ruetzleri* and *Gambierdiscus* ribotype 2 (Vandersea *et al*., 2012); *G. polynesiensis*, *G. toxicus*, *G. pacificus* and *G. australes* (Darius *et al*., 2018a). In addition, PCR assays have been published for: *G. lapillus* (Kretzschmar *et al*., 2017), *G. scabrosus*, *Fukuyoa* cf. *yasumotoi* (Nishimura *et al*., 2016); and *G. excentricus* and *G. silvae* (Litaker, Tester and Vandersea, 2019).

2.3.2 FLUORESCENCE *IN SITU* HYBRIDIZATION (FISH) PROBES

In situ hybridization with rRNA-targeted fluorescently labelled oligonucleotides has been reported to be a reasonable and rapid method for the detection, identification and enumeration of species (Pitz, 2016). Species-specific FISH probes can be used in combination with epifluorescence microscopy to obtain data on the abundance and diversity of *Gambierdiscus* species in field samples. Different species have probes of different wavelengths; thus, the use of multiple filter sets on the epifluorescence microscope allows species-specific abundance counts of up to six species in any given sample. As this technology develops, additional species and automated through-flow cytometry may be possible.

2.3.3 RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) TYPING

This technique can be used either as a screening method prior to the selection of species and strains for further study, or in combination with other methods of community diversity profiling (e.g. quantitative polymerase chain reaction [qPCR]) (Lyu *et al*., 2017). It is based on the comparison of DNA-extract profiles obtained from different strains/species of *Gambierdiscus* and *Fukuyoa* following their digestion using appropriate restriction enzymes. The RFLP digestion products are further separated and analysed by electrophoresis on agarose gel.

2.3.4 HIGH-THROUGHPUT SEQUENCING (HTS) METABARCODING

High-throughput sequencing (HTS) metabarcoding uses universal PCR primers to mass-amplify specific gene sequences from environmental samples and enables the characterization of all species or specific taxa present in the sample (Smith *et al*., 2017). This approach allows greater resolution of microbial community composition than do traditional morphological and molecular methodologies. The use of HTS metabarcoding for characterizing microbial communities is rapidly increasing due to the adaptability of the methods and a continual lowering of cost per sample (Lallias *et al*., 2015).

2.4 SAMPLING STRATEGIES

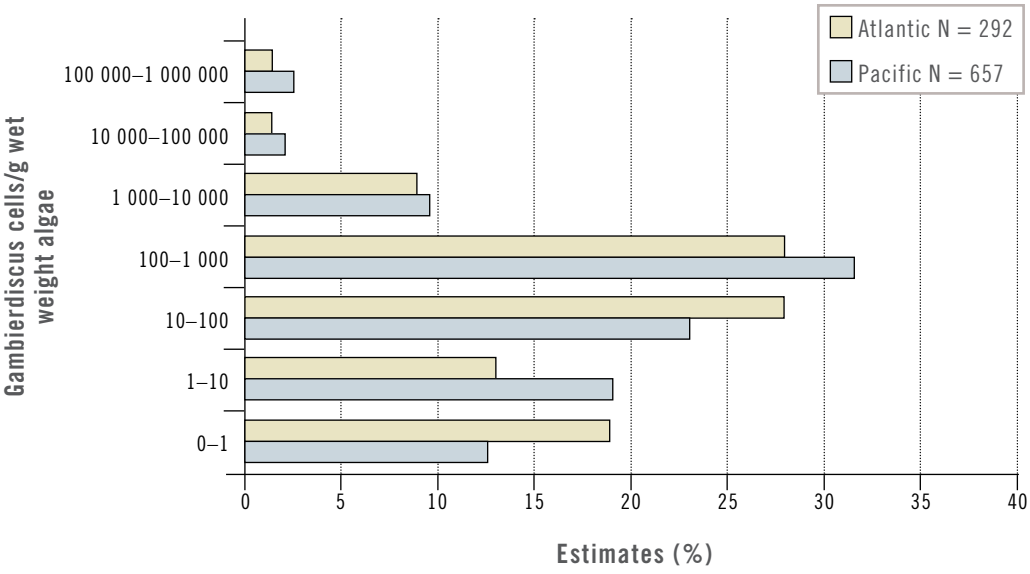
Several sampling methods to confirm the presence of *Gambierdiscus* species are available (Steidinger and Castillo, 2018). For example, sampling of natural substrates such as representative macrophytes (e.g. turf algae, *Dictyota* spp. and *Halimeda* spp.) or seagrass (*Thalassia* spp. and *Halophila* spp.) in a region, or the *in situ* deployment of artificial supports (e.g. window screens or PVC tiles) for a given period (e.g. 24 h or 30 d). The use of either macroalgal samples and/or artificial substrates in combination with qPCR techniques can be useful for providing information on the diversity and relative abundance of the different species present in a given location (Tan *et al*., 2013; Tester *et al*., 2013; Tester *et al*., 2014; Darius *et al*., 2018b). Care should be taken in using artificial substrates in high-energy sites as these are less reflective of the natural community diversity and abundance under these conditions (Smith *et al*., 2017). However, the absence of toxigenic species does not necessarily reflect low risk. Due to the potential patchiness of *Gambierdiscus*, both macroalgal and artificial substrate collections should employ a robust sampling design that covers both spatial and temporal scales. When monitoring for the occurrence of *Gambierdiscus* and *Fukuyoa* species and cell abundance, long-term data series should be considered, if possible, to address the issue of seasonality as both the abundance and species compositions are known to vary over time. The sampling design should consider patchy distributions and include preliminary range finding exercises to determine the patch sizes (spatial scales) to capture inherent environmental variability with statistical rigour and the possibility of substrate preference.

2.5 *GAMBIERDISCUS* ABUNDANCE

Gambierdiscus cells are distributed in a very patchy manner, even over small distances (Ballantine *et al*., 1985; Ballantine, Tosteson and Bardales, 1988; Lobel, Anderson and Durand-Clement, 1988). Typically, coefficients of variation among adjacent samples range from 50 percent to > 150 percent. Even with this high variation, average *Gambierdiscus* abundance data from 46 published studies were used to estimate density distributions for the Atlantic and Pacific Oceans (Litaker *et al*., 2010) (Figure 6). The frequency distributions of average cell densities were similar in both the Atlantic and Pacific. Eighty-five percent of the abundance estimates were < 1 000 cells/g wet weight algae. About 10 percent of the abundance estimates were between 1 000 cells/g wet weight algae and 10 000 cells/g wet weight algae. Estimates exceeding 100 000 cells/g wet weight algae were fewer than 5 percent of the total. The only estimate that exceeded 1 000 000 cells/g wet weight algae was from the Pacific. It is likely that the highest 10 percent of the densities represent localized epibenthic blooms of *Gambierdiscus* (Nakajima, Oshima and Yasumoto, 1981; Withers, 1981; Darius *et al*., 2007) including a recent bloom in the Canary Islands (Spain) with 104 cells/g of *G. caribaeus* (Soler-Onís *et al*., 2016).

Collecting, counting (Tester *et al*., 2014) and identification methods for benthic microplankton are well documented (Steidinger and Castillo, 2018).

FIGURE 6 **MEAN *GAMBIERDISCUS* ABUNDANCES FROM 46 STUDIES**



Note: The 0–1 values are probably underestimated because samples were biased towards sites where *Gambierdiscus* cells were known to be present. N = number of samples analysed.

Source: Redrawn from Litaker *et al.*, 2010.

Early reports highlighted increased abundance of *Gambierdiscus* sp. following disturbance events and anthropogenic impacts in reef ecosystems (Rongo and van Woesik, 2013; Bagnis, 1994), including hurricanes, salinity fluxes due to heavy rain, rise in sea surface temperature, earthquakes, bomb testing, shipwrecks, pollution, sedimentation, and destructive fishing practices (Randall, 1958). Native Fijians documented that a 200 m section of reef became toxic after macroalgae coverage was destroyed on the coral following a severe hurricane in 1929. Fresh Creek, Andros Island, Bahamas, experienced several years of fishing closures following a series of ciguatera outbreaks after a severe storm in 1908 (Randall, 1958). Several outbreaks of ciguatera in the Pacific and Atlantic following hurricanes have also been described (de Sylva, 1994). In the Pacific Ocean, military activity, dredging of shipping channels, and waterside construction have all been implicated with increased ciguatera outbreaks, although this could be attributed to population increases and the improved reporting of CP (Ruff, 1989). Other regions have suffered fish poisoning events related to environmental pollution. Mariel Bay, Cuba, exceeded quality standards for safe human contact making it the most polluted waters of Cuba and macroalgae blanketed the coral reefs (Morrison *et al.*, 2008). The bay also experienced the highest rates of CP in Cuba, with 70 percent of nationwide cases occurring there from 1993 to 2002.

2.6 TOXICITY OF CP CAUSATIVE ORGANISMS

2.6.1 DETECTION OF CTXs IN *GAMBIERDISCUS* AND *FUKUYOA* AND IN THE ENVIRONMENT

The evaluation of CTX production in *Gambierdiscus* and *Fukuyoa* can be approached by establishing cultures from field isolates in the laboratory and evaluating the toxins produced (Table 1). It is also important to characterize the toxicogenic potency of natural populations. Where possible, direct sampling of *Gambierdiscus* and *Fukuyoa* blooms for further evaluation of toxin content should also be conducted in order to characterize both the toxin concentration and profile in wild cells. Generally, a known amount of *Gambierdiscus* or *Fukuyoa* cells are obtained through filtration or centrifugation of cultures or directly from field samples. Harvested cells are extracted using chemical methods to evaluate CTXs. Care should be taken to eliminate crossover between CTXs and maitotoxins (MTXs) (and related compounds) as both can invoke toxicity depending on the methodology used. Toxin cell quota can be calculated when the CTX content or toxicity is standardized to a known cell number. Very few studies have characterized the complete toxin profiles as many CTXs have not yet been structurally elucidated. This is an area of need that is somewhat hampered by the slow growth rates of toxigenic *Gambierdiscus* in culture, resulting in insufficient material for isolation and purification. Identification of previously elucidated CTXs such as the CTX 1B and 3 groups (see Section 3) in *Gambierdiscus* isolates is more straightforward and can be conducted by LC-MS/MS methods (see Section 4) but the lack of available standards for all elucidated congeners makes confirmation and quantification challenging.

The monitoring of toxins produced by *Gambierdiscus* and *Fukuyoa* in the water column with the use of passive adsorption approaches (e.g. solid phase adsorption toxin tracking [SPATT]) is worth considering. SPATT consists of the immersion in water of small devices holding resins within a planktonic mesh. Generally, SPATT bags are immersed for 3–10 days. Several groups of toxins including CTXs adsorb to the resin (e.g. HP20 resin). This approach has been implemented in the laboratory to detect and quantify CTXs in the medium of *Gambierdiscus* cultures (Fraga *et al.*, 2011) and also in the environment within ciguatera endemic areas (Roué *et al.*, 2016). The approach is a complementary strategy to the evaluation of CTXs in microalgal populations. SPATT may be useful as an early warning tool to detect CTXs in areas where *Gambierdiscus* or *Fukuyoa* may have not yet been reported or where molecular identification was not available. In addition, extraction of adsorbed toxins from SPATT bags reduces matrix effects that may interfere in the consequent analytical evaluation of CTXs.

This approach can provide complementary information on the presence not only of CTXs but also of other toxin classes adsorbing to the resin in a given site, as other toxin-producing benthic toxigenic species such as *Ostreopsis* and *Prorocentrum* are often present in high abundance in benthic assemblages of ciguateric biotopes.

In addition to the evaluation of CTXs, one may consider, for scientific purposes, identifying other toxins (MTXs1-4) and bioactive compounds not proved to be directly implicated in ciguatera (gambieric acid, gambierol, gambierone, etc.) produced by *Gambierdiscus* and *Fukuyoa* (Table 2).

When assessing the occurrence of microalgae involved in the production of CTXs, both *Gambierdiscus* and *Fukuyoa* have to be addressed. Whenever possible, data about *Gambierdiscus and Fukuyoa* cell abundance, species diversity and their respective toxin profiles should be documented, as these parameters can significantly impact the toxin profiles in surrounding fish and help explain variations in fish toxicity observed at a regional/local scale (Figures 2–5 and Section 3.2 Ciguatoxin classification).

TABLE 1 **BIOLOGICAL ACTIVITY REPORTED IN *GAMBIERDISCUS* AND *FUKUYOA* SPECIES**

SPECIES	CTX-LIKE ACTIVITY	MTX-LIKE ACTIVITY	OTHER ACTIVITY	COMPOUNDS (LC-MS/MS data)		
				CTXs	MTXs	Other
G. toxicus	MBA- (Chinain *et al.*, 1999) **RBA**+ (Chinain *et al.*, 2010a)	**MBA**+ (Chinain *et al.*, 1999)	no information available	no information available	MTX1, MTX2[1] (Nagai *et al.*, 1993; Holmes, Lewis and Gillespie, 1990)	**Gambieric acids A-D**[1] (Nagai *et al.*, 1993) **Gambierol**[1] (Satake, Murata and Yasumoto, 1993a) **Gambieroxide** (Watanabe *et al.*, 2013)
G. belizeanus	**RBA**+ (Chinain *et al.*, 2010a) **N2a**+ (Catania *et al.*, 2017; Litaker *et al.*, 2017) N2a (Xu *et al.*, 2014)	no information available	**haemolytic activity** (Holland *et al.*, 2013)	no information available	no information available	**Gambierone** (Rodriguez *et al.*, 2015) **44-methylgambierone** (Boente-Juncal *et al.*, 2019; Murray *et al.*, 2019)
F. yasumotoi	no information available	no information available	no information available	no (CTX3C, 3B, 4A, 4B) (Rhodes *et al.*, 2014b)[2]	no MTX1 (Rhodes *et al.*, 2014b)[2]	**MTX3**(+) (Rhodes *et al.*, 2014b)[2]
G. australes	**RBA**+ (Chinain *et al.*, 2010a) **N2a**+ (Reverté *et al.*, 2018)	**N2a**+ (Reverté *et al.*, 2018)	no information available	no information available	**MTX1**(+) (Rhodes *et al.*, 2014a)	**MTX3**(+) (Munday *et al.*, 2017)
G. pacificus	**MBA**+ (Chinain *et al.*, 1999) **N2a**+ (Xu *et al.*, 2014; Darius *et al.*, 2018a)	**MBA**+ (Chinain *et al.*, 1999)	no information available	no information available	no MTX1 (Rhodes *et al.*, 2014a)	**MTX3**(+) (Munday *et al.*, 2017)

(continues)

TABLE 1 (continued)

SPECIES	CTX-LIKE ACTIVITY	MTX-LIKE ACTIVITY	OTHER ACTIVITY	COMPOUNDS (LC-MS/MS data)		
				CTXs	MTXs	Other
G. polynesiensis	**MBA+** (Chinain *et al.*, 1999)	**MBA+** (Chinain *et al.*, 1999)	no information available	**(CTX3B,3C, 4A, 4B, M-Seco-CTX3C)**$^{(+)}$ (Chinain *et al.*, 2010a)	no MTX1 (Rhodes *et al.*, 2014a)	**MTX3**$^{(+)}$ (Munday *et al.*, 2017)
	RBA+ (Chinain *et al.*, 2010a)			**(CTX3B,3C, 4A, 4B)**$^{(+)}$ (Munday *et al.*, 2017)		
	N2a+ (Darius *et al.*, 2018a; Darius *et al.*, 2018b)			**(CTX3B,3C, 4A, 4B, 20H-CTX3C, M-Seco-CTX3C)**$^{(+)}$ (Sibat *et al.*, 2018b)		
G. caribaeus	**N2a+** (Litaker *et al.*, 2017)		**haemolytic activity** (Holland *et al.*, 2013)	no information available	no information available	no information available
G. carolinianus	**N2a+** (Litaker *et al.*, 2017)		**haemolytic activity** (Holland *et al.*, 2013)	no information available	no information available	no information available
G. silvae	N2a+ (Litaker *et al.*, 2017)	**N2A+**	no information available	no information available	no information available	no information available
G. carpenteri	**N2a+** (Litaker *et al.*, 2017)	(Kohli *et al.*, 2014b)	(Holland *et al.*, 2013)	no information available	no MTX1 (Munday *et al.*, 2017)	no MTX3 (Munday *et al.*, 2017)
	N2a- (Xu *et al.*, 2014; Darius *et al.*, 2018b)					
F. ruetzleri	no MBA or RBA data available	no information available	**lethal to brine shrimp** (Leung *et al.*, 2018)	no CTXs (Leung *et al.*, 2018)	no MTX1 (Leung *et al.*, 2018)	**MTX3**$^{(+)}$ (Leung *et al.*, 2018)
	N2a+ (Litaker *et al.*, 2017)		**haemolytic activity** (Holland *et al.*, 2013)			
G. excentricus	**N2a+** (Fraga *et al.*, 2011)	**N2a+** (Fraga *et al.*, 2011)	no information available	no information available	no information available	no information available
		N2a+; (N2a Ca2+ flux)+ (Pisapia *et al.*, 2017a)			**MTX4**$^{(+)}$ (Pisapia *et al.*, 2017a)	

(continues)

TABLE 1 (continued)

SPECIES	CTX-LIKE ACTIVITY	MTX-LIKE ACTIVITY	OTHER ACTIVITY	COMPOUNDS (LC-MS/MS data)		
				CTXs	MTXs	Other
G. scabrosus (formerly *Gambierdiscus* ribotype 1)	**MBA+** /no RBA or N2a data available (Nishimura *et al.*, 2013)	**MBA+** (Nishimura *et al.*, 2013)	no information available	no information available	no information available	no information available
F. paulensis	**MBA+** (Laza-Martinez *et al.*, 2016)	**MBA+** (Laza-Martinez *et al.*, 2016)	**toxic to mice by ip and gavage** (Laza-Martinez *et al.*, 2016)	**CTX3**$^{(+)}$ (Laza-Martinez *et al.*, 2016)	no MTX1 (Munday *et al.*, 2017)	**gambieric acid A; MTX3**$^{(+)}$ (Laza-Martinez *et al.*, 2016; Munday *et al.*, 2017)
G. balechii (formerly *Gambierdiscus* ribotype 6)	**MBA+** (Fraga *et al.*, 2016) **N2a+** (Dai *et al.*, 2017)	**MBA+** (Fraga *et al.*, 2016)	no information available	no information available	no information available	no information available
G. cheloniae	no MBA, RBA or N2a data available	no information available	**toxic to mice by ip and gavage** (Smith *et al.*, 2016)	no CTXs (Smith *et al.*, 2016; Munday *et al.*, 2017)	no MTX1 (Smith *et al.*, 2016)	**MTX3**$^{(+)}$ (Munday *et al.*, 2017)
G. lapillus	no MBA, RBA or N2a data available	no information available	**toxic to mice by ip and gavage** (Kretzschmar *et al.*, 2017)	no (CTX3C, 3B, 4A, 4B) (Kretzschmar *et al.*, 2017)	no MTX1 (Kretzschmar *et al.*, 2017)	**MTX3**$^{(+)}$ (Kretzschmar *et al.*, 2017)
G. honu	no MBA, RBA or N2a data available	no information available	**toxic to mice by ip and gavage** (Rhodes *et al.*, 2014a)	no CTXs (Rhodes *et al.*, 2014a; Munday *et al.*, 2017)	no MTX1 (Rhodes *et al.*, 2014a)	**MTX3**$^{(+)}$ (Munday *et al.*, 2017)

[1] A likely mis-identification of the species of *Gambierdiscus* involved should be considered for these compounds.
[2] The *Gambierdiscus* cf. *yasumotoi* strain tested in this study was later confirmed as being a *F. paulensis* strain (Argyle *et al.*, 2016).

TABLE 2 **RELATIVE CIGUATOXIN-LIKE TOXICITIES OF *GAMBIERDISCUS* AND *FUKUYOA* SPECIES REPORTED TO DATE**

SPECIES	TYPE LOCATION	DISTRIBUTION	RELATIVE TOXICITY EQ CELL^{-1}	PCR/qPCR
Gambierdiscus toxicus (Adachi and Fukuyo, 1979)	Gambier Islands, French Polynesia, South Pacific		**22–28 fg CTX3C eq** RBA (Chinain *et al.*, 2010a)	(Darius *et al.*, 2018b)
G. belizeanus (Faust, 1995)	Belize, Central America, Caribbean Sea	Kiribati (Xu *et al.*, 2014); Saudi Arabia (Red Sea) (Catania *et al.*, 2017)	**123 fg CTX3C eq** RBA (Chinain *et al.*, 2010a) **0.85 fg CTX3C** N2A (Litaker *et al.*, 2017)	(Vandersea *et al.*, 2012)
Fukuyo yasumotoi (Holmes, 1998; Gomez *et al.*, 2015)	Singapore harbour			(Nishimura *et al.*, 2016)

(continues)

TABLE 2 (continued)

SPECIES	TYPE LOCATION	DISTRIBUTION	RELATIVE TOXICITY EQ CELL^{-1}	PCR/qPCR
G. australes (Chinain, Faust and Pauillac, 1999)	Australes Archipelago, French Polynesia, South Pacific Ocean	Hainan Island (China) (Zhang *et al.*, 2016)	**17–30 fg CTX3C** RBA (Chinain *et al.*, 2010a) > 200–697 CTX1Beq N2A > Reverté *et al.* 2018	(Nishimura *et al.*, 2016; Darius *et al.*, 2018b)
G. pacificus (Chinain, Faust and Pauillac, 1999)	Tuamotu Archipelago, French Polynesia, South Pacific Ocean	Kiribati (Xu *et al.*, 2014), Hainan Island (China) (Zhang *et al.*, 2016)	**neg CTX3C** RBA (Chinain *et al.* 2010) **0.5–1 fg CTX3C** N2A (Darius *et al.*, 2018b)	(Darius *et al.*, 2018b)
G. polynesiensis (Chinain, Faust and Pauillac, 1999)	Tuamotu Archipelago, French Polynesia, South Pacific Ocean		**0.16 MU/1 000 cells** **2 800–4 400 fg CTX3C** RBA (Chinain *et al.*, 2010a) **18 200 fg CTX3C** LC-MS (Rhodes *et al.*, 2014a) **1 610–2 130 fg** N2A (Darius *et al.*, 2018b)	(Darius *et al.*, 2018b)
G. caribaeus (Litaker *et al.*, 2009)	Belize, Central America, Caribbean Sea	Thailand (Zhang *et al.*, 2016)	**0.66 fg CTX3C** N2A (Litaker *et al.*, 2017)	(Vandersea *et al.*, 2012)
G. carolinianus (Litaker *et al.*, 2009)	North Carolina, United States South Atlantic Bight	Globally distributed	**0.27 fg CTX3C** N2A (Litaker *et al.*, 2017)	(Vandersea *et al.*, 2012)
G. carpenteri (Litaker *et al.*, 2009)	Belize, Central America, Caribbean Sea	Australia (Kohli *et al.*, 2014b) Kiribati (Xu *et al.*, 2014)	**0.89 fg CTX3C** N2A (Litaker *et al.*, 2017)	(Vandersea *et al.*, 2012)
Gambierdiscus **ribotype 2** (Litaker *et al.*, 2009)	Belize, Central America, Caribbean Sea		**6.62 fg CTX3C** N2A (Litaker *et al.*, 2017)	(Vandersea *et al.*, 2012)
F. ruetzleri (Litaker *et al.*, 2009; Gomez *et al.*, 2015)	Belize, Central America, Caribbean Sea		**10.6 fg CTXC3** N2A (Litaker *et al.*, 2017)	(Vandersea *et al.*, 2012)
G. excentricus (Fraga *et al.*, 2011)	Canary Islands, eastern Atlantic Ocean		**469 fg CTX3C** N2A (Litaker *et al.*, 2017)	(Litaker, Tester *et al.*, 2020)
G. silvae (Fraga and Rodriguez, 2014)	Canary Islands, eastern Atlantic Ocean		**19.6 fg CTX3C** N2A (Litaker *et al.*, 2017)	(Litaker, Tester and Vandersea, 2019)
F. paulensis (Gomez *et al.*, 2015)	Ubatuba, Sao Paolo State, Brazil		**neg** N2A (Gomez *et al.*, 2015)	
G. balechii (Fraga *et al.*, 2016)	Celebes Sea, South Pacific	Kiribati (Dai *et al.*, 2017)	**1.1–19.9 fg CTX1B** (Dai *et al.*, 2017)	
G. cheloniae (Smith *et al.*, 2016)	Cook Islands, South Pacific Ocean		**MBA +, no CTX3C** (Smith *et al.*, 2016)	
G. lapillus (Kretzschmar *et al.*, 2017)	Great Barrier Reef, Australia		**MBA +, no CTX** (Kretzschmar *et al.*, 2017)	(Kretzschmar *et al.*, 2017)
G. scabrosus (Nishimura *et al.*, 2014)	Kashiwa-jima Island off southern Honahu, Japan		**20 MU/1 000 cells+** (Nishimura *et al.*, 2013) (Formerly Ribotype 1)	(Nishimura *et al.*, 2016)
G. honu (Rhodes *et al.*, 2017a)	Cook Islands & Kermadec Islands, South Pacific Ocean		**MBA +, no CTX3C** (Rhodes *et al.*, 2017a)	

Notes: RBA = receptor binding assay; N2A = neuro 2A functional assay; LC-MS = liquid chromatography and mass spectrometer; MBA = mouse bioassay.
Cross-comparisons of toxicity between species and strains cannot be made with confidence as no common certified reference standards were available for all of the studies reported.

2.7 OCCURRENCE OF CTXs IN SEAFOOD

More than 425 species of fish have been linked to ciguatera events (Perez-Arellano *et al*., 2005). Coral reef fishes are a premium sea product with global distribution, which contributes to the expansion of CP intoxications. Reef fish known to potentially accumulate these toxins are: barracuda (Sphyraenidae), amberjack (*Seriola*), grouper (Serranidae), snapper (Lutjanidae), po'ou (*Chielinus rodochrous*), jack (*Carangidae* spp.), trevally (*Caranx* spp.), wrasse (*Labridae* spp.), surgeon fish (*Acanthuridae* spp.), moray eel (*Muraenidae* spp.), roi (*Cephalopholis* spp.), and parrotfish (*Scaridae* spp.) (FDA, 2011) (see Tables 3 and 4, and Figure 7). A large variety of marine invertebrates including urchins, gastropods, bivalves, echinoderms, etc. have also been reported to contain CTXs and present an additional source of poisoning to seafood consumers.

TABLE 3 **MINIMUM FISH WEIGHT TO REQUIRE CTX ANALYSIS IN THE CANARY ISLANDS, SPAIN**

SPECIES	LATIN NAME	WEIGHT (KG) (equal to or larger than)
Amberjack	*Seriola* spp.	14
Wahoo	*Acanthocybium solandri*	35
Bluefish	*Pomatomus saltatrix*	9
Island grouper	*Mycteroperca fusca*	12
Dusky grouper	*Epinephelus spp.*	17
Atlantic blue marlin	*Makaira nigricans*	320
Swordfish	*Xiphias gladius*	320

Most of the tropical fish are territorial. Hence, those areas with toxicity usually remain toxic, while the areas without toxicity usually remain safe. This knowledge is used by local people of Pacific and Caribbean islands as a precautionary measure to avoid CP. Nevertheless, it is common that they have chronic levels of CTX that at a certain point reach a threshold resulting in disease (Dickey, 2008). *Gambierdiscus* has been also reported as free-swimming cell in the water column (Price *et al*., 2016), which further complicates a potential monitoring plan.

Due to international trade, there are reports of poisoning in many geographical areas without the risk of indigenous ciguatera (such as: Canada, California, New York, Rhode Island and Vermont in the United States of America (Graber *et al*., 2013); Hamburg and elsewhere in Germany (Schlaich *et al*., 2012; Gestal-Otero, 2014); and the Paris area in France, and also from patients returning to their countries after travelling. A report of CP poisoning after consumption of a farm-cultured salmon shows the complexity of the problem (Ebesu, Nagai and Hokama, 1994). It is also a matter of concern that the terminology as well as the denominations used for commercial fish change depending on the country.

The recent appearance of significant amounts of CTXs in the Canary Islands (Spain) (Boada *et al*., 2010; Perez-Arellano *et al*., 2005) and Madeira (Portugal) (Otero *et al*., 2010) suggest that CTXs are expanding their ecological niches, probably as a consequence of global warming (Kohli, Farrell and Murray, 2015). It has been reported that in the Caribbean Sea and the West Indies, where optimal growth of *Gambierdiscus* is above 29 °C, the number of days with sea surface temperatures above 29 °C has doubled, from 44 days to 86 days, in the last 30 years (Tester *et al*., 2010). Nevertheless, although global warming is facilitating the growth and expansion of *Gambierdiscus*, there are bodies of water that are warm enough to depress their growth, and this is a factor to take into account in understanding the role of global warming in ciguatera expansion (Llewellyn, 2010). Although several reports indicate the presence of *Gambierdiscus* in new areas (Brazil [Nascimento *et al*., 2012], Morocco [Ennaffah and Chaira, 2015] and Thailand [Tawong *et al*., 2015]), there is no solid link yet to this being caused by climate change (Botana, 2016; Silva *et al*., 2015). Another subject not yet clarified is how the change in pH and CO2 levels and in sea surface temperatures may affect toxin production, as these parameters influence the growth of microalgae (Schippers, Lürling and Scheffer, 2004; Hinga, 1992).

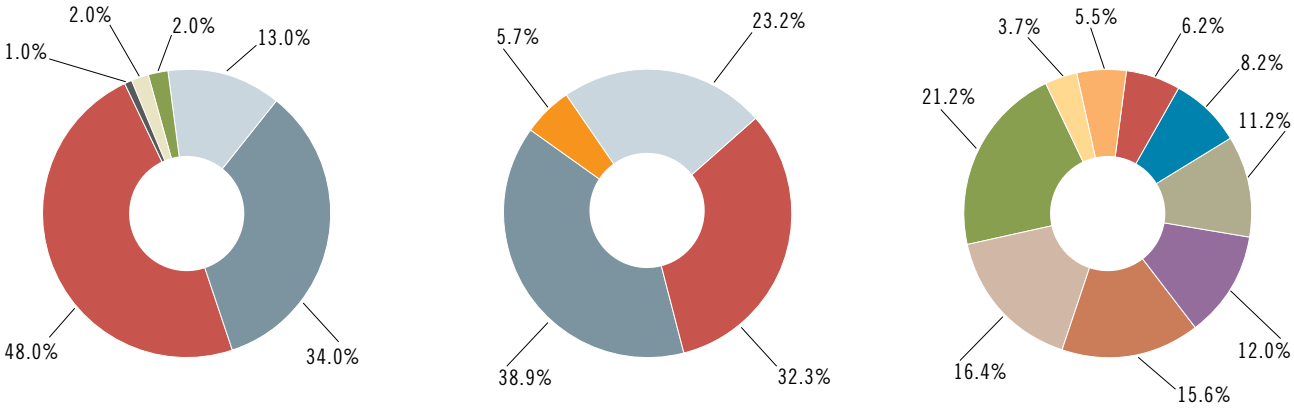
The poisoning of a fisher in Madeira (Portugal) was reported to be caused by *Seriola*, and the components showed a mixture of toxins identified as Caribbean or Indian and Pacific, namely CTX1B, CTX3C, and Caribbean or Indian Ocean analogues of 1 141.6 m/z (Otero *et al*., 2010). The concentrations reported were about 50 ng/g fish tissue. In the Canary Islands (Spain), several *Gambierdiscus* species have been identified (*G. silvae* and *G. australes*) (Fraga and Rodriguez, 2014), and the nine intoxications reported to date were associated to Caribbean C-CTX1 (Boada *et al*., 2010; Perez-Arellano *et al*., 2005; Nunez *et al*., 2012). In the Mediterranean Sea, there are reports of *Gambierdiscus* in several macroalgae (*Padina pavonica*, *Corallina elongate*, *Jania* spp. and *Cystoseira* spp.) in Crete (Greece) (Aligizaki and Nikolaidis, 2008), and although CP poisoning has never been reported in this region, ciguateric species have been proposed in the eastern Mediterranean (Chevaldonné, 1990; Bentur and Spanier, 2007).

The fact that CTXs enter the food chain from herbivores (both invertebrates and fish) to end up in carnivorous fish allows the accumulation of these toxins in hundreds of marine species (Yasumoto, 2001). The toxin profile in fish flesh, observed during a 20-year interval, was reported to be species-dependent – CTX1B and two deoxy congeners were found in snappers and groupers, CTX4A and CTX4B in spotted knifejaw (*Oplegnathus punctatus*) from Okinawan (Japan) waters, CTX3C-type toxins in spotted knifejaw from Miyazaki (Japan), and CTX1B-type and CTX3C-type toxins in red snapper (*Lutjanus bohar*) and amberjack (*Seriola dumerili*) (Yogi *et al*., 2011; Yogi *et al*., 2014). A possible explanation of species-specific toxin profiles was attributed to genetic differences in CTX bio-oxidation by the liver enzymatic complex CYP3A4. These changes in toxin profiles are the result of the bio-oxidative transformations that CTXs undergo as they move up the food chain, leading to more oxidized and also more toxic congeners (Lewis and Holmes, 1993).

For example, one study (Ikehara *et al.*, 2017) has shown that CTX1B, -2 and -3 actually derive from the enzymatic oxidation of CTX4A and -4B, whereas the biotransformation of CTX3C leads to CTX2A1 (see Figure 7).

This illustrates the complexity of a potential monitoring process, as depending on the species, the analytical challenge is different.

FIGURE 7 **CIGUATOXIN TOXIN PROFILES FOUND IN THE MICROALGA *GAMBIERDISCUS POLYNESIENSIS*, IN THE HERBIVOROUS FISH *CHLORURUS MICRORHINOS* (EX. *SCARUS GIBBUS*) AND IN THE CARNIVOROUS FISH *GYMNOTHORAX JAVANICUS***



MICROALGAE
Gambierdiscus polynesiensis RG-92

- P-CTX3C
- M-*seco*-CTX3C
- P-CTX3B (49-*epi*CTX3C)
- P-CTX4A (52-*epi*CTX4B)
- P-CTX4B
- miscellaneous

HERBIVOROUS FISH
Chlorurus microrhinos (Ex. *Scarus gibbus*)

- P-CTX3C
- M-*seco*-CTX4A
- P-CTX3B (49-*epi*CTX3C)
- P-CTX4A (52-*epi*CTX4B)
- miscellaneous

CARNIVOROUS FISH
Gymnothorax javanicus

- P-CTX3C
- P-CTX3C-2-ol
- A-*seco*-CTX3C-51-ol
- P-CTX-2A1 (2,3-dihydroxyCTX3C)
- P-CTX2 (52-*epi*-54-deoxyCTX1)
- *epi*CTX2
- P-CTX3 (54-deoxyCTX1)
- P-CTX1
- miscellaneous

Source: Institut Louis Malardé[1].

[1] The nomenclature of for the different toxins in Figure 7 might differ from the one used throughout the document.

TABLE 4 **FISH AND MARINE INVERTEBRATES REPORTED TO BIOACCUMULATE CTXs AND THEIR LOCATIONS**

COMMON NAME	SCIENTIFIC NAME	LOCATION WHERE FISH WAS FOUND
Greater amberjack / Kahala	*Seriola dumerili*	Canary Islands, Madeira Archipelago (Otero *et al.*, 2010), Hawaii (Hokama, Banner and Boylan, 1977; Hokama, Abad and Kimura, 1983; Campora *et al.*, 2008), Haiti (Poli *et al.*, 1997), Saint Barthélemy, Caribbean Sea (Vernoux and Abbad el Andaloussi, 1986), Saint Thomas, Caribbean Sea (Granade, Cheng and Doorenbos, 1976)
Lesser amberjack	*Seriola fasciata*	Selvagens Islands (Madeira Archipelago) (Otero *et al.*, 2010), West Africa (Canary Islands) (Boada *et al.*, 2010)
Almaco jack / Kahala	*Seriola rivoliana*	Canary Islands (Perez-Arellano *et al.*, 2005), Hawaii (Campora *et al.*, 2008), Saint Thomas, Caribbean Sea (Granade, Cheng and Doorenbos, 1976)
Angelfish	*Pomacanthus imperator*	Kiribati (Mak *et al.*, 2013)
Great barracuda	*Sphyraena barracuda*	Bahamas (O'Toole *et al.*, 2012), Cameroon (Bienfang, Oben and DeFelice, 2008), Florida Keys, the United States of America (Dechraoui *et al.*, 2005), French West Indies (Pottier *et al.*, 2003), Saint Barthélemy, Caribbean Sea (Vernoux and Abbad el Andaloussi, 1986; Kohli, Farrell and Murray, 2015), Guadeloupe (Pottier, Vernoux and Lewis, 2001), French Polynesia (Bagnis *et al.*, 1987)
Pickhandle barracuda	*Sphyraena jello*	Hervey Bay, Queensland, Australia (Lewis and Endean, 1984a)
Barracuda	*Sphyraena* sp.	California (Hokama, 1990)
Butterflyfish	*Chaetodon auriga*	Kiribati (Mak *et al.*, 2013)
Butterflyfish	*Chaetodon meyeri*	Kiribati (Mak *et al.*, 2013)
Butterflyfish	*Forcipiger longirostris*	Kiribati (Mak *et al.*, 2013)
Barracuda fish eggs	*Sphyraena* sp.	South Taiwan Province of China (Fenner *et al.*, 1997)
Green moray eel	*Gymnothorax funebris*	Saint Barthélemy, Caribbean Sea (Vernoux and Abbad el Andaloussi, 1986)
Moray eel	*Gymnothorax javanicus*	Tuamotu Archipelago and Tahiti (French Polynesia) (Murata *et al.*, 1990; Legrand *et al.*, 1989; Labrousse and Matile, 1996), Tarawa, Kiribati, central Pacific Ocean (Chan *et al.*, 2011; Lewis and Jones, 1997), Hawaii (Scheuer *et al.*, 1967), Kiribati (Mak *et al.*, 2013)
Moray eel	*Gymnothorax flavimarginatus*	Kiribati (Mak *et al.*, 2013)
Longface emperor bream	*Lethrinus olivaceus*	Nuku Hiva (Marquesas) (Darius *et al.*, 2007)
Trumpet emperor bream	*Lethrinus miniatus*	French Polynesia (Bagnis *et al.*, 1987)
	Lethrinus callopterus	Enewetak Island (Randall, 1980)
	Lethrinus miniatus	Enewetak Island (Randall, 1980)
Big-eye bream, emperor	*Monotaxis grandoculis*	French Polynesia (Bagnis *et al.*, 1987), Enewetak Island (Randall, 1980), Kiribati (Mak *et al.*, 2013)
Goldstriped goatfish	*Mulloidichthys auriflamma*	Hawaii (Hokama, 1990)
Yellow goatfish	*Mulloidichthys martinicus*	Saint Barthélemy, Caribbean Sea (Vernoux and Abbad el Andaloussi, 1986)
Twosaddle goatfish	*Parupeneus insularis*	Nuku Hiva (Marquesas) (Darius *et al.*, 2007)
Goatfish	*Parupeneus bifasciatus*	Kiribati (Mak *et al.*, 2013)
Cone snails	*Conus* spp.	Hawaii (Kohli, Farrell and Murray, 2015)

(continues)

TABLE 4 (continued)

COMMON NAME	SCIENTIFIC NAME	LOCATION WHERE FISH WAS FOUND
Giant clam (herbivorous)	*Tridacna maxima*	New Caledonia, French Polynesia (Roué *et al.*, 2016)
Giant clam (herbivorous)	*Hippopus hippopus*	Vanuatu (Roué *et al.*, 2016; Kohli, Farrell and Murray, 2015)
Blue-spotted grouper, roi	*Cephalopholis argus*	Nuku Hiva (Marquesas) (Darius *et al.*, 2007), Hawaii (Campora *et al.*, 2008), French Polynesia (Bagnis *et al.*, 1987), Kiribati (Mak *et al.*, 2013)
Coral cod / coral grouper	*Cephalopholis miniata*	Fiji (Dickey, 2008; Arnett and Lim, 2007), Arafura Sea, Australia (Lucas, Lewis and Taylor, 1997)
Orange-spotted grouper	*Epinephelus coioides*	China, Hong Kong SAR (Wong *et al.*, 2005)
	Epinephelus spp.	Canary Islands (CarlosIII, 2017)
Giant grouper	*Epinephelus lanceolatus*	China, Hong Kong SAR (Wong *et al.*, 2009)
Marble grouper	*Epinephelus microdon*	French Polynesia (Bagnis *et al.*, 1987), Enewetak Island, Bikini Island (Randall, 1980)
Misty grouper	*Epinephelus mystacinus*	Saint Thomas, Caribbean Sea (Granade, Cheng and Doorenbos, 1976)
Red grouper	*Epinephelus morio*	Saint Barthélemy, Caribbean Sea (Vernoux and Abbad el Andaloussi, 1986)
Large grouper	*Epinephelus fuscoguttatus*	Enewetak Island (Randall, 1980), Kiribati (Mak *et al.*, 2013)
Large grouper	*Epinephelus hoedtii*	Enewetak Island (Randall, 1980)
Large grouper	*Epinephelus maculatus*	Enewetak Island (Randall, 1980)
Large grouper	*Epinephelus tauvina*	Bikini Island (Randall, 1980), Kiribati (Mak *et al.*, 2013)
Large grouper	*Epinephelus coeruleopunctatus*	Kiribati (Mak *et al.*, 2013)
Large grouper	*Epinephelus multinotatus*	Kiribati (Mak *et al.*, 2013)
Large grouper	*Epinephelus polyphekadion*	Kiribati (Mak *et al.*, 2013)
Large grouper	*Epinephelus spilotoceps*	Kiribati (Mak *et al.*, 2013)
Small grouper	*Epinephelus merra*	Kiribati (Mak *et al.*, 2013)
Black grouper	*Mycteroperca bonaci*	Key Largo, Florida, the United States of America (Dickey, 2008)
Sawtail grouper	*Mycteroperca prionura*	Baja California, Mexico (Sierra-Beltran *et al.*, 1997)
Yellowfin grouper	*Mycteroperca venenosa*	Guadeloupe and Saint Barthélemy, Caribbean Sea (Pottier, Vernoux and Lewis, 2001)
	Mycteroperca fusca	Canary Islands (CarlosIII, 2017)
	Pamatomus saltatriz	Canary Islands (CarlosIII, 2017)
Squaretail coral grouper	*Plectropomus areolatus*	China, Hong Kong SAR (Wong *et al.*, 2005)
Blacksaddled coral grouper	*Plectropomus laevis*	China, Hong Kong SAR (Wong *et al.*, 2008)
Coral trout / leopard coral grouper	*Plectropomus leopardus*	French Polynesia, Tubuai (Australes) (Darius *et al.*, 2007), China, Hong Kong SAR (Wong *et al.*, 2005), Tahiti (Pompon and Bagnis, 1984), French Polynesia (Bagnis *et al.*, 1987), Enewetak Island (Randall, 1980)
	Plectropomus melanoleucus	Enewetak Island (Randall, 1980)
	Plectropomus truncatus	Enewetak Island (Randall, 1980)

(continues)

TABLE 4 (continued)

COMMON NAME	SCIENTIFIC NAME	LOCATION WHERE FISH WAS FOUND
Large grouper	*Cephalopholis argus*	Enewetak Island (Randall, 1980), Kiribati (Mak *et al.*, 2013)
	Pagrus pagrus	Selvagens Islands (CarlosIII, 2017)
Coral trout	*Plectropomus* sp.	Great Barrier Reef, Australia (Hamilton *et al.*, 2002a), French West Indies (Pottier *et al.*, 2002b, 2002a)
Gastropod	*Tectus niloticus*	French Polynesia (Gatti *et al.*, 2018)
Hawkfish	*Paracirrhites hemistictus*	Kiribati (Mak *et al.*, 2013)
Lyretail	*Variola albimarginata*	China, Hong Kong SAR (Wong *et al.*, 2008)
Large grouper	*Variola louti*	Enewetak Island (Randall, 1980), Kiribati (Mak *et al.*, 2013)
Blotched javelin grunt	*Pomadasys maculatus*	Platypus Bay, Queensland, Australia (Hamilton *et al.*, 2002a)
Tarry hogfish (a'awa)	*Bodianus bilunulatus*	Hawaii (Hokama, 1985)
Spanish hogfish	*Bodianus rufus*	Saint Barthélemy, Caribbean Sea (Vernoux and Abbad el Andaloussi, 1986), Hawaii (Hokama, 1990)
Giant trevally (ulua)	*Caranx ignobilis*	dice
Horse-eye jack	*Caranx latus*	French West Indies (Pottier *et al.*, 2002b, 2002a), Saint Barthélemy, Caribbean Sea (Vernoux and Lewis, 1997; Lewis, Vernoux and Brereton, 1998), Bahamas (Larson and Rothman, 1967), Saint Thomas, Caribbean Sea (Granade, Cheng and Doorenbos, 1976)
Black jack	*Caranx lugubris*	French West Indies (Pottier *et al.*, 2002b, 2002a)
Bluefin trevally	*Caranx melampygus*	Nuku Hiva (Marquesas) (Darius *et al.*, 2007), French Polynesia (Bagnis *et al.*, 1987)
Brassy trevally	*Caranx papuensis*	French Polynesia, Tubuai (Australes) (Darius *et al.*, 2007)
Trevally (ulua, papio)	*Caranx sp.*	Hawaii (Hokama, 1985, 1990)
Jellyfish (omnivorous)	*Cnidaria sp.*	American Samoa (Zlotnick *et al.*, 1995)
Lionfish	*Pterois volitans*	Virgin Islands (Robertson *et al.*, 2014)
Lionfish	*Pterois spp.*	Guadalupe, Caribbean (Solino *et al.*, 2015)
Lobster	*Panulirus penicillatus*	Kiribati (Mak *et al.*, 2013)
Moorish idol	*Zancius cornutus*	Kiribati (Mak *et al.*, 2013)
Mullet (herbivorous)	*Mugil cephalus*	(Ledreux *et al.*, 2014)
King mackerel "Coronado" (king fish) (omnivorous)	*Scomberomorus cavalla*	Florida, the United States of America (Dickey, 2008), Saint Barthélemy, Caribbean Sea (Pottier, Vernoux and Lewis, 2001; Vernoux and Abbad el Andaloussi, 1986), Guadeloupe (Pottier, Vernoux and Lewis, 2001)
Spanish mackerel (omnivorous)	*Scomberomorus commerson*	Hervey Bay, Queensland, Australia (Lewis and Endean, 1984a), (Lewis and Endean, 1984a, 1983)
Fringelip mullet (omnivorous)	*Crenimugil crenilabis*	Nuku Hiva (Marquesas) (Darius *et al.*, 2007), French Polynesia (Bagnis *et al.*, 1987)
Thinlip grey mullet (omnivorous)	*Liza vaigiensis*	Nuku Hiva (Marquesas) (Darius *et al.*, 2007), Miyazaki, Japan (Yogi *et al.*, 2011)
Spotted knifejaw (omnivorous)	*Oplegnathus punctatus*	Miyazaki, Japan (Yogi *et al.*, 2011)
Parrotfish	*Hipposcarus longiceps*	Kiribati (Mak *et al.*, 2013)

(continues)

TABLE 4 (continued)

COMMON NAME	SCIENTIFIC NAME	LOCATION WHERE FISH WAS FOUND
Porcupinefish	*Diodon liturosus*	Kiribati (Mak *et al.*, 2013)
Porcupinefish	*Diodon hystrix*	Kiribati (Mak *et al.*, 2013)
Pufferfish	*Arothron nigropunctatus*	Kiribati (Mak *et al.*, 2013)
Pacific slopehead parrotfish (herbivorous)	*Chlorurus frontalis*	French Polynesia, Tubuai (Australes) (Darius *et al.*, 2007)
Steephead parrotfish (herbivorous)	*Chlorurus microrhinos*	French Polynesia, Tubuai (Australes) (Darius *et al.*, 2007)
Filament-finned parrotfish (herbivorous)	*Scarus altipinnis*	French Polynesia, Tubuai (Australes) (Darius *et al.*, 2007)
Heavy beak parrotfish (herbivorous)	*Scarus gibbus*	French Polynesia (Satake *et al.*, 1996), Tahiti (Pompon and Bagnis, 1984), French Polynesia (Bagnis *et al.*, 1987), Enewetak Island (Randall, 1980)
Blue-barred parrotfish (herbivorous)	*Scarus ghobban*	French Polynesia, Tubuai (Australes) (Darius *et al.*, 2007)
Ember parrotfish (herbivorous)	*Scarus rubroviolaceus*	Nuku Hiva (Marquesas) (Darius *et al.*, 2007)
Parrotfish (herbivorous)	*Scarus ghobban*	Kiribati (Mak *et al.*, 2013)
Parrotfish (herbivorous)	*Scarus russelii*	Kiribati (Mak *et al.*, 2013)
Rabbitfish (herbivorous)	*Siganus argenteus*	Kiribati (Mak *et al.*, 2013)
Marbled spinefoot rabbitfish (herbivorous)	*Siganus rivulatus*	Eastern Mediterranean (Bentur and Spanier, 2007)
Farmed salmon (omnivorous)	Oncorhynchus kisutch	Chile (Ebesu, Nagai and Hokama, 1994)
Blue sea chub (omnivorous)	*Kyphosus cinerascens*	French Polynesia, Tubuai (Australes), Nuku Hiva (Marquesas) (Darius *et al.*, 2007), Enewetak Island (Randall, 1980)
Sea cucumber (herbivorous)	*Holothuria spp.*	Hawaii (Park, 1999; Kohli, Farrell and Murray, 2015)
Hawaiian monk seal	*Monachus schauinslandi*	Hawaii (Bottein *et al.*, 2011)
Black forktail snapper (wahanui)	*Aphareus furca*	Hawaii (Hokama, 1990)
Blue-green snapper	*Aprion virescens*	French Polynesia (Bagnis *et al.*, 1987), Enewetak Island, Bikini Island (Randall, 1980)
Mangrove red snapper	*Lutjanus argentimaculatus*	China, Hong Kong SAR (Wong *et al.*, 2008)
Two-spot red snapper (red bass)	*Lutjanus bohar*	Mauritius (Hamilton *et al.*, 2002b; Hamilton *et al.*, 2002a), Minamitorishima (Marcus) Island, Japan (Yogi *et al.*, 2011), French Polynesia, Tubuai (Australes) (Darius *et al.*, 2007), Nuku Hiva (Marquesas) (Darius *et al.*, 2007), Hawaii (Hokama, 1990), French Polynesia (Bagnis *et al.*, 1987), Enewetak Island, Bikini Island (Randall, 1980), Kiribati (Mak *et al.*, 2013), India, Indonesia, Viet Nam (Friedemann, 2019)
Snapper	*Lutjanus fulvus*	Kiribati (Mak *et al.*, 2013)
Blackfin snapper	*Lutjanus buccanella*	Saint Croix, United States Virgin Islands (Hoffman, Granade and McMillan, 1983)
Humpback red snapper	*Lutjanus gibbus*	Nuku Hiva (Marquesas) (Darius *et al.*, 2007), French Polynesia (Bagnis *et al.*, 1987), Enewetak Island, Bikini Island (Randall, 1980)

(continues)

TABLE 4 (continued)

COMMON NAME	SCIENTIFIC NAME	LOCATION WHERE FISH WAS FOUND
Bluestripe snapper (taape)	*Lutjanus kasmira*	Hawaii (Hokama, 1985)
One-spot snapper	*Lutjanus monostigma*	Nuku Hiva (Marquesas) (Darius *et al.*, 2007), Enewetak Island, Bikini Island (Randall, 1980)
Red emperor	*Lutjanus sebae*	Mauritius (Nazareth, Saya de Malha, Soudan) (Hamilton *et al.*, 2002b; Hamilton *et al.*, 2002a)
Grey snapper	*Lutjanus griseus*	French West Indies (Pottier *et al.*, 2002b, 2002a)
Snapper	*Lutjanus spp.*	Antigua (Hokama, 1990), Okinawa, Japan (Yogi *et al.*, 2011), West Africa (Bienfang, Oben and DeFelice, 2008), Baja California, Mexico (Kohli, Farrell and Murray, 2015), Saint Thomas, Caribbean Sea (Granade, Cheng and Doorenbos, 1976)
Star snapper	*Lutjanus stellatus*	China, Hong Kong SAR (Wong *et al.*, 2008)]
Epaulette soldier fish (squirrelfish)	*Myripristis kuntee*	Hawaii (Hokama, 1985)
Soldier fish	*Myripristis berndti*	Kiribati (Mak *et al.*, 2013)
Sabre squirrelfish	*Sargocentron spiniferum*	Nuku Hiva (Marquesas) (Darius *et al.*, 2007)
Squirrelfish	*Sargocentron tiere*	Kiribati (Mak *et al.*, 2013)
Ophiuroids (brittle stars) starfish (omnivorous)	*Ophiocoma spp.*	Hawaii (Kohli, Farrell and Murray, 2015)
Dussumier's surgeon fish (palani) (herbivorous)	*Acanthurus dussumieri*	Hawaii (Hokama, 1985)
Bluelined surgeon fish (maiko) (herbivorous)	*Acanthurus nigroris*	Hawaii (Hokama, 1985)
Orangeband surgeon fish (naenae) (herbivorous)	*Acanthurus olivaceus*	Hawaii (Hokama, 1985)
Yellowfin surgeon fish (herbivorous)	*Acanthurus xanthopterus*	Hawaii (Hokama, 1990), Nuku Hiva (Marquesas) (Darius *et al.*, 2007)
Surgeonfish (herbivorous)	*Acanthurus lineatus*	Kiribati (Mak *et al.*, 2013)
Surgeonfish (herbivorous)	*Acanthurus maculiceps*	Kiribati (Mak *et al.*, 2013)
Surgeonfish (omnivorous)	*Acanthurus gahhm*	Kiribati (Mak *et al.*, 2013)
Surgeonfish (omnivorous)	*Acanthurus nata*	Kiribati (Mak *et al.*, 2013)
Surgeonfish (omnivorous)	*Acanthurus striatus*	Kiribati (Mak *et al.*, 2013)
Whitebar surgeonfish	*Acanthurus leucopareius*	French Polynesia
Striped bristletooth surgeon fish (herbivorous)	*Ctenochaetus striatus*	Nuku Hiva (Marquesas) (Darius *et al.*, 2007), Tahiti (Bagnis *et al.*, 1985a)
Sand tilefish	*Malacanthus plumieri*	Saint Barthélemy, Caribbean Sea (Vernoux and Abbad el Andaloussi, 1986)
Dogtooth tuna	*Gymnosarda unicolor*	Nuku Hiva (Marquesas) (Darius *et al.*, 2007), French Polynesia (Bagnis *et al.*, 1987), Enewetak Island (Randall, 1980)
Humpback unicorn fish	*Naso brachycentron*	Nuku Hiva (Marquesas) (Darius *et al.*, 2007)
Spotted unicorn fish	*Naso brevirostris*	Nuku Hiva (Marquesas) (Darius *et al.*, 2007)

(continues)

TABLE 4 (continued)

COMMON NAME	SCIENTIFIC NAME	LOCATION WHERE FISH WAS FOUND
Sleek unicorn fish	*Naso hexacanthus*	Nuku Hiva (Marquesas) (Darius *et al.*, 2007)
Orangespine unicorn fish	*Naso lituratus*	Nuku Hiva (Darius *et al.*, 2007), (Marquesas) (Bagnis *et al.*, 1987)
Bluespine unicorn fish	*Naso unicornis*	Nuku Hiva (Marquesas) (Darius *et al.*, 2007)
Humphead wrasse	*Cheilinus undulatus*	French Polynesia (Bagnis *et al.*, 1987), China, Hong Kong SAR (Wong *et al.*, 2005), Enewetak Island (Randall, 1980)
Clown coris (wrasse)	*Coris aygula*	French Polynesia, Tubuai (Australes) (Darius *et al.*, 2007), Enewetak Island (Randall, 1980), Kiribati (Mak *et al.*, 2013)
Shark	*Carcharhinus leucas*	Madagascar (Diogene *et al.*, 2017)
	Carcharhinus amblyrhinchos	Enewetak Island (Randall, 1980)
	Carcharhinus limbatus	Enewetak Island (Randall, 1980)
	Lycodontis javanicus	Enewetak Island (Randall, 1980)
	Sphyraena barracuda	Enewetak Island (Randall, 1980)
Snapper	*Macolor niger*	Enewetak Island (Randall, 1980), Kiribati (Mak *et al.*, 2013)
	Caranx ignobilis	Enewetak Island (Randall, 1980)
	Caranx lugubris	Enewetak Island (Randall, 1980)
	Caranx melampygus	Enewetak Island (Randall, 1980)
	Hipposcarus harid	Enewetak Island (Randall, 1980)
Triggerfish	*Balistapus undulatus*	Kiribati (Mak *et al.*, 2013)
Wrasse	*Epibulus insidiator*	Kiribati (Mak *et al.*, 2013)
Starfish	*Ophidiaster ophidianus*	Madeira, Azores (Silva *et al.*, 2015)
Starfish	*Marthasterias glacialis*	Madeira, Azores (Silva *et al.*, 2015)
Sea urchin	*Tripneustes gratilla*	French Polynesia (Darius *et al.*, 2018a)

One issue that has been identified as a potential problem for CP prevention is the mislabelling of fish species, either unintentionally or by fraudulent initiatives. This may lead to ciguateric species that replace others being put on the market (Stewart *et al.*, 2009). Moreover, the identification of ciguateric fish and their catch location is often difficult, and this adds to the difficulty of establishing the real toxic levels that did cause the poisoning, due to analytical limitations (Farrell *et al.*, 2017).

The approach followed by many countries is to impose fish size restrictions as a ciguatera risk management action (Clausing, Chinain and Dechraoui Bottein, 2016). As an example, the Sydney Fish Market (Market, 2013) is to warn of possible ciguatoxic fish that may be sold (surgeonfish [all Acanthuridae members], flowery rockcod [*Epinephelus fuscoguttatus*], yellowtail kingfish and samsonfish [*Seriola* spp.], narrow-barred Spanish mackerel [*Scomberomorus commerson*], coral trout [*Plectropomus* spp. and *Variola* spp.) and parrot fish [all Scaridae members]), and to forbid others (Chinamanfish [*Symphorus nematophorus*], tripletail Maori wrasse [*Cheilinus trilobatus*] and humphead Maori wrasse [*Cheilinus undulatus*], red bass [*Lutjanus bohar*], paddletail [*Lutjanus gibbus*], giant moray [*Gymnothorax*

javanicus]) obtained in the regions of Kiribati, certain Queensland areas, Marshall Islands, Northern Australian Territory waters (Bremer Island, Bonner Rocks, Miles Island, Cape Arnhem, etc), and Fijian waters. For high-risk species, a maximum size is allowed, with a range that varies from, for example, 3 kg for coral rockcod (*Cephalopholis* spp.) to 10 kg for mackerel (*Scomberomorus* spp.).

The approach taken in the Canary Islands (Spain), is to check for the presence of CTXs in fishes of certain species above a threshold weight, and always excluding viscera (Table 3).

The method for routine control is the neuroblastoma assay (Canarias, 2017; Caillaud *et al*., 2012) (see Section 4).

The toxicity of some species is associated to seasonal variations. *Sphyraena barracuda* was reported to increase in toxicity in winter and autumn (60–70 percent toxic fish), while toxic fish is only 10 percent in summer and December (Tosteson, Ballantine and Durst, 1988). This observation, taken in Puerto Rico, might be associated to changes in the toxicity of fish prey.

Some countries count on reporting systems. In the United States of America, the reporting of cases should be made to the surveillance system of Centers for Disease Control and Prevention (CDC; www.cdc.gov/nors/index.html). In the European Union, the Rapid Alert System for Food and Feed (RASFF; https://ec.europa.eu/food/safety/rasff_en) provides alerts to food safety agencies and consumers. In French Polynesia, the Institut Louis Malardé (www.ilm.pf) provides information and issues alerts about CP.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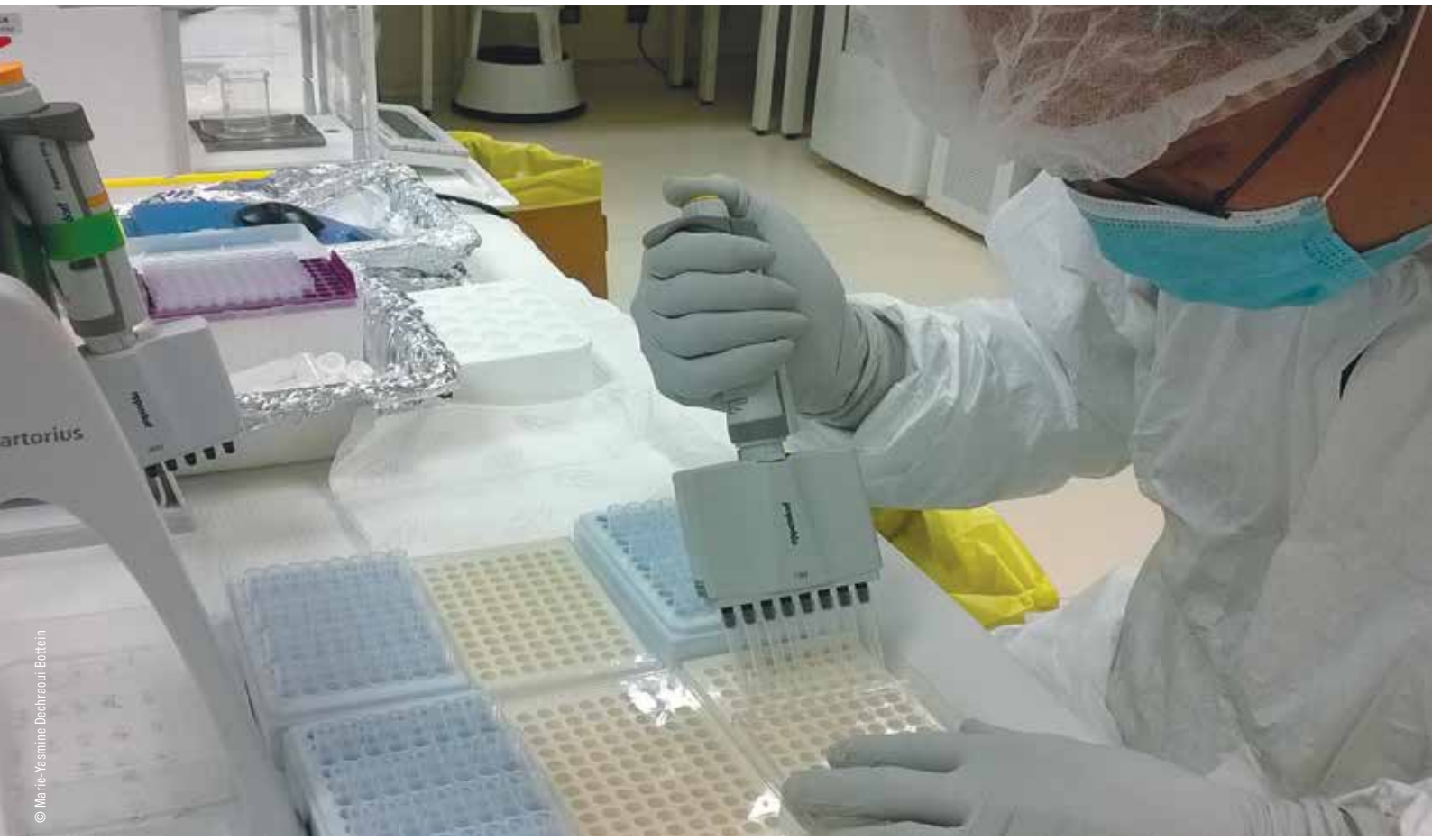
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CHAPTER 3
CHEMISTRY AND BIOSYNTHETIC PATHWAYS

3.1 CHEMISTRY

3.1.1 STRUCTURES

Ciguatoxins (CTXs) are a class of large polyether ladder-like structures that contain 13–14 fused rings (Nicolaou, Frederick and Aversa, 2008). Representative backbone structures of CTXs identified so far are represented by CTX4A, CTX3C and C-CTX1.

After the initial description of "ciguatoxin" in 1967 (Scheuer *et al.*, 1967), the structure of "CTX" (= CTX1B) and its analogue (CTX4A) were reported in 1989 following significant isolation and purification efforts from moray eel and *Gambierdiscus* cultures, respectively (Murata *et al.*, 1989). The diastereoisomers 52-*epi*-54-deoxyCTX1B and 54-deoxyCTX1B (CTX2 and CTX3, respectively) were subsequently described, and these represent less oxidized forms of CTX1B (Lewis *et al.*, 1991). The only difference between CTX1B, 52-*epi*-54-deoxyCTX1B and 54-deoxyCTX1B involves modification at one end of the CTX structure with no hydroxyl group on the M-ring, and this manifests in modest differences in potency (Lewis *et al.*, 1991). The structure of CTX3C was first elucidated in 1993 after extraction from a culture of what was at that time described as *G. toxicus* and is now thought to have been a strain of *G. polynesiensis.* Subsequent structures of the oxidized forms of the toxin were determined in 1998 from fish (Satake *et al.*, 1998). The backbone structure of CTX3C toxins differs from the CTX4A group on the E-ring (i.e. an eight-membered ring in CTX3C and a seven-membered ring in CTX4A) and absence of an aliphatic side chain on the A-ring (Satake *et al.*, 1998; Satake *et al.*, 1996; Satake, Murata and Yasumoto, 1993b). Several toxic Caribbean CTX analogues have been identified and isolated from fish for structural characterization (Crouch *et al.*, 1995). The structure of the major Caribbean toxin C-CTX1 and its 56-epimer C-CTX2 were first described by Lewis and Vernoux in 1997 and 1998 (Vernoux and Lewis, 1997; Lewis, Vernoux and Brereton, 1998). C-CTX2 was found as a minor analogue in fish, rearranging to C-CTX1 in solution. The C-CTX backbone shares characteristics with CTX4A and CTX3C but does not possess the aliphatic side chain on the A-ring and contains an additional ring on the right wing of the molecule. Additional Caribbean CTXs have been reported

in several studies but are yet to be structurally elucidated (Pottier *et al*., 2002b). An Indian Ocean group has also been described (Hamilton *et al*., 2002b), and although masses have been reported in the literature (Diogene *et al*., 2017), no molecular structures have been determined to date. Yasumoto and Satake (1996) provide a concise summary of the chemistry and aetiology of CTXs and other related toxins that could be associated with ciguatera poisoning.

3.1.2 CHEMICAL CHARACTERISTICS

Ciguatoxins are lipid-soluble compounds that are thermostable and resistant to mild pH changes (Guzmán-Pérez and Park, 2000). The most toxic analogue described to date is CTX1B, which is stable at 100 °C, 1 N NaOH, and in sunlight for 1 h, but loses toxicity in 1 N HCl after 10 min (Guzmán-Pérez and Park, 2000; Nukina, Koyanagi and Scheuer, 1984). Ciguatoxins are odourless, tasteless, heat stable and present at very low (typically < ppb) levels in contaminated seafood, making them difficult to detect without advanced detection methods (see Section 4 for more details on detection methods). The toxic potency of CTXs has been shown to increase as they become more oxidized (Lewis *et al*., 1991). Analogues originally isolated from the Pacific are currently thought to be the most potent and have been well characterized. Some CTXs are metabolites generated through the process of enzyme-mediated biotransformation in invertebrates and fish. For example, one study has demonstrated the oxidation of algal toxins CTX4A, CTX4B and CTX3C *in vitro* to the analogues found in fish using human liver CYP enzymes and fish liver microsomes (S9 fractions) (Ikehara *et al*., 2017). However, the low yields reported using this approach make large-scale production of standards impractical at present. Biotransformation contributes to the large number and structural diversity of CTXs observed in fish, with more than 30 analogues reported to date (Yasumoto *et al*., 2000; Vernoux and Lewis, 1997; Hamilton *et al*., 2002b; Diogene *et al*., 2017). Ciguatoxins are lipophilic, a property recognized in early studies (1950–1960), based on bioassay-guided separation of components in toxic fish (Hashimoto, 1956; Banner *et al*., 1960). Ciguatoxin lipophilicity has been estimated from the partition coefficient of CTX1B between ethyl acetate and HEPES-buffered Ringer solution in a 1:1 ratio at 37 °C (Lewis, Hoy and Sellin, 1993). As the ratio was determined to be > 2.0, it was confirmed that CTX1B was a lipophilic compound and suggested that it could cross cellular membranes (Lewis, Hoy and Sellin, 1993). Experimental CTX1B oral and intraperitoneal (ip) dosing studies in mice confirmed the rapid absorption capacity of CTXs, and ability to cross the blood–brain barrier as evidenced by CTX1B levels in post-mortem brain tissue (Bottein, Wang and Ramsdell, 2011). Reports in wild-caught fish contaminated with CTXs showed similar compartmentalization of toxins across tissues into liver, spleen, brain, muscle, gonads, fat and bone (Vernoux *et al*., 1985a). While these studies confirm the lipophilic nature of CTX1B, no detailed studies of partitioning coefficients or cellular transport mechanisms have been conducted on this CTX analogue to date, largely due to the lack of available standards. The polarity of other CTXs has been estimated based on the order of elution (i.e. retention time) when using

reversed-phase chromatography (Satake *et al*., 1998; Yasumoto *et al*., 2000; Hessel, Halstead and Peckham, 1960; Scheuer *et al*., 1967; Hashimoto, 1956). It would be advantageous to explore quantitative structure activity relationship modelling from known and newly elucidated structures as they become available in order to help predict the absorption of CTXs in humans and aquatic organisms, particularly fish.

3.1.3 BIOSYNTHETIC PATHWAYS

A remarkable feature of *Gambierdiscus* is its unique biochemical machinery, responsible for the production of multiple structurally complex polyether toxins, including CTXs, gambierol, gambierone, gambieroxide, gambieric acids and maitotoxins. Different *Gambierdiscus* spp. produce varying amounts of toxin, generate different toxin profiles, and strain differences are also observed. For example, a recent toxicity study of various *Gambierdiscus* species, and strains, showed that they all produce MTX-like activity, in the range 1.5–86 pg MTX eq/cell, and CTX-like activity in the range 0.6–50 fg CTX3C eq/cells (Pisapia *et al*., 2017a). In addition, a strain of *G. excentricus* isolated from the Canary Islands (Spain) was reported to produce 1 426 fg CTX3C eq/cell (Pisapia *et al*., 2017a), and a strain of *G. polynesiensis* from the South Pacific produced 18.2 pg/cell (Rhodes *et al*., 2016). A comprehensive table of *Gambierdiscus* toxicity can be found in Section 2.6 Toxicity of CP causative organisms. In the Pacific, the link between algal CTX production and those observed in fish has been established (Ikehara *et al*., 2017). However, more evidence is needed before this information can be used to predict the presence of CTXs in seafood from a specific region.

The size of dinoflagellate genomes is enormous, with more than 100 chromosomes and up to 80-fold the human haploid genome (Lin, 2011), and this large genome is suspected to be one of the causes of their slow growth. Dinoflagellates possess some of the largest genomes known from eukaryotes, from 1.85 Gbp to 112 Gbp (LaJeunesse *et al*., 2005). Some of the toxins they produce are among the largest non-polymeric compounds reported in nature. For example, MTX is produced by several *Gambierdiscus* spp. and has a molecular weight of 3 422 Da, containing 32 rings, with 99 elements of stereochemistry. It is the largest and most toxic natural product characterized to date (Murata *et al*., 1993; Nicolaou, Frederick and Aversa, 2008). Therefore, *Gambierdiscus* has the genetic and biochemical machinery for synthesis of extremely large and complex natural compounds.

The genes involved in *Gambierdiscus* toxin production are not known, and application of molecular methods to detect toxic species is therefore not currently possible. The enzymes needed for construction of these complex molecules are thought to be extender unit polyketide synthases (PKSs), which are one of the most important and diverse class of enzymes occurring in plants, bacteria and fungi. They are a large multimodular enzymatic group that participate in the synthesis of many natural compounds (Barrios-Gonzalez and Miranda, 2010), and are classified into three types, according to their domain organization: (i) Type I PKS are large multifunctional enzymes with modular or iterative activity, where multiple catalytic

sites exist on a single polypeptide; (ii) Type II PKSs are organized in complexes of smaller monofunctional enzymes with iterative activity, where each gene encodes a single catalytic enzyme; and (iii) Type III PKSs are similar to Type II but smaller. To date, transcriptome data are available for four species of *Gambierdiscus*: *G. polynesiensis*, *G. belizeanus*, *G. australes* and *G. excentricus* (Pawlowiez, *et al.*, 2014; Kohli *et al.*, 2017; Kohli *et al.*, 2015); and PKS genes-related sequence data show similarity to type I PKSs, as reported in other dinoflagellates (Van Dolah *et al.*, 2017). The biosynthesis of MTX by PKS would require multiple steps that include polyepoxidation, cyclization and sulfonation (Kohli *et al.*, 2015). Some species, such as *G. australes*, produce MTX and not CTX (Rhodes *et al.*, 2014a). Several studies report low CTX production in cultures (Holmes, Lewis and Gillespie, 1990; Murata *et al.*, 1990), and it has been shown that environmental manipulation can supress toxin production (Morton *et al.*, 1993). Nevertheless, each strain has its own unique toxic profile, which is dependent on the optimal growth conditions. *G. polynesiensis* produces similar amounts of MTX and CTX in culture, but as the cells age, CTX becomes dominant (Chinain *et al.*, 2010a). Some investigators have reported that *Gambierdiscus* cultures require an acclimation time to produce toxins, between 16 weeks and 52 weeks (Chinain *et al.*, 2010a). The rate of toxin production is inverse to the rate of growth. Hence, maximum toxin levels correspond to a stationary phase, which also suggests that CTXs are secondary metabolites (Sperr and Doucette, 1996). Further research is required to determine the toxin profile of the different *Gambierdiscus* spp., and to understand why toxin production changes when in culture and how this process is modulated.

3.2 CIGUATOXIN CLASSIFICATION

Three families of CTXs have commonly been classified according to their geographical location, i.e. Pacific (P-CTXs), Caribbean (C-CTXs) and Indian Ocean (I-CTXs). Thanks to advances in structural elucidation, it is now possible and appropriate to classify CTXs based on the known chemical structures. The structural characteristics of P-CTXs actually allow further classification into two separate groups based on their chemical structure, i.e. CTX3C vs CTX4A backbones and their derivatives. The Caribbean CTXs represent a third group. To date, only two Caribbean CTXs have been structurally elucidated (C-CTX1 and C-CTX2) (Lewis, Vernoux and Brereton, 1998; Vernoux and Lewis, 1997). Several further compounds have been identified as potential C-CTXs; however, there is little evidence concerning their structure or relative toxicity (Pottier *et al.*, 2002b, 2002a). Figure 8 shows the backbone structures and associated analogues of these three groups. An Indian Ocean group has also been referred to; however, their structures have not been elucidated (Hamilton *et al.*, 2002a; Hamilton *et al.*, 2002b). More research is needed to isolate the toxins observed in the Indian Ocean region in order to determine their structural characteristics and potency, and whether they are related to CTXs found in other regions. Tables 5 and 6 shows the classification and abbreviation synonyms of CTXs.

TABLE 5 **CLASSIFICATION OF CTXs AND ABBREVIATION SYNONYMS**

COMPOUND (accepted synonym)	OTHER SYNONYMS	REFERENCES	MOLECULAR WEIGHT (Da)
Ciguatoxin 4A group (CTX4A and derivatives)			
CTX1B	CTX	Vernoux and Lewis, 1997; Legrand *et al.*, 1989; Murata *et al.*, 1989; Murata *et al.*, 1990; Satake *et al.*, 1996; Inoue *et al.*, 2006; Hamajima and Isobe, 2009	1110.6
	CTX1b	Gaudry-Talarmain *et al.*, 1996; Benoit *et al.*, 1996	
	CTX1B	Satake *et al.*, 1997	
	CTX1	Lewis *et al.*, 1991	
	P-CTX-1	Vernoux and Lewis, 1997	
	P-CTX1B	Caillaud *et al.*, 2009	
CTX1A	52-epiCTX, 52-*epi*CTX1B	Yasumoto, 2001	1110.6
54-deoxyCTX1B	CTX3	Lewis *et al.*, 1991; Lewis *et al.*, 1993	1094.6
	P-CTX-3	Vernoux and Lewis, 1997	
	54-deoxyCTX	Yasumoto, 2001	
	54-deoxyCTX1B	Yogi *et al.*, 2011	
52-*epi*-54-deoxyCTX1B	CTX-2	Lewis, *et al.*, 1991; Lewis *et al.*, 1993	1094.5
	P-CTX2	Vernoux and Lewis, 1997	
	52-*epi*-54-deoxyCTX	Yasumoto, 2001	
	52-*epi*-54-deoxyCTX1B	Yogi *et al.*, 2011	
CTX4A (52-*epi*-CTX4B)	CTX4A, scaritoxin (possibly a mixture of CTX4A and CTX4B)	Satake *et al.*, 1997; Satake *et al.*, 1996	1060.6
	P-CTX4A	Vernoux and Lewis, 1997	
CTX4B	no name	Murata *et al.*, 1989	1060.6
	(gambiertoxin-4B) GTX4B	Murata *et al.*, 1990	
	CTX4B	Satake *et al.*, 1996	
	scaritoxin	Satake *et al.*, 1997	
	P-CTX4B	Vernoux and Lewis, 1997	
CTX4C	Analogue of CTX	Legrand, *et al.*, 1989; Legrand *et al.*, 1990; Legrand, 1991	Personal communication from T. Yasumoto: possible artefact from preparative chromatography (it is a mixture of CTX4A and 4B)
M-*seco*-CTX4A/4B	M-*seco*-CTX4A	Yasumoto, 2001	1078.6
	M-*seco*-CTX4A/4B	Yogi *et al.*, 2011	

(continues)

TABLE 5 (continued)

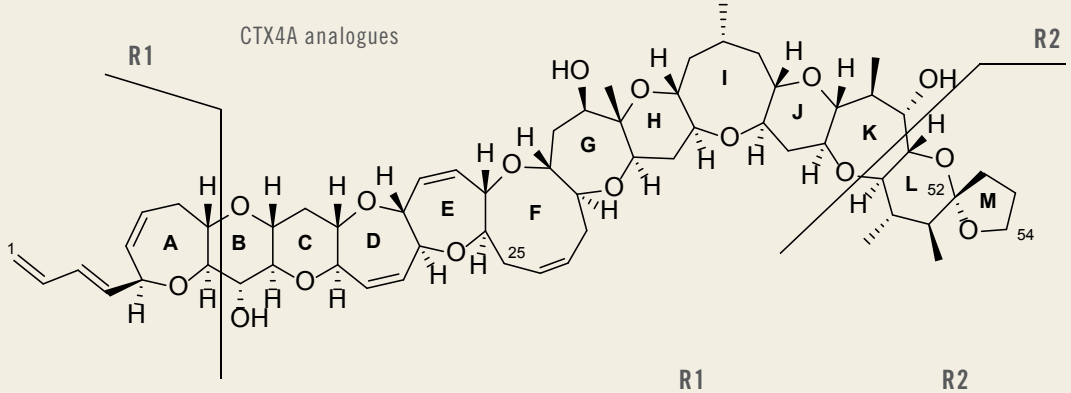
COMPOUND (accepted synonym)	OTHER SYNONYMS	REFERENCES	MOLECULAR WEIGHT (Da)
Ciguatoxin 4A group (CTX4A and derivatives)			
7-oxoCTX1B	7-oxoCTX	Yasumoto, 2001	1126.6
7-hydroxyCTX1B	7-hydroxyCTX	Yasumoto, 2001	1128.6
4-hydroxy-7-oxoCTX1B	4-hydroxy-7-oxoCTX	Yasumoto, 2001; Ikehara *et al.*, 2017	1144.6
54-deoxy-50-hydroxyCTX1B	54-deoxy-50hydroxyCTX	Yasumoto, 2001	
Ciguatoxin 3C group (CTX3C and derivatives)			
CTX3C	CTX3C	Satake, Murata and Yasumoto, 1993b; Hirama, *et al.*, 2001	1022.6
	P-CTX3C	Vernoux and Lewis, 1997	
CTX3B	49-*epi*CTX3C	Chinain *et al.*, 2010a Yasumoto, 2001	
51-hydroxyCTX3C		Satake *et al.*, 1998; Inoue *et al.*, 2006	1038.6
2,3-dihydro-2,3-dihydroxyCTX3C	2,3,-dihydroxyCTX3C	Satake *et al.*, 1998	1056.6
2,3-dihydro-2-hydroxyCTX3C		Yasumoto *et al.*, 2000	1040.6
2,3-dihydro-51-hydroxy-2-oxo CTX3C	51-hydroxy-2-oxoCTX3C	Yasumoto *et al.*, 2000	1054.6
2,3-dihydro-2,3,51-trihydroxy CTX3C	2,3,51-trihydroxyCTX3C	Yasumoto *et al.*, 2000	1072.6
A-*seco*-2,3-dihydro-51-hydrox yCTX3C	A-seco-51-hydroxyCTX3C	Yasumoto *et al.*, 2000	1058.6
M-*seco*-CTX3C		Yasumoto *et al.*, 2000; Yogi *et al.*, 2011	1040.6
M-*seco*-CTX3C methylacetal		Yasumoto *et al.*, 2000; Yogi *et al.*, 2011	1054.6
Caribbean ciguatoxin group (C-CTX1 and derivatives)			
Caribbean ciguatoxin-1	C-CTX1	Vernoux and Lewis, 1997	1140.7
		Lewis, Vernoux and Brereton, 1998	1140.6
Caribbean ciguatoxin-2	C-CTX2	Vernoux and Lewis, 1997	1140.7
		Lewis, Vernoux and Brereton, 1998	
C-CTX-analogues	10 additional analogues C-CTX3-12	Vernoux and Lewis, 1997; Pottier *et al.*, 2002b, 2002a; Abraham *et al.*, 2012a; Estevez *et al.*, 2019	1126.6 (1) 1140.6 (3) 1142.6 (2) 1156.6 (3) 1158.6 (1)
Indian ciguatoxin group (I-CTX1 and derivatives)			
Indian Ocean ciguatoxins 1-6	I-CTX1-6	Hamilton *et al.*, 2002b; Diogene *et al.*, 2017	1140.6 (2) 1156.6 (2) 1138.6 (1) 1154.6 (1)

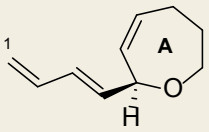
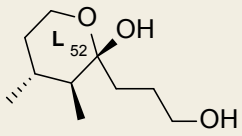
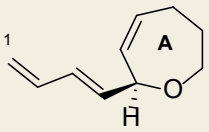
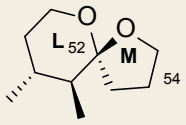
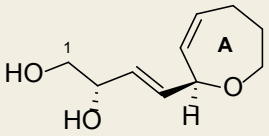
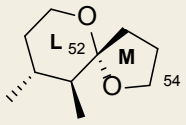
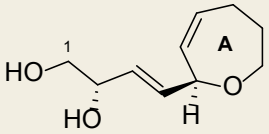
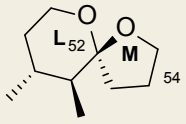
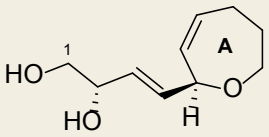
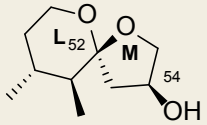
TABLE 6 ***GAMBIERDISCUS*** **METABOLITES OTHER THAN CTXs**

COMPOUND (accepted synonym)	OTHER SYNONYMS	REFERENCE	MOLECULAR WEIGHT
MTX	MT (maitotoxin)	Yasumoto, Bagnis Vernoux, 1976	
	MTX	Murata *et al.*, 1993; Murata *et al.*, 1994	3422
	MTX-1	Sasaki *et al.*, 1996; Nonomura *et al.*, 1996; Zheng *et al.*, 1996	
MTX2	MTX-2	Holmes, Lewis and Gillespie, 1990	3298
MTX3	MTX-3 (44-methyl gambierone)	Holmes and Lewis, 1994; Murray *et al.*, 2019; Boente-Juncal *et al.*, 2019	1060.5
MTX4	MTX-4	Pisapia *et al.*, 2017b	3292.5 (free acid form)
Gambieric acids A-D	GA-A	Nagai *et al.*, 1992; Morohashi *et al.*, 2000	1056.6389 (mono-isotopic mass of neutral molecule)
	GA-B		1070.6546 (mono-isotopic mass of neutral molecule)
	GA-C		1187.7098 (mono-isotopic mass of neutral molecule)
	GA-D		1201.7254 (mono-isotopic mass of neutral molecule)
Gambierol		Satake, Murata and Yasumoto, 1993a	756.4451 (mono-isotopic mass of neutral molecule)
Gambieroxide		Watanabe *et al.*, 2013	1194.5648 (mono-isotopic mass of neutral molecule)
Gambierone		Rodriguez *et al.*, 2015	1024.4704 (mono-isotopic mass of neutral molecule)

FIGURE 8 **CIGUATOXIN CLASSIFICATION**

Group 1: CTX4A analogues



	R1	R2
M-seco-CTX4A/B		
CTX4B		
52-epi-54-deoxyCTX1B		
54-deoxyCTX1B		
CTX1B		

Group 2: CTX3C analogues

CTX-3C

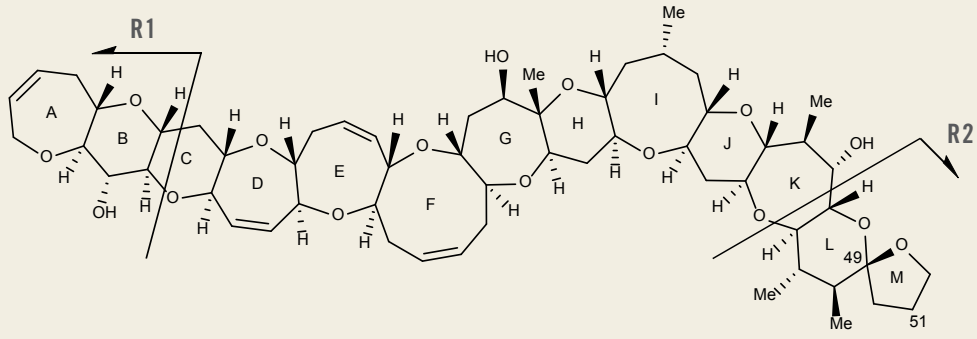


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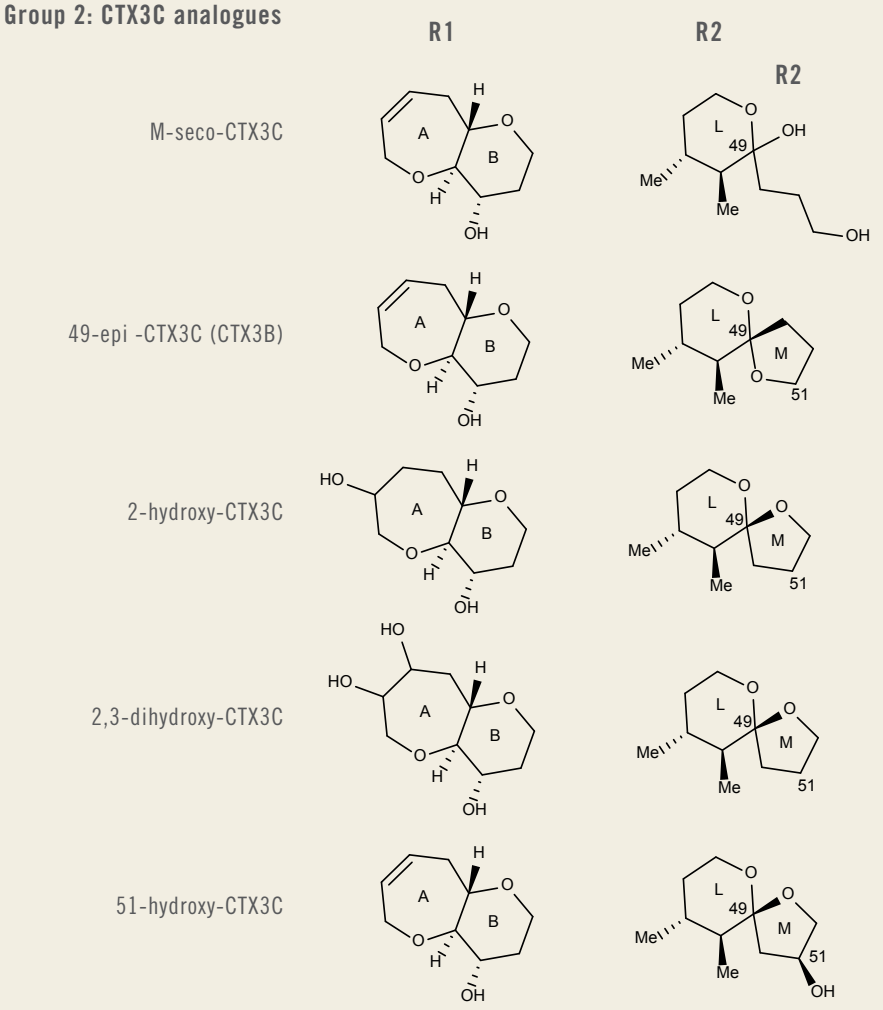
FIGURE 8 (continued)

Group 2: CTX3C analogues



Group 3: C-CTX1 and C-CTX2

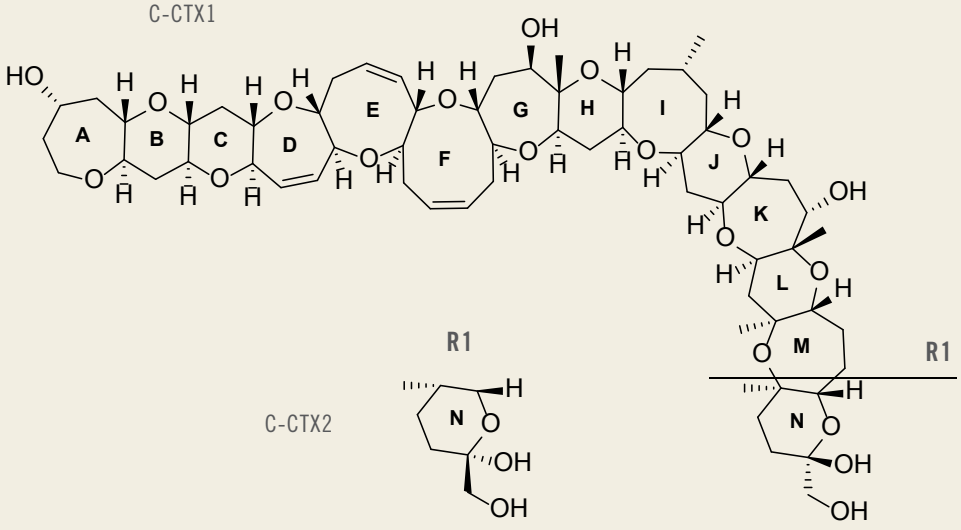
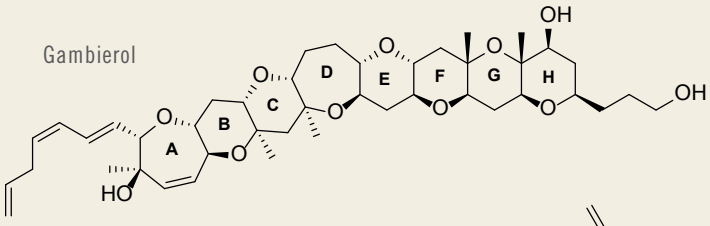
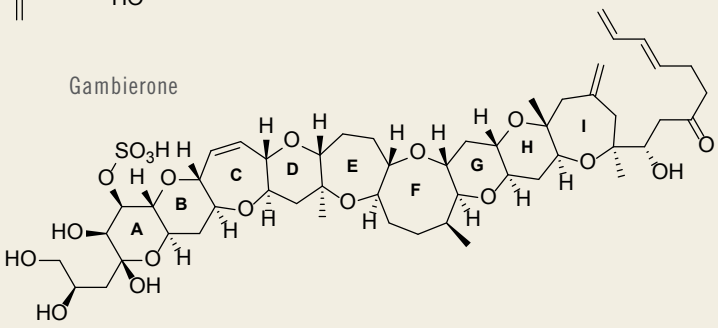


FIGURE 9 ***GAMBIERDISCUS*** **METABOLITES OTHER THAN CTXs**

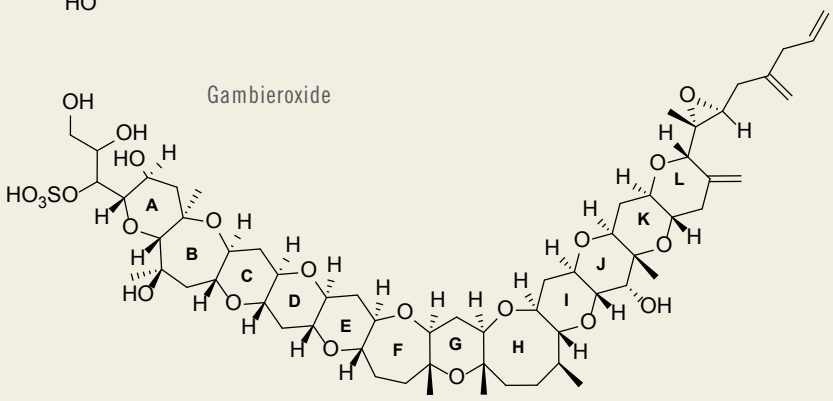
Gambierol



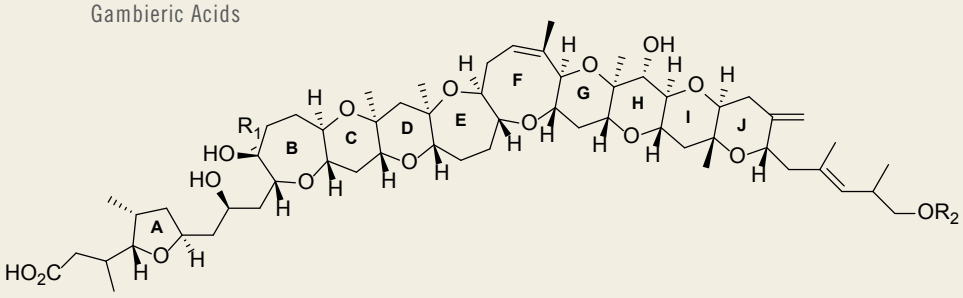
Gambierone



Gambieroxide



Gambieric Acids



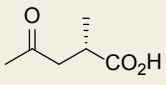
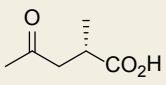
	R1	R2
Gambieric Acid A	H	H
Gambieric Acid B	CH_3	H
Gambieric Acid C	H	
Gambieric Acid D	CH_3	

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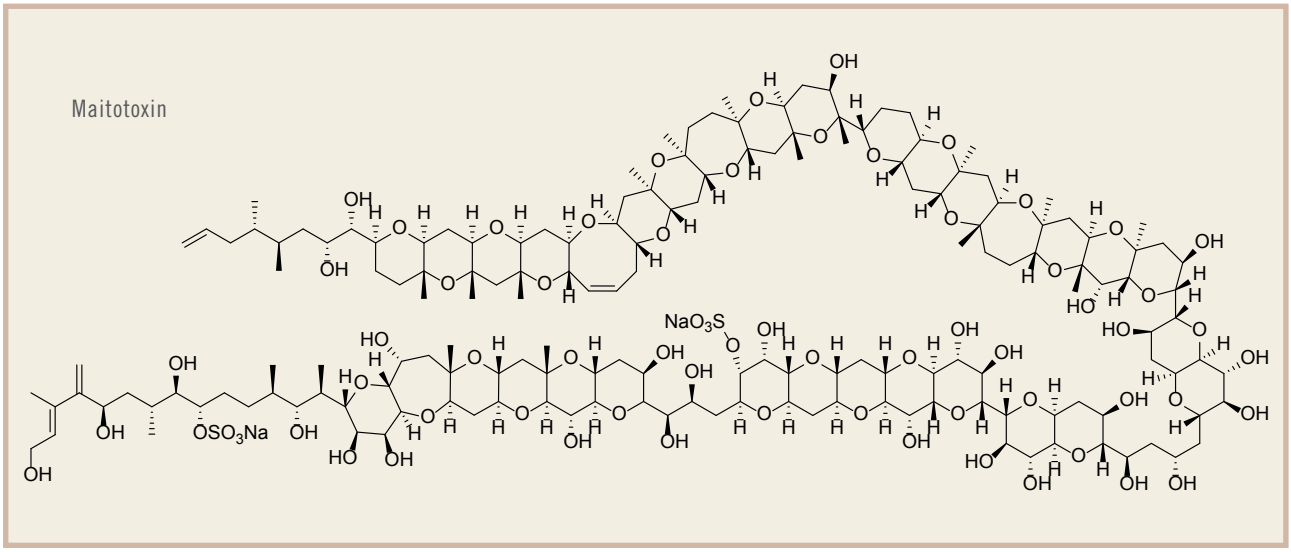
FIGURE 9 (continued)



While the biotransformation of algal toxins into metabolites through the food web, and in particular fish, has been investigated for CTX3C and CTX4A analogues (Yasumoto, 2001; Ikehara *et al*., 2017), no such information is available for the C-CTX group or Indian Ocean CTXs at this time.

There are other known bioactive compounds produced by *Gambierdiscus* spp. that potentially have relevance to human health, including maitotoxin 1–4, gambierone, gambieroxide, gambierol and gambieric acids; structures of these compounds are shown in Figure 9. While for some compounds (e.g. MTX1) a different mode of action has been determined, the toxicity mechanisms remain to be ascertained for the other compounds. There is no clear evidence that these compounds accumulate in fish flesh to significant levels, while they have been consistently found at higher levels in visceral tissues (Kohli *et al*., 2014a; Diogene *et al*., 2017). However, in processing environments, it cannot be excluded that cross-contamination of fish flesh could occur from poorly handled visceral tissues. In many areas of the world, invertebrates and fish are eaten whole, and for a variety of fish species many organs are targeted for direct consumption (e.g. liver, gonads and roe) and eaten on a regular basis. Thus, the contribution of these other bioactive compounds in the human health risk associated with ciguatera cannot be discounted.

CHAPTER 4
DETECTION METHODOLOGIES FOR CTXs

Due to their high potency, their complex structures, trace occurrence, and the scarce availability of analytical standards, detecting CTXs in seafood and algae presents many analytical challenges (Suzuki *et al.*, 2017; Vilarino *et al.*, 2018). Proposed analytical strategies for detecting CTXs can be divided into screening and confirmation methods. To date, none of the methods described has been reported to have undergone single- or multi-laboratory validation. Table 7 provides an overview of the existing published methods along with the advantages and disadvantages of each strategy. While some laboratories have produced reference materials and quantified standards on a small scale, recent data have most consistently reported CTX3C equivalents due to commercial availability. While not commercially available, some quantified reference materials are available through a variety of laboratories.

TABLE 7 **AVAILABLE SCREENING ASSAYS AND CONFIRMATION METHODS**

ASSAYS	APPROXIMATE DETECTABILITY (µg/kg)	DEVELOPMENT TIME	SAMPLE USAGE	PROS	CONS	REFERENCES
N2A-MTT	LOD: [0.0096–0.17] ng CTX1B eqv.g-1 LOD: 0.02 ng CTX3C eqv.g-1 LOD: [0.002–0.032] ng C-CTX1 eqv.g-1 LOQ: [0.4–17] pg CTX1B eqv.g-1 LOQ: [0.006–0.039] ng C-CTX1 eqv.g-1 EC50 CTX1B = [0.078–19.0] pg.ml-1 CTX3C = [0.57–3.1] pg.ml-1 C-CTX1 = [0.74–20.0] pg.ml-1	48 h (24 h following 24 h to plate the cells)	1–5 g	Activity-based;[3] Low sample and standard consumption; High throughput capacity; Quantitative composite CTX-like activity determination, can differentiate between VGSC blocker toxins (STX, TTX) and VGSC activator toxins (e.g. CTXs, PbTxs), highly sensitive detection; non-specific neurotoxicity can be detected with appropriate control dose-response curves; No radioisotopes required.	Cell culture skills and facilities needed; Extensive sample cleanup required; Time intensive to obtain results due to incubation periods (24 h)	Bienfang, DeFelice and Dowling, 2011; Caillaud *et al.*, 2012; Darius *et al.*, 2018a; Darius *et al.*, 2018b; Diogene *et al.*, 2017; Ledreux *et al.*, 2014; Chan *et al.*, 2011; Bottein Dechraoui *et al.*, 2005; Sanchez-Henao *et al.*, 2019; Bottein Dechraoui, Wang and Ramsdell, 2007; Manger *et al.*, 1995; Pisapia *et al.*, 2017a; Roué *et al.*, 2016; Pawlowiez *et al.*, 2013

(continues)

TABLE 7 (continued)

ASSAYS	APPROXIMATE DETECTABILITY (µg/kg)	DEVELOPMENT TIME	SAMPLE USAGE	PROS	CONS	REFERENCES
RBA H[3] Based on competitive binding of CTXs with H3-brevetoxin-3 [3]H	LOD: 0.155 ng CTX3C eqv.g-1 LOQ: [0.31–0.33] ng CTX3C eqv.g-1 EC50 CTX1B = 0.26 ± 0.14 ng. ml-1 CTX3C = [0.35–0.66] ng. ml-1 C-CTX1 = 0.34 ± 0.11 ng. ml-1	3–5 h	5 g	Activity based;[3] Low sample and standard consumption; Quantitative composite site 5 sodium channel-based activity determination; High throughput parallel format capacity; Simple assay format; Robust detection and stability due to radiometric measurement; rapid assay.	Regulation and radiation protection skills required; Specialized lab with dedicated radiation space needed; Reduced sensitivity compared with CBA-N2A; No differentiation between site 5 sodium channel toxins; Requires specialized equipment.	Chinain *et al.*, 2010a; Clausing *et al.*, 2018; Darius *et al.*, 2007; Gaboriau *et al.*, 2014; Darius *et al.*, 2013; Dechraoui *et al.*, 2005; Bottein Dechraoui *et al.*, 2005; Diaz-Asencio *et al.*, 2018; Hardison *et al.*, 2016; Pawlowiez *et al.*, 2013
RBA Fluorescence Based on competitive binding of CTXs with BODIPY-brevetoxin-2	LOD: 0.075 ng CTX3C eqv.g-1 LOQ: 0.1 ng CTX3C eqv.g-1 EC50 CTX3C = 0.66 ± 0.16 ng. ml-1 C-CTX1 = 0.87 ± 0.12 ng. ml-1	2 h	5 g	Activity based;[3] High throughput parallel format; Rapid assay; Quantitative composite binding affinity determination of the sample to site 5 of voltage gated sodium channels; Commercially available; No radioisotopes required.	No information about compounds (only site-5 activity).	Hardison *et al.*, 2016
ELISA (direct-sandwich)	0.018–0.032 CTX1B[1]	2.5 h	5 g	High specificity, user friendly, detection of CTX1B, CTX3C, 51-hydroxyCTX3C, and 54-deoxyCTX1B; High throughput parallel format; Commercially available from Fujifilm Wako; No radioisotopes required.	Preliminary studies show no cross-reactivity with C-CTX1; Sample cleanup requirement.	Tsumuraya *et al.*, 2018
MBA	LD_{50}: 0.25 CTX1B[1]	24 h observation[2]	40 g	Composite toxicity determination; Relevance to human health effects; Model organism also used historically for other toxin classes.	Institutional Animal Care and Use Committee (IACUC) authorization required; No longer recommended by the EFSA. Specialist animal facilities and training; Access and supply of specific mouse breed, non-specific (i.e. no differentiation between toxic components); Multiple animals needed per sample; Ethical concerns limit use in many regions.	Lewis and Sellin, 1993; Banner *et al.*, 1960; Lewis, 1995; Dechraoui *et al.*, 1999

(continues)

TABLE 7 (continued)

ASSAYS	APPROXIMATE DETECTABILITY (µg/kg)	DEVELOPMENT TIME	SAMPLE USAGE	PROS	CONS	REFERENCES
LC-MS/MS	LOQ: 0.01–0.1 CTX3C	15–20 min per sample[2]	2–20 g	High specificity and sensitivity; Toxin profiling possible; Quantification of individual analogues; Rapid method; No radioisotopes required.	Lack of available standards limits quantification of congeners; Extensive sample cleanup required; High cost of instrumentation; Trained operator needed; Serial format.	Dickey, 2008; Moreiras, Leao and Gago-Martinez, 2018; Yogi *et al.*, 2014; Murray *et al.*, 2018
LC-HRMS	LOQ: 1.25 CTX3C	13 min per sample[2]	20 g	High degree of specificity; Toxin profiling; Detection of unknown toxins; Rapid analysis; Reliable confirmation of analytes; No radioisotopes required.	Unavailability of standards; Extensive sample cleanup requirement; Lower sensitivity; High cost of instrumentation; Highly trained operator; Serial format.	Sibat *et al.*, 2018a

[1] Results for these tests are expressed as toxin equivalents.

[2] Excluding sample preparation time.

[3] Does not require structural information.

Note: No methods have been validated, and, in the majority of studies, certified reference materials were not available; hence, reports and comparisons of EC_{50}, LOD, LOQ, and/or LD_{50} cannot be compared or verified.

4.1 SCREENING ASSAYS FOR CIGUATOXINS

Several assays for the screening of fish samples for CTXs have been described, based on *in vitro* assays, *in vivo* (mouse) bioassays, and immunoassays. These assays all have high-throughput capacity (apart from the mouse bioassay), which allow parallel measurements at the respective endpoints of each assay. However, reliable quantification of the bioactivities and/or toxicity of CTXs in seafood extracts requires a full dilution series (6–8 concentrations) of a certified reference and, where possible, matrix-matched reference.

4.1.1 *IN VITRO* ASSAYS

The *in vitro* assays for CTXs make use of selective binding to site 5 (Catterall, Trainer and Baden, 1992; Trainer *et al.*, 1993) of the voltage-gated sodium channel (VGSC). Although several *in vitro* assays have been proposed, the most widely used in screening are the N2A-MTT assay and receptor binding assays. (RBA).

4.1.1.1 N2A-MTT assay for ciguatoxins

Development of cell assays for marine toxins was stimulated by early work by Kogure *et al*. (1988) using mouse neuroblastoma (N2a) cells to detect marine toxins active at VGSCs. Their use of a morphological endpoint limited the assay's utility, and the assay was directed at saxitoxins (STXs) and tetrodotoxins (TTXs) rather than CTXs. The neuroblastoma N2A assay (N2A-MTT) proposed by Manger *et al*. (Manger *et al.*, 1993; Manger *et al.*, 1995) is based on detection of mitochondrial dehydrogenase activity using MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium]) to detect viability colorimetrically. Since its introduction by Mossman (Mosmann, 1983), cell biologists have used MTT across many different cell lines for the evaluation of cytotoxicity. However, direct cytotoxicity is not readily observed with CTXs or other sodium channel active toxins, and this is how the N2A-MTT assay differs. A key aspect of the assay that has been optimized for CTXs, brevetoxins and site 5 sodium channel toxins is the synergistic effect between site 2 and site 5 toxins of VGSCs described by Catterall and Risk (1981). Cells are treated with veratridine, which targets site 2 of the sodium channel, and blockade of the sodium-potassium ATPase by ouabain forces sodium accumulation and cell death. Site 5 toxins such as the CTXs and brevetoxins are detected with very high-sensitivity due to the site 2 – site 5 synergism. The N2A-based methods require only low quantities of CTX calibrants (25–50 pg CTX1B or C-CTX1 is sufficient for an eight-point curve) and samples (1–2 g). Measuring the absorbance at 570 nm is used for detecting mitochondrial metabolism of MTT and omission of veratridine and ouabain in (-O/V) control experiments reveals any (non-specific) cell death not caused by site 5 toxins. The -O/V control experiments, performed in parallel to the assay as full dose-response curves, are crucial to data interpretation and success. Other important parameters are: pre-treatment of Neuro-2a (N2a) cells obtained from culture collections (e.g. American Tissue Type Collection, and Sigma); plating consistency (i.e. standardized number of cells seeded per assay well); stable growth rates in maintenance cultures (which can be obtained by cell enumeration during passaging and tracking following propagation from cryopreservation); among other factors. These considerations will be important parameters for future harmonization.

As with all analytical methods for CTXs, their use for commercial trading would require a validation that has not been performed to date due to the absence of certified reference materials. There are other drawbacks. As activity-based assays, N2A assays do not supply structure information but simply respond to site 5 activity of any component within the sample extract. Many investigators have improved the specificity of CBA-N2a by optimizing different concentrations of OV treatment for the specific detection of either VGSC activators (brevetoxins [PbTxs] and CTXs) or blockers (STXs and TTXs), as they have opposite mode of action (Canete and Diogene, 2008).

The N2A-MTT can be sensitive to matrix effects such as protein and lipid content and other sample matrix components (e.g. phytosterols, and free fatty acids). Refinements of the method have addressed matrix effects and included sample conditioning and cleanup steps. Although maximum sensitivity can require a 24 h

incubation, the assay can be performed more rapidly following sample matrix conditioning. These requirements vary depending on fish matrix lipid content. Interest in the N2A-MTT assay remains high, and the need for standardization of the assay has been asserted (Caillaud *et al*., 2012).

Related methods using voltage-sensitive fluorescent dyes for high throughput have also been reported using neuroblastomas (Louzao *et al*., 2004) or synaptosomes (David *et al*., 2003). This latter approach does not require use of ouabain or MTT as it is based on cell membrane depolarization rather than cell death. This approach is promising as depolarization can be observed in minutes versus the overnight incubations typically used for the viability end point of the N2A-MTT assays. Flow cytometric detection (Manger *et al*., 2014) allows selection of the most responsive cells using electronic gating. Assays based on voltage-sensitive dyes (including N2A-Flow) have not been optimized or practised by other laboratories to the same extent as the N2A-MTT format, with the latter used extensively and refined over the past 25 years.

4.1.1.2 Receptor binding assay (RBA) for ciguatoxins

Following study of an RBA for brevetoxins by Poli *et al*. (Poli, Mende and Baden, 1986) and Sharkey *et al*. (1987) the first RBA for CTXs was demonstrated by Lombet *et al*. (Lombet, Bidard and Lazdunski, 1987). The first application to an outbreak of ciguatera was by Poli *et al*. (1997). This radioreceptor assay using rat brain synaptosomes, is based on the competition for the receptor between a brevetoxin and CTXs, has since been refined (Diaz-Asencio *et al*., 2018). The assay allows CTX quantitation by means of using tritiated brevetoxin-3 or tritiated brevetoxin-9 (Pawlowiez *et al*., 2013; Gaboriau *et al*., 2014; Darius *et al*., 2013; Chinain *et al*., 2010b; Poli, Mende and Baden, 1986; Dechraoui *et al*., 1999; Darius *et al*., 2007). The Radioligand RBA for CTXs is operational in several countries impacted by ciguatera, however the need to use radiolabelled compounds is a drawback to its use. A comparison of RBA and N2a assay has shown that N2a was 12-fold more sensitive for ciguatoxins, whereas RBA was 3–24-fold more sensitive for brevetoxins (Dechraoui *et al*., 2005). A similar approach with brevetoxin-2 conjugated with fluorescent labels (Texas Red-PbTx-2, 6-TAMRA-PbTx-2, and BODIPY®-PbTx-2) has also been proposed for detection of PbTxs and applied for CTXs and Caribbean fish by Hardison *et al*. (Hardison *et al*., 2016) with a performance equivalent to the radioligand RBA assay (McCall *et al*., 2014) and semi-quantitative limit of detection of 0.075 µg/kg CTX3C equivalent (0.0975 µg/kg C-CTX1). Recently, improvements have been presented concerning chemiluminescent probes (Yasumoto, 2018). Preliminary results using chemiluminescent acridinium ABTX probes have shown superior sensitivity compared with tritium and fluorescent probes. However, more research on this probe is needed before making final conclusions.

The method based on the RBA with the fluorescent probe is part of the programme of the National Oceanic and Atmospheric Administration (NOAA) for detection of CTXs, and it is currently being used to screen invasive lionfish in the Caribbean (https://coastalscience.noaa.gov/news/habs/noaa-improves-monitoring-ciguatera-fish).

The fluorescent radioreceptor assay has been announced to be a commercially available kit by SeaTox Research, Inc. The International Atomic Energy Agency (IAEA) has been providing support for the establishment of the radioligand RBA. It is used by the Caribbean regional network for early warning (Cuellar-Martinez *et al*., 2018).

4.1.1.3 Immunological assays

Although attempts have been made to develop immunoassays kits for CTXs, until recently none has been reported in the literature with sufficient performance to meet the FDA advisory levels of 0.01 ppb for Pacific CTXs (as CTX1B equivalents). Campora *et al*. reported an enzyme-linked immunosorbent assay (ELISA) with a detection limit of 0.078 ng/ml CTX1 in crude fish extract (Campora *et al*., 2008). This level is already at least ten times the advisory level even before accounting for dilution during extraction. However, they concluded that their ELISA was not suitable for commercial purposes. More recent synthetic haptens have provided a route to improved immunoassays that have resulted in a highly sensitive, direct sandwich ELISA format (Tsumuraya, Fujii and Hirama, 2014). ELISAs based on the multiple antibodies developed from these fragments now allow detection of the Pacific CTXs below 0.01 ppb CTX1B, and allow the simultaneous detection of multiple Pacific CTXs (CTX1B, CTX3C, 51-hydroxy CTX3C, and 54-deoxyCTX1B) (Tsumuraya, Fujii and Hirama, 2014). An ELISA kit for the simultaneous detection of CTX1B and 54-deoxy CTX1B is now commercially available (FUJIFILM Wako Chemicals, Japan) (Tsumuraya *et al*., 2018). Users of the assay should keep in mind that these assays are specific for the Pacific congeners and do not cross-react with Caribbean CTXs.

It should be noted that a commercial immunoassay distributed as Cigua-Check was once available, and is still in circulation in some regions, but it had significant rates of false positives and false negatives, so cannot be recommended (Lehane and Lewis, 2000; Bienfang, DeFelice and Dowling, 2011).

4.1.2 *IN VIVO* ASSAYS

The mouse bioassay (MBA) was used for years until *in vitro* and analytical methods started to replace it (FAO, 2004). In the United States of America, it was recommended in 1994 that this bioassay be replaced for regulatory analyses by *in vitro* assays (Dickey, 2008). The method was described by Banner *et al*. (Banner *et al*., 1960) and later improved (Lewis and Sellin, 1993; Lewis, 1995). It is based on an acetone extraction and partitioning into hexane, ethanol-water and diethyl ether. The extraction method is designed to eliminate the interference of maitotoxin. A nitrogen-dried ether extract is injected in 0.5 ml 1–5 percent Tween 60/0.9 percent saline solution to two mice. The observation time is 24 h (continuous observation for the first 2 h), with animals of standard weight (18–21 g) housed at 23 ± 2 °C. The lethality is expressed in mouse units (MU); an MU is the intraperitoneal LD_{50} at 24 h, equivalent to 5 ng (CTX1B), 48 ng (52-*epi*-54-deoxyCTX), 18 ng (CTX3C) or 80 ng for CTX4B (Guzmán-Pérez and Park, 2000; Lewis, Hoy and McGiffin,

1992; FAO, 2004). The LD_{50} is 0.25 µg CTX1B/kg bw and 3.7 µg C-CTX1/kg bw (Lewis *et al.*, 1991; Vernoux and Lewis, 1997). The relationship between MU and time of death is log MU = 2.3 log (1 + T-1) (T being the time to death in hours). The observable signs of toxicity are diverse, such as inactivity, piloerection, jumping, cyanosis, diarrhoea, weight loss, hypersalivation, lacrimation and dyspnoea with gasping (Vernoux, 1994; Caillaud *et al.*, 2010). Because CTXs induce hypothermia in mice, rectal body temperature may be measured, as the drop in temperature is of about 5 °C after 60 min (Lewis and Sellin, 1993; Lewis and Hoy, 1993). The bioassay was further improved by including a Florisil solid-phase extraction cleanup to further eliminate lipid contaminants (Wong *et al.*, 2009) – the recovery improves up to 96.7 percent, while the standard procedure provides a 49–77 percent recovery (Lewis and Sellin, 1993).

The need for a dose response curve, required for the calibration and calculation of MU, makes this method difficult to implement on a routine base, given the limited commercial supply of CTXs. Although the symptoms could identify the toxin group (Vernoux, 1994), the bioassay does not inform about the toxin causing the effect. Therefore, its lack of selectivity is a problem, along with the ethical and legal acceptability that animal experiments poses in many countries (European Communities, 1986).

Several other methods described, not currently used, have been proposed as alternatives to the bioassay, such as contraction of guinea pig atria (Miyahara, Akau and Yasumoto, 1979), brine shrimp (Granade, Cheng and Doorenbos, 1976), cat (Hashimoto, 1956), mosquito (Bagnis, *et al.*, 1985b) or Diptera larvae (Labrousse and Matile, 1996) assays. Chicken bioassay was also used as a feeding test, and its significant symptoms included hypersalivation along with an important drop in rectal temperature and weight losses, as in mice. Accumulation of toxins was proved: repeated administration, once a day, of toxic extracts at a subsymptomatic level, induced lethality. For the same cumulative dosage, lethality decreased to zero through repeated doses given once a day during a week. However, when a single sublethal dosage eliciting the lowest response 48 h after oral feeding was given, the subsequent feeding at the same dose level given to the same chicken had to be retarded by at least 7 days in order to have no lethal effect. This showed that detoxification is low in chickens (Vernoux *et al.*, 1985b, Vernoux and Lahlou, 1986; Pottier and Vernoux, 2003).

4.2 STRUCTURE-BASED CONFIRMATORY METHODS

Liquid chromatography coupled with mass spectrometry (LC-MS) is a suitable approach for the identification and confirmation of CTXs, and related compounds, in a range of matrices. All liquid chromatographic methods use a serial analysis format that ultimately limits throughput in screening applications, in contrast to most of the assays described above. However, this approach allows accurate quantitation of individual CTX analogues, provided availability of analytical standards. All approaches described here involve the use of liquid chromatography coupled with

electrospray ionization mass spectrometry, either in low-resolution tandem mass spectrometry (MS/MS) or high-resolution (HRMS) modes. Key limitations for the implementation and validation of these techniques are the lack of pure toxin standards and tissue reference materials. While most laboratories employ these methods for confirmatory purposes, they cannot be used for screening, especially in the absence of toxin standards for many analogues.

The first attempt to identify CTXs and MTXs using ion spray was by Lewis *et al.* in 1994 (Lewis, *et al*., 1994; Lewis and Jones, 1997). Due to the low concentrations of CTXs in fish that are relevant to human health (Moreiras, Leao and Gago-Martinez, 2018), it is necessary to significantly concentrate them from crude extracts. Sample preparation and the removal of matrix co-extractives haves also been shown to be very important for the performance of LCMS-based methods (as reviewed in Harwood, Murray and Boundy, 2017). As CTXs are lipophilic, it is important to eliminate potential interfering compounds (e.g. lipids) from the sample, to improve the efficiency of the extraction and to minimize matrix effects during MS analysis. Further refinements of the instrumentation have improved the detection and quantitation of CTXs in the past 25 years (Moreiras, Leao and Gago-Martinez, 2018; Otero *et al*., 2010; Dickey, 2008; Lewis, Yang and Jones, 2009; Yogi *et al*., 2014; Pottier and Vernoux, 2003). This has included the use of new generation LC-MS systems allowing faster separation (ultra-high-pressure liquid chromatography [UHPLC]), improved sensitivity (lower detection limits) and a greater level of specificity (through use of HRMS systems). These represent the main advantages of this approach. A key challenge associated with LC-MS methods is extensive sample cleanup, resulting in low recoveries that may compromise the ability to meet the guidance levels established by the Food and Drug Administration of the United States of America (FDA) (0.01 µg CTX1B eq/kg; 0.1 µg C-CTX1 eq/kg).

Given the large number of CTX analogues, the need for standards represents one of the most critical aspects of CTX research and control. The only CTX standard currently available commercially is CTX3C. The lack of calibrants also limits the ability to perform the required validation. Recent improvements include: the detection of CTXs using low- or high-resolution mass spectrometry (Sibat *et al*., 2018a); the systematic assessment of parameters for MS optimization (Moreiras, Leao and Gago-Martinez, 2018); and a method combining MTXs and CTXs within the same analysis (Murray *et al*., 2018). This has allowed the profiling of toxins in microalgal and environmental samples and seafood (fish, bivalves, sea urchins, starfish and gastropods). It has also been successfully applied to confirm the presence of CTXs and profiles in fish from the Pacific and Caribbean areas (Estevez *et al*., 2019; Yogi *et al*., 2011; Yogi *et al*., 2014; Abraham *et al*., 2012a) and the enzymatic biotransformation in fish (Ikehara *et al*., 2017). Recent implementations on LC-MS/MS also allowed the confirmation of the presence of C-CTX1 in fish from Atlantic coasts of Spain and Portugal (Estevez *et al*., 2019; Costa *et al*., 2018). A new hydroxyl metabolite of C-CTX1 was also confirmed in samples from the Canary Islands (Spain). However, this remains to be elucidated and confirmed. Further

implementation on the LC-MS/MS methods for Caribbean CTX analogues described by Pottier *et al*. (Pottier *et al*., 2003; Pottier, Vernoux and Lewis, 2001; Pottier *et al*., 2002b) allowed investigators to conclude that the CTX profiles of CTX implicated fish from the Canary Islands (Spain) and Madeira (Portugal) were similar to those observed in *Sphyraena barracuda* and *Caranx latus* from the French West Indies.

4.3 CONCLUDING REMARKS

Quantitative LC-MS methods and *in vitro* bioassays need to be validated internationally based global standards and guidelines. Such validation will ensure harmonization and method comparability. At this stage, formal validation is not possible due to the lack of certified calibrants and matrix reference materials. Method comparability is also needed in order to give confidence in the testing methods used for analysis of CTXs. To achieve comparability between LC-MS (analogue-specific method) and screening assays (e.g. N2A or RBA), it is necessary that all toxicologically relevant analogues be accurately quantified and have reliable toxicity equivalency factors (TEFs). Several recent publications have shown a correlation between screening methods and LC-MS. Nevertheless, more data are required in order to strengthen the comparability of the various methods employed.

As toxin calibrants are scarce, a double-tiered approach is recommended; first, using a screening method, followed by a further interrogation of positive samples with a confirmatory method such as LC-MS/MS. The United States of America has defined advisory toxin levels for consideration in monitoring and control (FDA, 2011). The system utilizes screening based on semi-quantitative *in vitro* mouse neuroblastoma cell assay (N2A-MTT) and later toxin confirmation by LC/MS-MS (Dickey and Plakas, 2010). The composite toxicity of the sample is determined by N2A-MTT assay, and samples positive by N2A are analysed by LC-MS/MS for confirming the presence of CTX analogues. A similar approach has been proposed in Australia (Stewart *et al*., 2009). One of the main challenges for CTX detection in a surveillance system is the low amount of toxin in fish that, combined with matrix effects and number of analogues present in a single sample, make the analysis very complex (Friedman *et al*., 2017). For this reason, it is also important to have procedures for the rapid identification of CTX in the blood and liver of patients (Hamilton *et al*., 2010; Bottein Dechraoui *et al*., 2005; Bottein Dechraoui, Wang and Ramsdell, 2007). Although there has been some progress, given the high potency of the toxins and the large number of analogues, this is still a problem to be resolved (Matta *et al*., 2002).

Even more powerful than using the methods separately is the combination of N2A-MTT assay and mass spectrometry. Ciguatoxin profiles can be studied in detail by performing fractionation of samples by LC and performing N2A-MTT assay (Manger *et al*., 1993) to provide cytotoxicity profiles (Dechraoui *et al*., 2005; Abraham *et al*., 2012b). In this way, detailed structural confirmation can be combined with detection of toxin bioactivity.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CHAPTER 5
HAZARD IDENTIFICATION

5.1 ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME)

5.1.1 ANIMAL DATA

Available data on absorption, distribution, metabolism and excretion (ADME) are scarce, mainly limited to rats and mouse models, and to one purified CTX analogue, one of the most toxic, the CTX1B (also known as CTX or CTX1) found in the Pacific region. Two major studies from Dechraoui Bottein *et al.* (Bottein, Wang and Ramsdell, 2011) and Ledreux and Ramsdell (Ledreux and Ramsdell, 2013) performed in rats using CTX1B (> 90 percent purity), LD_{50} 0.25 µg/kg bw in mouse (Lewis, *et al.*, 1991) yielded reliable data on CTX toxicokinetics. Due to the lack of 14C-radiolabelled CTXs and of sufficiently sensitive analytical methods, toxin levels in blood, excreta and tissues were determined using *in vitro* bioassays such as the N2a neuroblastoma cell-based assay. Sprague Dawley rats were exposed by ip or oral (gavage) route to a high dose of 0.26 µg CTX1B/kg bw or by intravenous (iv) route to 0.13 µg CTX1B/kg bw (Bottein, Wang and Ramsdell, 2011; Ledreux and Ramsdell, 2013).

Results indicated a rapid absorption following ip and oral exposure (Tmax values of 25 min and 2 h, respectively; alpha half-lives of 1.15 h and 2.61 h, respectively) and by combining ip and oral blood data study with the iv study, results suggested a "bioavailability" (of the parent CTX but also bioactive metabolites, given the bioassay used) of 75 percent and 39 percent, respectively. The Cmax after ip exposure was found to be 3.3-fold higher than the Cmax following administration of the same dose via oral route (6.40 ± 0.43 and 1.95 ± 0.15 pg/ml, respectively). However the areas under the blood concentration–time curve were not statistically different (282.22 ± 106.63 and 144.78 ± 52.45 pg/ml, respectively) indicating a similar toxin bioavailability regardless of the route of administration.

The volume of distribution (Vd) was comparable between ip and oral routes (77 litres/kg and 78 litres/kg, respectively) but lower for the iv route (35 litres/kg). Considering that no first-pass metabolism is taking place after iv administration, this Vd could represent that of the parent CTX (i.e. the CTX1B), whereas ip and oral route Vd would also include distribution of its potential metabolites. The very large Vd, especially for ip and oral routes, is indicative of additional extravascular distribution. Indeed, CTX1B was still detected in muscle, liver and brain tissues

four days after administration (as measured by CBA at the end of the study), with 96 percent of the recovered total CTX activity found in muscle and 2–3 percent in the other tissues, regardless of the administration route. This is further evidenced by the biphasic elimination phase of the time course of CTX in blood following oral, ip and iv administration.

The Vd, which exceeds the total body water volume of the rat (~0.7 litres/kg), indicates a larger distribution of CTXs than in aqueous compartments. As CTXs are not soluble in very non-polar solvents such as hexane (Amzil, Vernoux and Pottier, 2001), they are unlikely to be stored in fat (neutral lipids) of adipose tissue. Rather, CTXs are expected to bind to tissue proteins, primarily to Na_v channels (Au *et al*., 2016) but possibly also to muscular proteins as reported in fish (Hahn, Capra and Walsh, 1992), and/or to blood serum lipoproteins as shown for brevetoxins (Woofter, Spiess and Ramsdell, 2005).

Regarding CTX metabolism, toxin analysis in liver by CBA at termination of the ip and oral experiment revealed presence of CTX1B with two additional less polar metabolites (Bottein, Wang and Ramsdell, 2011). Ciguatoxin metabolization is further evidenced by the induction of gene expression of numerous cytochrome and glutathione S-transferases in mouse liver, 4 h post ip exposure to the same CTX1B dose (Morey *et al*., 2008), indicative of a complex biotransformation through both phase I and II enzymes. The induction of the expression of many members of the solute carrier family in the liver argues for a CTXs hepatobiliary excretion. In rat faeces, three main bioactive less-polar metabolites were found 2 days (oral route) or 3 days (ip route) after CTX1B exposure (Bottein, Wang and Ramsdell, 2011), thus confirming the remaining activity previously reported in blood 3 days after ip exposure to the same dose (Bottein-Dechraoui *et al*., 2008). Biotransformation in mammals to more polar metabolites may be a strategy to favour elimination of CTXs. However, it could lead to a toxification of the compounds as it occurs in fish.

The beta half-lives following oral, ip and iv administration were 81.82 h, 112.27 h and 35.5 h, respectively. The elimination of CTXs occurred predominantly via faeces (hepatobiliary route), and in lower quantities into the urine, regardless of the administration route.

CTX1B was undetectable at day 7 after a single administration by gavage at 0.26 µg/kg bw (Zhang *et al*., 2013). The excitatory neurotoxic actions on ACC neurons after acute single-dosage CTX exposure in rats could be recovered after 7 days of exposure.

In another rat study (Wang *et al*., 2017), where Sprague Dawley rats were exposed to CTX1B (purity > 95 percent) by gavage at an initial dose of 0.26 µg/kg bw, followed by administration of a fourth of this dose (0.065 µg/kg bw) every 72 h for 8 weeks, CTX1B concentration in blood samples ranged from 0.006 µg/litre to 0.015 µg/litre at 24 h, 48 h and 72 h after the final exposure to the toxin at week 8, and no difference was detected among these three time points ($F = 2.72$, $p > 0.05$, $n = 4$ rats for each time point). Higher concentrations were detected in the brain samples of rats after repetitive administration of CTX1B (0.034 ± 0.010, 0.030 ± 0.009 and 0.035 ± 0.005 µg/kg brain sample at 24 h, 48 h and 72 h, respectively). No difference was detected among

these three time points ($F = 0.11$, $p > 0.05$, $n = 4$ rats for each time point). In this experiment, a low dose of CTX1B was administrated every 72 h; thus the toxin level at 72 h was the baseline concentration for the next toxin administration. These data suggest that accumulative quantities of the toxin are present in the brain and blood of rats as a result of chronic low-dose CTX1B treatment.

In mice, no quantitative assessment of the bioavailability was found. However, LD_{50} studies conducted (ip and oral) using semi purified toxins (> 85 percent) on male Institute of Cancer Research (ICR) mice (four-weeks-old), suggest a very high oral uptake as identical LD0 values and almost similar signs of intoxications were reported for the two routes of administration (Ito, Yasumoto and Terao, 1996; Lewis and Hoy, 1993; Lewis, Hoy and Sellin, 1993; Lehane and Lewis, 2000; Terao *et al.*, 1991). Diarrhoea was only caused by CTX when ip administered; with a dose of 4/5 MU (10.4 ng/28 g). Diarrhoea started within 10 min after administration and lasted until 30 min, associated with accelerated mucus secretion and peristalsis in the colon. It is likely that CTX given by oral route was absorbed and metabolized in a slightly different manner from that of ip route, and therefore did not cause diarrhoea. Another study, ip or orally administering CTX1B or a mixture CTX-m (CTX1B, 52-*epi*-54-deoxyCTX1B and 54-deoxyCTX1B) found no apparent differences in signs between CTX1B and CTX-m. However, signs induced by oral administration, including hind-limb paralysis, swollen tongue, delayed effects and the absence of diarrhoea, do not occur following ip administration (Lewis, Hoy and Sellin, 1993). Such differences were not evident in previous studies on the effects of orally administered CTX (Ogura, Nara and Yoshida, 1968; Terao *et al.*, 1991).

In conclusion, CTXs are efficiently absorbed and rapidly distributed to the tissues after ingestion in laboratory animal models.

5.2 TOXICOLOGICAL STUDIES

5.2.1 ACUTE TOXICITY

Species highly sensitive to CP have been described and used efficiently to assess ciguateric fish by feeding or gavage test: cat (Hessel, Halstead and Peckham, 1960; Lewis and Endean, 1983); mongoose (Banner *et al.*, 1960; Hamilton *et al.*, 2002b); and chicks (Kosaki and Stephens, 1968; Vernoux *et al.*, 1985b). Cat, which is very sensitive, was also used to study CTX mode of action (Legrand, Galonnier and Bagnis, 1982).

In the laboratory, mouse bioassay was used to monitor extracted CTXs and to assess their LD_{50} (Vernoux, 1994), by either oral or ip route. Ciguatoxins, regardless of the route of administration, induce a set of signs, which, as a whole, hallmarks acute ciguatoxicity: loss of activity, piloerection, hypothermia, profuse diarrhoea, hypersalivation, lachrymation cyanosis (especially of the penis during transient pre-erection state), motricity disorders, dyspnea with gasping, possible hind-limb paralysis, and death due to respiratory distress. Surviving mice recover in hours, except from a significant weight loss induced by sublethal doses, whose recovery takes a few days. As shown by studies performed with pure CTXs from the Pacific (Lewis

et al., 1993; Lewis and Hoy, 1993), Caribbean (Vernoux and Lewis, 1997; Lewis, Vernoux and Brereton, 1998) and Indian Ocean (Hamilton *et al*., 2002a; Hamilton *et al*., 2002b) areas, this typical symptomatology in mice is common to all tested CTX analogues, regardless of the geographic origin. Clinical signs in mouse induced by pure congeners isolated from microalgae or carnivorous fish are also similar (Terao *et al*., 1991; Lewis, 1995). Ciguateric extracts obtained from accepted extraction procedures induced similar effects in mice (Vernoux and Talha, 1989; Lewis and Sellin, 1993) to those from purified substances. Limited data suggest that effects observed are similar following ip and oral administration (Terao *et al*., 1991; Lewis and Hoy, 1993).

As documented by mouse ip LD_{50} data (see Table 8), variability in the potencies exists between some different analogues of CTXs (Nicholson and Lewis, 2006; Solino and Costa, 2018). However, many data are lacking for a number of identified congeners. On the basis of LD_{50} estimates in mice, oral CTX1 (a.k.a. CTX1B) (0.22 µg/kg) was similar in potency to ip CTX1B (0.25 µg/kg [Lewis, *et al*., 1991]). This publication proposes the use of CTX1B (also known as CTX1) as a worldwide reference CTX due to its commercial availability.

An original study by Terao *et al*. (Terao *et al*., 1991) reported both clinical signs and histopathological changes observed in mice from 10 min to 24 h following ip injection or gavage of 0.7 µg/kg bw of pure (> 99 percent purity) CTX1B or CTX4C (i.e. above LD_{50}, which is 0.25 µg/kg bw). Clinical signs included laborious movements, lumbar muscle contraction, defecation followed by severe watery diarrhoea then severe dyspnea, cyanosis and death within 24 h in 70 percent of the mice, whereas paw paralysis and/or penis erection occurred in the survived mice. Macroscopical examination of the organs of deceased mice showed dilated heart, lung oedema and congestion of all the other organs. Microscopical examination indicated heart, lung, medulla of adrenal glands, autonomic nerves and penis as target organs. Histopathological changes included mainly necrotizing and swollen cardiac muscle cells, and plasma effusion in the heart and lung, and swollen neuromuscular synapses in the small intestine. There were no significant differences in clinical signs or histopathological symptoms induced by the two toxins and the two administration routes. Atropine pre-treatment prevented diarrhoea, indicating that it was caused by actions on the autonomic nerve system in the intestinal wall. However, atropine did not prevent cardiac injuries.

In a second study conducted by the same team, a single ip sublethal dose of 0.1 µg/kg bw of CTX1B or CTX4C caused no discernible morphological changes in macroscopic, microscopic and at even ultrastructural level in hearts of mice (Terao, Ito and Yasumoto, 1992).

Symptomatology in rats was reported (Zhang *et al*., 2013) for acute toxicity obtained by gavage of pure (> 95 percent) CTX1B at a single dose of a sublethal dosage of 0.26 µg/kg bw given to adult male Sprague–Dawley rats (250–350 g) . It induced decreased body weight, reduced food consumption, and severe visceral pain after 5 h exposure was observed, mimicking the clinical gastrointestinal symptoms noted in patients with acute ciguatera poisoning, especially visceral allodynia and hyperalgesia. Neurotoxicity was also observed (Asthana *et al*., 2018).

TABLE 8 **ACUTE TOXICITY AND RELATIVE POTENCY OF CTXs**

TOXIN[1]	ACUTE TOXICITY (mouse ip, µg/kg)	MEAN ACUTE TOXICITY (mouse ip, µg/kg)	RELATIVE POTENCY (CTX1B = 1.0)
CTX1B (CTX, CTX1)[6]	0.25 (Lewis *et al.*, 1991)[2] 0.36 (Yogi *et al.*, 2014)[3] 0.35 (Murata *et al.*, 1990)[4] 0.33 (Dechraoui *et al.*, 1999)[b]	0.32	1.0
52-*epi*-54-deoxyCTX1B (CTX2, CTX2A2)[6]	0.7 (Yogi *et al.*, 2014)[3] 2.3 (Lewis *et al.*, 1991)[2] 1.9 (Dechraoui *et al.*, 1999)[2]	1.6	0.2
54-deoxyCTX1B (CTX3)[6]	0.9 (Lewis *et al.*, 1991)[2]	1.6	0.2
CTX3C	1.3 (Satake, Murata and Yasumoto, 1993b)[4] 1.3 (Inoue *et al.*, 2009)[2] 2.5 (Dechraoui *et al.*, 1999)[2] 1.2 (Yogi *et al.*, 2014)[3]	1.6	0.2
51-hydroxyCTX3C	0.27 (Satake *et al.*, 1998)[4] 0.20 (Yogi *et al.*, 2014)[c]	0.24	1.3
2,3-dihydroxyCTX3C (CTX2A1)[6]	1.8 (Satake *et al.*, 1998)[4] 3.5 (Dechraoui *et al.*, 1999)[2]	2.7	0.1
CTX4A	2 (Satake *et al.*, 1996)[4] 1.4 (Yogi *et al.*, 2014)[3]	1.7	0.2
CTX4B	4 (Satake *et al.*, 1996; Murata *et al.*, 1990)[4] 10 (Dechraoui *et al.*, 1999)[2] 3.6 (Yogi *et al.*, 2014)[3]	5.9	0.05
C-CTX1	3.6 (Vernoux and Lewis, 1997)[5]	3.6	0.1
C-CTX2	1 (Vernoux and Lewis, 1997)[5]	1	0.3

[1] The toxin nomenclature used in this table and toxin synonyms are described in Section 1 of the current report.

[2] LD_{50}.

[3] Mouse bioassay.

[4] Mouse lethality.

[5] LD_{50} from mouse bioassay.

[6] As also named in cited references.

5.2.2 SHORT-TERM STUDIES

In four-week-old male C57BL/6 mice, a second ip administration of a sublethal dose of 0.26 µg/kg bw of purified CTX1B, (CTX1, provided by R.J. Lewis) three days after an initial ip dose of 0.26 µg/kg bw prolonged the hypothermic response, and enhanced the reduced motor activity and the antinociceptive effect (by the tail flick assay) seen after the first administration. In addition, 30 percent (n=2 over 6) of the mice died within 7 h after the second injection whereas no deaths were observed after the first injection at the same dose. The second exposure also caused a twofold greater body weight loss after 7 h. The CTX1B concentration in blood measured by N2a 1h post-exposure was higher after the second injection compared with the first injection (16.6 ± 1.0 pg/ml vs 9.0 ± 1.0 pg/ml, respectively) (Bottein-Dechraoui *et al.*, 2008).

Repeated ip and oral administrations of purified CTX1B and CTX4C (99 percent pure) to four-week-old male ICR mice at a dose of 0.1 µg/kg bw for 15 days resulted

in marked swelling of cardiac cells and endothelial lining cells of blood capillaries in the heart. Single doses caused no discernible pathological changes. After 15 doses, both ventricles were dilated at necropsy; the rest of the bodies were normal. Damage to the capillaries was followed by prominent effusion of serum and erythrocytes into the interstitial spaces of the myocardium. Swelling of the endothelial lining cells of capillaries caused narrowing of the lumen and accumulation of blood platelets in capillaries, which resulted in multiple single-cell necrosis of cardiac muscle cells. According to the authors, the ultrastructural changes of the heart were similar to those observed after a single dose of 0.7 µg/kg bw.

About one month after the final administration, myocytes and capillaries appeared to be normal. Effusion in the interstitial spaces resulted in formation of bundles of dense collagen, which persisted for 14 months. Up to the fifth month, considerable hypertrophy of cardiac muscle cells was also noted. The mean weight of the hearts of CTX-treated mice at 14 months (280 ± 36 mg) was significantly heavier than that of controls (207 ± 15 mg), ($P < 0.05$).

No differences in clinical signs or histopathology between CTX1B and CTX4C were observed.

This study shows that CTX1B and CTX4C have a cumulative effect on cardiac tissue. After 15 injections of CTX1B or CTX4C at a dose of 0.1 µg/kg bw, marked swelling of the myocardia and the endothelial lining cells of blood capillaries was observed, whereas there were no discernible changes after a single administration of the same dose (Terao, Ito and Yasumoto, 1992).

5.2.3 MEDIUM-TERM STUDIES

Adult Sprague Dawley male rats (280–300 g) received purified CTX1B (purity ≥ 95 percent) dissolved in saline 1 percent Tween 60 by gavage at an initial dose of 0.26 µg/kg bw followed by administration of one-quarter of this dose (0.065 µg/kg bw) every 72 h for 8 weeks. This protocol was designed to mimic exposure in endemic regions. The author selected the dose of 0.065 µg/kg bw because it did not affect the average food intake per unit body weight nor the average body weight increase. The control group was treated with saline containing 1 percent Tween 60 of the same dosage and at the same time points as the CTX1B group. Body weight and food intake were monitored and reported every week. Behavioural tests were performed between week 5 and week 8 (open field test, elevated plus maze, Morris water maze, and rat gambling task). Rats were sacrificed at different time intervals (24 h, 48 h and 72 h) after the final exposure at week 8. Blood and brain were collected and analysed by Neuro-2a assay to quantify CTX with CTX1B standard (in-house standard, based on CTX1B provided by R.J. Lewis).

In the open field test, no difference between the treated and control rats was observed for the locomotor function (total distance moved and mean speed). Spontaneous exploratory activity in CTX1B-treated rats was suppressed compared with control rats, as indicated by a reduced time spent in the centre area (23.95 ± 2.43 s vs 13.31 ± 2.26 s;

$p < 0.01$), and a decreased number of entries into the centre area (10.25 ± 0.91 s vs 7.50 ± 1.24 s; $p < 0.05$). These results suggest that chronic low CTX1B exposure leads to increased anxiety-like behaviour in rats.

Results in the elevated plus maze test confirmed the absence of effects on locomotor function, and increased anxiety-like behaviour in rats, expressed as the decreased time spent in the open arms and the lower number of entries into the open arms.

In the Morris water maze, CTX1B rats showed longer escape latency than control rats during the training process ($p < 0.05$), suggesting spatial learning ability was affected in these rats. Moreover, in the probe test, chronic CTX1B rats showed significantly shorter duration in the target quadrant (39.57 ± 3.46 s vs 23.46 ± 3.01 s; $p < 0.01$) and shorter duration in the platform region (2.76 ± 0.51 s vs 1.40 ± 0.34 s; $p < 0.05$) compared with control rats. These results suggest that chronic low dose CTX1B exposure impaired spatial learning and reference memory in rats.

In the rats gambling task, the proportion of good decision makers decreased in the CTX1B group (65.0 percent vs 41.2 percent), and the proportion of poor decision makers increased (15.0 percent vs 41.2 percent) compared with controls. Similar results in the proportion of undecided rats were detected between the two groups (20.0 percent vs 17.6 percent). The difference in the proportions of the three types of decision-making behaviours between the two groups was significant (Mann-Whitney U test, $U = 102.5$; $p < 0.05$). The mean food reward obtained during the task by CTX rats was significantly lower than controls (159.6 ± 18.3 vs 109.3 ± 12.0; $p < 0.05$). These data indicate that rats developed a decision-making deficit after chronic low dose CTX1B treatment (Wang *et al*., 2017).

In conclusion, after repeated exposures of rats (every 3 days for 8 weeks) to low dose of CTX1B (0.065 µg/kg bw) after an initial high dose (0.26 µg/kg bw), this study observed the development of anxiety-like behaviour (by open field test and elevated plus maze test), learning and memory deficits (by the Morris water maze) and decision-making impairment (by the rats gambling task).

This study showed neurotoxic effects in rats relevant with symptoms reported in humans. However, only one dose of CTX1 was tested, so it is not possible to identify a lowest observed adverse effect level (LOAEL) or a non-observable adverse effect level (NOAEL).

5.2.4 LONG-TERM STUDIES

Terao *et al*. (1994) conducted a long-term toxicity of CTX1B in mice focused on heart effects (Terao *et al*., 1994). CTX1B isolated and purified (purity and composition not known) from contaminated fish (Micronesia, Okinawa [Japan]) were given orally by intubation into male ICR mice (4 weeks of age) once a week at a dose of 0.1 µg/kg bw (n=20) for 25 weeks or 0.05 µg/kg bw (n=15) for 40 weeks. A control group of five mice received saline once a week for 40 weeks. Two mice from the group treated with CTX1B at 0.1 µg/kg bw were sacrificed 5 h after administration at the 12th,

14th, 18th and 23rd week from the beginning of the treatment. Two mice from the group treated with CTX1B at 0.05 µg/kg bw were sacrificed 5 h after administration at the 18th and 40th week from the beginning of the treatment.

Until about ten weeks, the mice showed no abnormal clinical symptoms. After about 18 weeks, mice treated with 0.1 µg/kg bw showed marked hypertrophy of the heart. Histopathological analysis showed swelling and rupture of the endothelium of the capillaries and widening caused by exudation or collagen fibres in the interstitial space. Occasionally, degenerated or swollen mitochondria were prominent in the myocardium. Accumulations of platelets in the capillaries were frequently observed. No effects were observed in mice treated with 0.05 µg/kg bw, even ultrastructural changes at 40 weeks.

This study provides a NOAEL for heart toxicity of 0.05 µg/kg bw (after 40 weeks, once a week).

5.2.5 REPEATED VERSUS ACUTE TOXICITY

Cardiotoxicity in mice was studied after a single dose of purified CTX1B of 0.1 ug/kg bw and a similar daily dose during 15 days. While no cardiotoxicity was observed after a single dose, severe morphological effects on the heart were observed after the multiple dose exposure, indicating an accumulative process for this endpoint.

A single dose of 0.7 ug/kg of purified CTX1B caused similar cardiotoxic symptoms as observed after multiple daily administrations during the 15 day period.

Thus, cardiotoxicity is similar for a single high dose versus multiple lower daily dosages (Terao, Ito and Yasumoto, 1992).

A long-term study for 25 weeks and 40 weeks with oral administration of 0.1 and 0.05 ug/kg once a week also observed similar cumulative cardiotoxic effects for the mice with the highest dose level. No cardiotoxicity was observed for the low dose group over a period of 40 weeks (Terao, Ito and Yasumoto, 1992).

Two single ip dosages of 0.26 ug/kg of purified CTX1B were given to mice, and bodyweight, thermoregulation, motor activity and tail flicking were studied. These effects were more pronounced after the second dose, but from a qualitative point of view similar effects could be found after a single or double dose (Bottein-Dechraoui *et al*., 2008).

In rats, an initial dose of 0.26 ng/kg of purified CTX1B follow by a dose of 0.065 ug/kg every three days during an eight-week period was used to study neurobehavioural effect. This study showed that after an initial high dose of CTX1B followed by a long-term study, neurobehavioural deficits could be observed. These effects included anxiety-like behaviour, and learning and memory deficits. The study indicates that chronic low-level exposure to CTXs after a clinically relevant poisoning period may cause neurobehavioural deficits (Wang *et al*., 2017; Bottein-Dechraoui *et al*., 2008).

Overall, these studies indicate that, in mice and rats, subchronic exposure may lead to similar toxic effects, e.g. cardiotoxicity, as found with a single higher dose level.

Cardiotoxicity was the only endpoint studied in both acute and repeated toxicity studies (by the same team). However, similar symptoms are not reported in human.

5.2.6 CONCLUSION FROM TOXICOLOGICAL STUDIES IN RODENTS

There are very few oral studies in mice or rats, and only using purified CTX1B or CTX4C. None is suitable to establish a health-based guidance value (acute or chronic).

In a limited study in mice, a single oral dose of 0.1 µg/kg bw of purified CTX1B caused no clinical or histopathological changes, whereas an oral dose of 0.7 µg/kg bw caused severe toxicity in the heart, adrenal gland and penis. Repeated oral doses of 0.1 µg/kg bw also caused severe toxicity (Terao, Ito and Yasumoto, 1992; Terao, *et al*., 1994). However, the experts of this Expert Meeting highlighted that the quantification of the doses in the Terao study is probably not accurate. In addition, CTX4C is an old terminology, and in the absence of chemical information in the paper, it is not possible to identify this compound. Repeated low oral dose of purified CTX1B (0.065 µg/kg bw) after an initial high dose (0.26 µg/kg bw) caused anxiety-like behaviour, learning and memory deficits, and decision-making impairment in rats (Wang *et al*., 2017).

5.3 MODE OF ACTION

The main molecular target of ciguatoxins is the voltage-gated sodium channel (VGSC, Na_v) (Bidard *et al.*, 1984), to which they bind to the "receptor-site 5" of the alpha subunit. This binding site is shared with brevetoxins (Gawley *et al*. 1992; Dechraoui *et al*. 1999), although with lower affinity. The receptor-site 5 is located in the region of interaction of certain residues of segments S5 of domain IV and S6 of domains I (Trainer *et al.*, 1993). The binding of CTXs leads to a shift in the voltage dependence of the channel activation to more negative membrane potentials (hyperpolarizing shift in the thresholds of activation) and an increase in the recovery rate from inactivation. The overall effect is a persistent activation of Na_v (i.e. Na^+ influx) at the resting membrane potential and, as a consequence in excitable cells, CTXs cause an increase in the membrane excitability, eliciting depolarization and even spontaneous and repetitive actions potentials at the resting potential (Bidard *et al*. 1984; Benoit *et al*. 1986; Benoit and Legrand 1994; Strachan *et al*. 1999; Hogg *et al*. 2002; Ghiaroni *et al*. 2006). This action, especially in peripheral nerves where binding of CTXs to Na_v is long-lasting (Strachan *et al*. 1999; Au *et al*. 2016), explains most of the effects of the group. CTXs show affinity for all the VGSC alpha subunit isoforms (Na_v 1.1 to 1.9; Inserra *et al*. 2017), with difference in potency between the different congeners of CTXs.

The membrane hyperexcitability induced by CTXs is primarily due to the Na_v activation but also to a blockade of voltage-gated potassium channels (VGPCs, K_v), although the potency of this effect varies according to congeners and study models. In frog myelinated axons, CTX4B reduced potassium currents (Benoit and Legrand 1994) and was shown to be more effective in blocking K_v than Na_v and,

compared to CTX1B, 4 times more potent at blocking K_v but 50 fold less potent at activating Na_v (Schlumberger *et al.*, 2010a). Low nanomolar concentrations of CTX1B partially blocked potassium currents in cultured primary rat (mammalian) skeletal muscle cells (Hidalgo *et al.* 2002). In rat sensory neurons, CTX1B blocking effect on K_v was shown to involve the delayed-rectified and A-type potassium channels (Birinyi-Strachan, Gunning, *et al.*, 2005b). CTX3C failed to inhibit potassium currents in taste cells (Ghiaroni *et al.* 2006), whereas it inhibited them more potently than 51-hydroxy-CTX3C and CTX1B in cerebellar granule cells (Pérez *et al.* 2011).

Na^+ influx induced by CTXs elicits several secondary cell events, including the water entry in nervous structures leading to swelling. CTXs cause the volume increase of frog myelinated axons (nodes of Ranvier) and motor nerve terminals (Benoit *et al.* 1996; Mattei *et al.* 1997, 1999a, b). The CTX1B-induced nodal swelling of frog myelinated axons was shown to be the combined result of the Na^+ influx subsequent to the direct Na_v activation by the toxins and a K^+ efflux subsequent to the depolarization-induced opening of K_v channels (Mattei *et al.* 2014). An opening of calcium-activated potassium channels following the activation of the nitric oxide-cGMP pathway was involved in the swelling of frog erythrocytes induced by CTXs (Sauviat *et al.* 2006).

Another consequence triggered by the increased Na_v gating and conductance is an increase in cytosolic calcium concentration shown in several neuronal or myocardial models, by mechanisms involving either the reverse mode of the Na^+-Ca^{2+} exchanger (Molgó *et al.* 1993a; Gaudry-Talarmain *et al.* 1996) or the inositol triphosphate (InsP3)-dependent mobilization of internal Ca^{2+} stores (Molgó *et al.* 1993b; Hidalgo *et al.* 2002). Consequently, calcium-dependent responses, such as exocytosis of neurotransmitter and neuromodulators, are induced: acetylcholine release from motor nerve terminals, cholinergic models (Molgo *et al.* 1990, 1992) and intestine vagal innervation (Lewis and Endean 1984; Tatsumi *et al.* 1985; Lewis and Hoy 1993), noradrenaline release from sympathetic innervation of the smooth muscle of artery (Brock *et al.* 1995, 1997), heart (Lewis *et al.* 1992; Lewis and Hoy 1993) or the vas deferens (Ohizumi *et al.* 1981; Lewis and Endean 1984; Tatsumi *et al.* 1985), GABA release from cortical neurons (Martin *et al.* 2015a) and release of the neuropeptides CGRP (calcitonin gene-related peptide) and substance P (SP) release from sensory neurons (Zimmermann *et al.* 2013; Le Garrec *et al.* 2016; Touska *et al.* 2017).

In mice following intraplantar administration of CTX, the induced cold allodynia was demonstrated to involve the activation of the $Na_v1.8$ isotype in CGRP-positive sensory neurons that triggers a calcium influx through the cold-sensitive Transient Receptor Potential Ankyrin 1 (TRPA1) cation channel. CTX1B does not directly activate TRPA1 but sensitizes it to cold, leading to cold allodynia (Vetter *et al.*, 2012). The neuropeptide CGRP is a marker of peptidergic sensory neurons that, together with SP, mediates neurogenic inflammation, pain and also itch (Andoh *et al.* 1998; McCoy *et al.* 2012), which is a typical symptom of CFP.

The human intracutaneous injection of (sub)nanomolar concentrations of CTX elicited the release of enough CGRP to induce a lasting axon reflex flare, i.e. a neurogenic skin inflammation sign, which was accompanied by localized pruritus, burning pain and cold allodynia (Zimmermann *et al.*, 2013).

Another secondary effect of CTX following Na_v activation is gene expression modulation (Rubiolo *et al*. 2018), including upregulation of the immediate early genes *Arc* and *Egr* and downregulation of glutamate NMDA and AMPA receptors in cortical neurons (Martin *et al*. 2015b). The inducible nitric oxide synthase was also upregulated in macrophages (Kumar-Roine *et al.*, 2008).

Regarding water-soluble compounds produced by *Gambierdiscus* spp., which can be present in digestive viscera (see CTX classification), the mode of action of MTX is by inserting in the plasma membrane to increase massive influx of calcium (Reyes *et al.*, 2014). Gambierol potently blocked K_v channels in several models, whereas it had mild or no effect on Na_v channels (Inoue *et al*. 2003; Ghiaroni *et al*. 2005, 2006; Louzao *et al*. 2006; Cuypers *et al*. 2008; Schlumberger *et al*. 2010b; Cao *et al*. 2014). Gambieric acid and gambierone are mild inhibitors of the Na_v channels (Inoue *et al.*, 2003; Rodriguez *et al.*, 2015).

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CHAPTER 6
HUMAN DATA

6.1 ADME

Data on CTX toxicokinetics in humans are very limited. Aside from one experiment showing that recombinant human CYP3A4 enzyme can oxidize CTX4A and CTX4B to CTX1B, 54-deoxyCTX1B, and 52-*epi*-54-deoxyCTX1B (Ikehara *et al.*, 2017), toxicokinetic findings are deduced from clinical observations. As systemic symptoms may appear soon (in some cases in less than one hour) after toxin ingestion (Friedemann, 2016; Bagnis, 1968; Chateau-Degat *et al.*, 2007), it appears that systemic distribution in humans may occur quickly after ingestion. Ciguatoxin resorption via mucosa appears possible, based on a case report of systemic effects in a female after vaginal sexual intercourse with a ciguatera-affected male (Lange, Lipkin and Yang, 1989; Geller, Olson and Senecal, 1991; Ting, Brown and Pearn, 1998) and another case report of systemic effects in a male after sexual intercourse with a female (Hevia Pumariega and Hernández Mullings, 2008). Other reports of symptoms among sexual partners of ciguatera cases further support mucosal elimination (Lange, Lipkin and Yang, 1989; Geller, Olson and Senecal, 1991). Many ciguatera cases express central nervous system symptoms (Allsop *et al.*, 1986; Friedman *et al.*, 2017; Gatti, Oelher and Legrand, 2008), suggesting that CTXs can enter the brain. Ciguatoxins have been measured in blood several hours after ingestion – i.e. CTX1B and CTX3B/3C measured by mass spectrometry hours after consumption of red snapper (*Lutjanus campechanus*) (Mendoza *et al.*, 2013). However, toxins may not persist long in blood. In a case series of four tourists who had eaten the same barracuda in Cuba and developed cold allodynia (Butera *et al.*, 2000), CTXs were undetectable in serum, plasma or urine 90 h after poisoning using HPLC or the cell-based, synaptosome receptor binding assay. Ciguatoxins were detected in human liver in an autopsy of a lethal case six days after fish consumption (Hamilton *et al.*, 2010), indicating that CTXs may persist in tissues after exposure. Case reports describing symptoms among infants of ciguatera-affected mothers suggest that women may eliminate toxins via breast milk, and that toxins may be resorbed through breast milk (Bagnis and Legrand, 1987; Blythe and de Sylva, 1990). However, the importance of this mode of toxin elimination may vary across individuals. In another case report describing a mother who presented with ciguatera-related symptoms, CTXs were detected in fish remnants but not in breast milk, and the breastfed infant remained healthy (CDC, 2009). Case reports of apparent toxicity among foetuses of ciguatera-affected women suggest that

transplacental toxin transfer is possible (Rivera-Alsina *et al.*, 1991; Pearn *et al.*, 1982). Other reports of apparently uncomplicated pregnancies of ciguatera-affected women suggest heterogeneity in the extent of transplacental toxin transfer (Fenner *et al.*, 1997; Rivera-Alsina *et al.*, 1991; Geller, Olson and Senecal, 1991; Senecal and Osterloh, 1991).

6.2 CLINICAL FEATURES AND TOXICITY

Drawing on the case definition provided in a recent epidemiological review (Friedman *et al.*, 2017), which adapted definitions provided by the CDC Yellow Book, the European Food Safety Authority (EFSA) Framework Agreement, and the FDA Bad Bug Book, a possible case definition for ciguatera may be defined (as described in Box 1): the consumption of ciguatoxic fish followed by the onset within 48 hours of specific, incident neurological symptoms: cold allodynia (which may be considered as nearly pathognomonic), paraesthesia, dysaesthesia, pruritus, myalgia, arthralgia, and/or dizziness.

BOX 1

CIGUATERA CASE DEFINITION AND CORROBORATING EVIDENCE OF DISEASE

Ciguatera case definition:
Patient presenting with a recent history of consumption of marine fish[1] known to be associated with Ciguatera poisoning (CP), prior to the onset of symptoms (exposure criteria),

And:
Reporting neurological symptoms within 48 hours postprandial (clinical criteria), which may include any set of cold allodynia (which may be considered as nearly pathognomonic), paraesthesia, dysaesthesia, pruritus, myalgia, arthralgia or dizziness,
Possibly preceded or accompanied by:
Gastrointestinal and/or cardiovascular symptoms (e.g. nausea, vomiting, diarrhoea, hypotension, bradycardia).

Ciguatera diagnosis corroborated by:
- Confirmation of ciguatoxin(s) presence in fish meal remnant with laboratory test (laboratory criteria).
- Appearance of symptoms in a context of outbreak (epidemiological criteria)

Exclusion criteria:
- Pre-existing neurological pathology.
- Simultaneous fever (ciguatera does not cause fever).

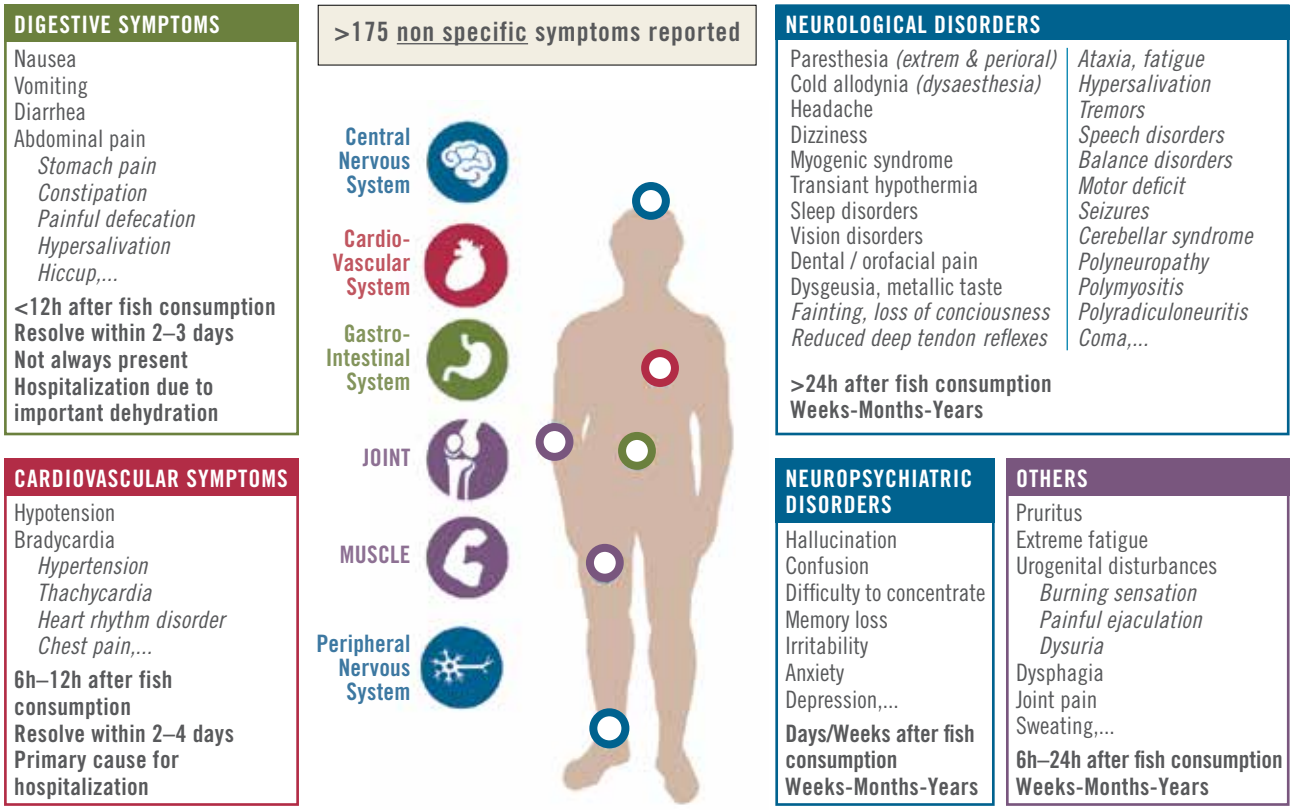
[1] Ciguatera diagnosis may also be considered in a patient with the above-mentioned symptoms following to the consumption of marine invertebrates

Source: Adapted from Friedman, M.A., Fernandez, M., Backer, L.C., Dickey, R.W., Bernstein, J., Schrank, K., Kibler, S., *et al.* 2017. An updated review of Ciguatera poisoning: clinical, epidemiological, environmental, and public health management. Marine Drugs, 15(3): 72.

6.2.1 ACUTE SYMPTOMS

Ciguatera poisoning, although defined by its neurological symptoms, is characterized by a combination of non-specific symptoms including possible gastrointestinal, neurological, cardiovascular and other systemic manifestations (Friedman *et al.*, 2017). General, unspecific symptoms such as severe fatigue and any kind of pain (e.g. myalgia, arthralgia and dentalgia) are very common. More than 175 different symptoms have been reported to date (Sims, 1987) (see Figure 10).

FIGURE 10 **EXAMPLE OF REPORTED ACUTE SYMPTOMS OF CIGUATERA POISONING**



Source: FAO/WHO.

The organ systems most commonly affected during the acute phase of the poisoning are (Bagnis, Kuberski and Laugier, 1979; Palafox *et al.*, 1988; Pearn, 2001; Dickey and Plakas, 2010; Friedman, *et al.*, 2017):

- the **gastrointestinal tract,** e.g. nausea, vomiting, abdominal pain, diarrhoea;
- the **cardiovascular system,** e.g. bradycardia, hypotension (Geller, Olson and Senecal, 1991);
- the **peripheral nervous system,** e.g. paraesthesia (tingling of the mouth and digits, numbness, pruritus), dysaesthesia (cold allodynia), hyporeflexia, dysphagia (Bagnis *et al.*, 1977; Friedman *et al.*, 2007; Oehler *et al.*, 2009).

- the **central nervous system,** e.g. headache, dizziness, anxiety, tremor, ataxia, changes of behaviour (Bagnis *et al.*, 1977; Friedman *et al.*, 2007).
- the **autonomic nervous system,** e.g. disturbances of thermoregulation, hypersalivation, mydriasis, meiosis, altered sense of smell, laryngeal spasm.
- the **arthro-muscular system,** e.g. muscle weakness, myalgia, arthralgia.

The most characteristic manifestations of ciguatera, found in all patients, are neurological symptoms. Paraesthesia of the extremities and other body surfaces generally appears within 12 h postprandial, may begin perioral a few minutes after ingestion of marine food products (Bagnis, 1968; Chateau-Degat *et al.*, 2007; Friedemann, 2016), and can last days, month or even years (Friedemann, 2016). Paraesthesia causes great discomfort in ciguatera poisoning patients and may worsen symptoms of anxiety or depression (Friedemann, 2016).

Ciguatoxins may act on the central thermoregulation centre in the hypothalamus (Peng *et al.*, 1995). Hypothermia is observed during the first stage of the disease in many human patients, consistent with symptomatology described in animals (Gatti, Oelher and Legrand, 2008; Boucaud-Maitre *et al.*, 2018). Shivering and chills are often present in the acute phase (Boucaud-Maitre *et al.*, 2018; Gatti, Oelher and Legrand, 2008; Gatti *et al.*, 2018; Mattei *et al.*, 2014).

Cold allodynia is nearly pathognomonic for ciguatera (Mattei *et al.*, 2014). This modification of thermal sensation (dysaesthesia) makes victims feel an unpleasant or painful "burning cold" sensation, tingling or electric discharges (Bagnis, 1968; Palafox and Buenconsejo-Lum, 2001; Pearn, 2001) as consequence of any contact with cold fluids, cold surfaces (e.g. bathroom floors, door handles of buildings or vehicles), or cold wind. It has been suggested that this pathological thermal sensation is mediated by bilateral responses in the medial insula, medial cingulate cortex, secondary somatosensory cortex, frontal areas, and cerebellum (Eisenblatter *et al.*, 2017). Vetter *et al.* presented TRPA1-dependent $Ca2^{+}$ influx in response to mild cooling as responsible for the development of pain and cold allodynia (Vetter, Zimmermann and Lewis, 2014).

Unlike the gastrointestinal symptoms of ciguatera (e.g. nausea, vomiting, diarrhoea and abdominal pain), which may appear within 12 h and last for up to 1 or 2 days, or the cardiovascular symptoms (e.g. bradycardia and hypotension) that remain for 3–4 days (Butera *et al.*, 2000), neurological manifestations can last for weeks, months or even years (Friedman, *et al.*, 2007; Friedman, *et al.*, 2017).

Psychiatric symptoms have also been reported, such as depression, anxiety, and memory disturbances (Pearn, 2001; Arena *et al.*, 2004; Dickey, 2008).

Symptoms in the urogenital tract (pain, paraesthesia, dyspermia, dyspareunia) sometimes occur and are often primarily misdiagnosed (Lange, Lipkin and Yang, 1989; Pearn, 1989; Müller and Majer, 2018).

Worldwide occurrence of gastrointestinal, neurological, and cardiovascular ciguatera symptoms:

Gastrointestinal, neurological and cardiovascular symptoms can all occur after consumption of contaminated seafood from any of the major areas where ciguatera is endemic (i.e. Pacific Ocean, Indian Ocean, and the Caribbean) (Friedman, *et al*., 2017).

Ciguatera poisoning severity criteria:

The Poison Severity Score (PSS) is a standardized inventory for grading the severity of acute poisonings in adults and children (Persson *et al*., 1998), and has been recommended as a guideline for the evaluation of poisonings regardless of causative agent by WHO since 2011 (WHO, 2011). According to the PSS, poisoning cases presenting with an acute bradycardia (< 40 BPM) should be considered severe, consistent with past ciguatera studies where authors suggested using cardiovascular symptoms as a ciguatera poisoning severity indicator (Morris *et al*., 1982; Katz, Terrell-Perica and Sasaki, 1993; Chateau-Degat *et al*., 2007; Friedemann, 2016). Other symptoms of ciguatera poisoning that should be considered as severe include: intense abdominal pain (Friedemann, 2016; Chan, 2016), extreme agitation, areflexia, generalized seizures (Chan, 2016), paralysis (Chan, 2016; Oehler *et al*., 2009), ophthalmoplegia (Chan, 2016; Bagnis, 1979), decreased function or paralysis of respiratory tract muscles (Chan, 2016). Often, deep coma precedes death (Chan, 2016; Oehler *et al*., 2009; Bagnis, 1979).

6.2.2 CHRONIC SYMPTOMS

Chronic ciguatera symptoms are those that persist beyond three months after the initial poisoning, in accordance with the definition of chronic pain (Treede *et al*., 2015), and concern at least 20 percent of ciguatera-affected persons (Pearn, 1996; Palafox and Buenconsejo-Lum, 2001; Friedemann, 2016). Gatti *et al.* reported persistent symptoms in 100 percent, i.e. 9 persons, 6 months after a single exposure linked to the consumption of the gastropod *Tectus niloticus* in French Polynesia (Gatti *et al*., 2018), where high amounts of CTXs were detected in *Tectus niloticus* samples collected in the same area shortly after this outbreak (Darius *et al*., 2018a).

Ciguatera poisoning may have neurological, psychiatric and/or general symptoms that can persist months or years after the initial poisoning (Blythe *et al*., 1992; Baumann, Bourrat and Pauillac, 2010; Friedemann, 2016; Gatti *et al*., 2018) (Table 9). Smoking has been suggested as a potential risk factor for ciguatera symptom persistence (Chateau-Degat *et al*., 2007).

TABLE 9 **COMMON CHRONIC SYMPTOMS OF CIGUATERA POISONING**

NEUROLOGICAL SYMPTOMS	NEUROPSYCHIATRIC SYMPTOMS	UNSPECIFIC/GENERAL SYMPTOMS
Paraesthesia	Depression	Malaise
Dysaesthesia	Anxiety	Fatigue
Cold allodynia	Inability to concentrate	Weakness
Pruritus	Subjective memory loss	
Hypersomnia	Irritability	
Headache	Attention disorder	

The mechanism underlying the long persistence of symptoms in ciguatera poisoning patients is not confirmed, but it has been suggested that one of the possible mechanisms could be that CTXs may be stored in deep tissue and occasionally released into the blood stream following lipid metabolism activation (Nicholson and Lewis, 2006). Another hypothesis is that immune dysregulation could be involved (Ryan, Wu and Shoemaker, 2015; Shoemaker, House and Ryan, 2010).

6.2.3 ACUTE RECURRENCE/EXACERBATION OF CIGUATERA SYMPTOMS

The acute exacerbation of specific symptoms in chronic courses of ciguatera poisoning is characterized by the transient recurrence of neurological and possibly other systemic symptoms, following the consumption of certain foods, exposure to certain environmental factors, or enactment of specific behaviours (see Table 10) (Bagnis, Kuberski and Laugier, 1979; Gillespie *et al.*, 1986; Lange, Snyder and Fudala, 1992; Fleming and Blythe, 1997; Lewis, 2001; Vigneau *et al.*, 2008; Gatti *et al.*, 2018; Friedman *et al.*, 2017). Ciguatera-specific symptoms may reappear even in cured patients when they are exposed to certain triggers, e.g. to ciguatoxic and non-toxic fish consumption (Pottier, Vernoux and Lewis, 2001).

TABLE 10 **FACTORS CONTRIBUTING TO THE RECURRENCE OR EXACERBATION OF SYMPTOMS**

FOOD-BASED FACTORS	BEHAVIOURAL AND EXTERNAL FACTORS
Alcohol (Gillespie *et al.*, 1986; Lange, Snyder and Fudala, 1992; Barton *et al.*, 1995; Lewis, 2001; Vigneau *et al.*, 2008; Friedman *et al.*, 2017; Gatti *et al.*, 2018)	Intense physical activity (Gatti *et al.*, 2018; Lewis, 2001; Barton *et al.*, 1995)
Marine/freshwater related products (Vigneau *et al.*, 2008; Lewis, 2001; Gatti *et al.*, 2018; Friedemann, 2019)	Exposure to cold air (Gatti *et al.*, 2018)
Pork (Gillespie *et al.*, 1986; Lewis, 2001; Gatti *et al.*, 2018)	Dehydration (Lange, Snyder and Fudala, 1992)
Beef (Gatti *et al.*, 2018)	Fatigue (Gatti *et al.*, 2018; Bagnis, 1993)
Chicken (Gillespie *et al.*, 1986; Lewis, 2001; Fleming and Blythe, 1997; Friedemann, 2019)	Stress (Bagnis, 1993; Barton *et al.*, 1995)
Nuts (Fleming and Blythe, 1997; Gatti *et al.*, 2018; Vigneau *et al.*, 2008; Lewis, 2001)	Lack of sleep (Gatti *et al.*, 2018)
Canned products (Gillespie *et al.*, 1986)	Rapid weight loss (Gatti *et al.*, 2018; Barton *et al.*, 1995)
Dairy products (Gatti *et al.*, 2018)	Sun exposure (Gatti *et al.*, 2018)
Caffeine (Lewis, 2001; Fleming and Blythe, 1997)	Sexual intercourse (Lange, Lipkin and Yang, 1989)
Chocolate (Vigneau *et al.*, 2008)	

6.2.4 LETHALITY

Although ciguatera poisoning usually has a benign prognosis, there have been reports of lethal cases in all endemic regions (Rabenjarison *et al.*, 2016; Chan, 2016; Friedman, *et al.*, 2017). The ciguatera poisoning case fatality rate has been estimated as < 0.5 percent (Bagnis, Kuberski and Laugier, 1979; Allsop *et al.*, 1986), but in some contexts may exceed 10 percent (Bagnis 1970; Rabenjarison *et al.*, 2016). Death due to CTX exposure often follows cardiovascular and/or complications of the central nervous system. It might be preventable by avoiding consumption of fish heads, liver and viscera or possibly through better clinical management practices (Hamilton *et al.*, 2010; Rabenjarison *et al.*, 2016).

6.3 EPIDEMIOLOGY

Ciguatera poisoning is a global health problem. Although CP is endemic to countries from intertropical area around the globe, "imported cases" are reported in countries far from the tropics through tourists being poisoned during travel in ciguatera-endemic regions and/or poisoning cases with imported toxic fishes from ciguatera endemic regions (Table 11). Population-specific estimates of incidence rates have been published for some locations, from 0.002/10 000 persons in Kakeroma Island, Japan (Chan, 2015b) to 280/10 000 persons in Marquesas, French Polynesia (Chateau-Degat *et al.*, 2007). However, epidemiological understanding of the global burden of disease is extremely limited, in part due to systematic under-reporting (Begier *et al.*, 2006; Tester *et al.*, 2010; Skinner *et al.*, 2011). Recent initiatives to consolidate global monitoring of harmful algal events, such as ciguatera poisonings, have been implemented, for example, as the Harmful Algae Information System operated by the Intergovernmental Oceanographic Commission (http://haedat.iode.org).

TABLE 11 **CIGUATERA AS A GLOBAL HEALTH PROBLEM**

ENDEMIC AREAS (Ciguatera poisoning due to consumption of autochthonous fish)	NON-ENDEMIC COUNTRIES (Ciguatera poisoning due to the consumption of toxic imported fish; ciguatera poisoning of tourists during a stay in endemic region)
American Samoa (Tester *et al.*, 2010)	Australia (Farrell *et al.*, 2017; Brett and Murnion, 2015)
Anguilla (Bourdeau and Bagnis, 1989; Tester *et al.*, 2010)	Canada (Frenette, MacLean and Gyorkos, 1988; Muecke *et al.*, 2015)
Antigua and Barbuda (Pottier, Vernoux and Lewis, 2001; Tester *et al.*, 2010)	China, Hong Kong SAR (Wong *et al.*, 2005)
Aruba (Tester *et al.*, 2010)	France (Moulignier, Binet and Frottier, 1995)
Australia (Farrell *et al.*, 2017; Farrell *et al.*, 2016; UNESCO-IOC-ICES-PICES, 2018)	Germany (Mattei *et al.*, 2014; Yalachkov *et al.*, 2019; Friedemann, 2019)
Bahamas (Pottier, Vernoux and Lewis, 2001; Tester *et al.*, 2010)	Italy (Chan, 2015b; Bavastrelli *et al.*, 2001)
Barbados (Tester *et al.*, 2010)	Netherlands (Slobbe, van Genderen and Wismans, 2008)
Belize (Tester *et al.*, 2010)	New Zealand (Armstrong *et al.*, 2016)
Bermuda (Tester *et al.*, 2010; Government of Bermuda, 2016)	Portugal (Puente *et al.*, 2005)
British Virgin Islands (Tester *et al.*, 2010)	Spain (Gascón, *et al.*, 2003)
Canary Islands (Spain) (Boada *et al.*, 2010; Nunez *et al.*, 2012)	United Kingdom of Great Britain and Northern Ireland (Friedemann, 2019)
Cayman Islands (Tester *et al.*, 2010)	United States of America (Winter, 2009; CDC, 2013; Radke, Reich and Morris, 2015)
Chagos Islands (British Indian Ocean Territory) (Lebeau, 1978)	
China (Fujian, Hainan, China, Hong Kong SAR, Guangdong) (Wong, Hung and Lo, 2014; Chan, 2014; UNESCO-IOC-ICES-PICES, 2018)	
Colombia (Tester *et al.*, 2010)	
Cook Islands (Tester *et al.*, 2010; Rongo and van Woesik, 2011; Skinner *et al.*, 2011)	

(continues)

TABLE 11 (continued)

ENDEMIC AREAS (Ciguatera poisoning due to consumption of autochthonous fish)	NON-ENDEMIC COUNTRIES (Ciguatera poisoning due to the consumption of toxic imported fish; ciguatera poisoning of tourists during a stay in endemic region)
Costa Rica (Winter, 2009)	
Cuba (Pottier, Vernoux and Lewis, 2001; Tester *et al.*, 2010; Bagnis, Kuberski and Laugier, 1979)	
Dominican Republic (Tester *et al.*, 2010)	
Egypt (Abd-Elhaleem and Abd-Elkarim, 2011)	
Fiji (Tester *et al.*, 2010; Dalzell, 1992; Skinner *et al.*, 2011)	
Florida (United States of America) (Tester *et al.*, 2010; CDC, 1986, 2000, 2006; de Sylva, 1994)	
French Polynesia (Tester *et al.*, 2010; Bagnis, Kuberski and Laugier, 1979; Chateau-Degat *et al.*, 2007; Skinner *et al.*, 2011)	
Grenada (Tester *et al.*, 2010)	
Guadeloupe (Pottier, Vernoux and Lewis, 2001; Tester *et al.*, 2010; Boucaud-Maitre *et al.*, 2018)	
Guam (Tester *et al.*, 2010; Skinner, *et al.*, 2011)	
Guatemala *(UNESCO-IOC-ICES-PICES, 2018)*	
Haiti (Pottier, Vernoux and Lewis, 2001; Tester *et al.*, 2010; Poli *et al.*, 1997)	
Hawaii (United States of America) (Tester *et al.*, 2010; Skinner *et al.*, 2011; Copeland, Palmer, and Bienfang, 2014)	
India (Karunasagar *et al.*, 2018; Rajisha *et al.*, 2017; UNESCO-IOC-ICES-PICES, 2018)	
Indonesia (IOC-UNESCO-WESTPAC, 2018)	
Israel (Bentur and Spanier, 2007)	
Jamaica (Pottier, Vernoux and Lewis, 2001; Tester *et al.*, 2010)	
Japan (Oshiro, *et al.*, 2010; Chan, 2015b)	
Kiribati (Tester *et al.*, 2010; Skinner *et al.*, 2011; Dalzell, 1992)	
Madagascar (Diogene *et al.*, 2017)	
Malaysia (Chan, 2015b)	
Maldives (Lagraulet, 1975)	
Marshall Islands (Skinner *et al.*, 2011)	
Martinique (Pottier, Vernoux and Lewis, 2001; Tester *et al.*, 2010)	
Mauritius (Lebeau, 1978; Quod and Turquet, 1996; Glaizal *et al.*, 2011)	
Mayotte (Hossen *et al.*, 2013; UNESCO-IOC-ICES-PICES, 2018)	
Mexico (Pottier, Vernoux and Lewis, 2001; Tester *et al.*, 2010; Núñez-Vázquez *et al.*, 2019)	
Micronesia (Federated States of) (Tester *et al.*, 2010; Dalzell, 1992)	
Montserrat (Tester *et al.*, 2010)	
Nauru (Tester *et al.*, 2010)	

(continues)

TABLE 11 (continued)

ENDEMIC AREAS (Ciguatera poisoning due to consumption of autochthonous fish)	NON-ENDEMIC COUNTRIES (Ciguatera poisoning due to the consumption of toxic imported fish; ciguatera poisoning of tourists during a stay in endemic region)
New Caledonia (Dalzell, 1992; Skinner *et al.*, 2011; Baumann, Bourrat and Pauillac, 2010)	
Niue (Tester *et al.*, 2010; Dalzell, 1992; Skinner *et al.*, 2011)	
Northern Mariana Islands (Tester *et al.*, 2010; Skinner *et al.*, 2011)	
Owia (Saint Vincent and the Grenadines) (Morris, 1992, cited in Pottier, Vernoux and Lewis, 2001)	
Pakistan (Wasay *et al.*, 2008)	
Palau (Tester *et al.*, 2010; Skinner *et al.*, 2011)	
Papua New Guinea (Tester *et al.*, 2010)	
Philippines (Mendoza *et al.*, 2013; UNESCO-IOC-ICES-PICES, 2018)	
Puerto Rico (Tester *et al.*, 2010; Azziz-Baumgartner *et al.*, 2012)	
Réunion (Lebeau, 1978; Quod and Turquet, 1996; UNESCO-IOC-ICES-PICES, 2018)	
Republic of Korea (Chan, 2015b, Cha *et al.*, 2007)	
Rodrigues Island (Mauritius) (Lebeau, 1978)	
Selvagens Islands (Portugal) (Gouveia, *et al.*, 2009; Boada *et al.*, 2010; Otero *et al.*, 2010; Costa *et al.*, 2018)	
Solomon Islands (Tester *et al.*, 2010)	
Saint Barthélemy (Vernoux, 1988; Bourdeau and Bagnis, 1989)	
Saint Kitts and Nevis (Tester *et al.*, 2010)	
Saint-Martin (Pottier, Vernoux and Lewis, 2001; Tester *et al.*, 2010; Bourdeau and Bagnis, 1989)	
Samoa (Tester *et al.*, 2010; Skinner *et al.*, 2011)	
Taiwan Province of China (Chan, 2015a)	
Thailand (Saraya *et al.*, 2014; Chan, 2015a)	
Timor-Leste (Pottier, Vernoux and Lewis, 2001)	
Tokelau (Tester *et al.*, 2010; Skinner *et al.*, 2011)	
Tonga (Tester *et al.*, 2010; Skinner *et al.*, 2011)	
Trinidad and Tobago (Tester *et al.*, 2010)	
Turks and Caicos Islands (, 2010)	
Tuvalu (Dalzell, 1992; Skinner *et al.*, 2011)	
United States Virgin Islands (Tester *et al.*, 2010; UNESCO-IOC-ICES-PICES, 2018)	
Vanuatu (Skinner *et al.*, 2011; Vienne, 1982)	
Venezuela (Bolivarian republic of) (Tester *et al.*, 2010)	
Viet Nam (Chan, 2015b; Ha, *et al.*, 2018)	
Wallis and Futuna Islands (Tester *et al.*, 2010; Hossen *et al.*, 2013)	

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Inferences regarding the likely impacts of climate change on the global burden of ciguatera poisoning are limited by the dearth of high-quality quantitative data on the historical burden of ciguatera poisoning. A retrospective analysis of telephone calls to the United States Poison Control System identified direct associations between major storms and upticks in ciguatera reports about 18 months later and suggested that there could be dramatic increases in the ciguatera burden if the temporal trends continue (Gingold, Strickland and Hess, 2014). These results coincide with observations in the Caribbean, where intervals of 1–2 years between the occurrence of severe storms and upsurges in ciguatera incidence or toxicity of fish were reported (Pottier, Vernoux and Lewis, 2001). However, there have not been consistent time trends identified in 30-year time trends analysis of reported cases in the Marshall Islands (Radke *et al*., 2013). It has been suggested that the evolution of the ciguatera burden under climate change may be more nuanced than a simple increase with rising temperature, as ciguatera-causing algae may have preferred environmental conditions (Llewellyn, 2010).

The European Rapid Alert System for Food and Feed (RASFF) has issued 8 alerts for ciguatoxins in the past 5 years. In all eight cases the notifying country was France (https://webgate.ec.europa.eu/rasff-window/portal), and the poisoning includes; snapper fillets (27 July 2017) and chilled kingfish (22 August 2016) from India; chilled great barracuda from Senegal (22 August 2016); frozen whole barracuda from India (19 August 2016); wild-caught fish from Sri Lanka (27 January 2015); frozen red snapper from Viet Nam (17 March 2017) and India (14 July 2016 and 16 November 2012).

Outbreaks of CP have also been reported from tropical or subtropical regions, such as Australia (Gillespie *et al*., 1986), China, Hong Kong SAR (Chan, 2014; Wong *et al*., 2008; Wong *et al*., 2005), French Polynesia (Gaboriau *et al*., 2014; Chinain *et al*., 2010b), Okinawa, Japan (Chan, 2015a; Oshiro *et al*., 2010), and the United States of America (Barrett, *et al*., 2017).

Because CP is associated with tropical marine environments between latitudes 35°N and 35°S, most of the intoxications are located in these areas, although, as mentioned above, international trade is increasing the poisoning cases of non-native countries. In the Atlantic, it is common in Florida (the United States of America), the Bahamas, throughout the Caribbean (Boucaud-Maitre *et al*., 2018), particularly in Cuba, the Dominican Republic, Haiti, Puerto Rico, and the Leeward Islands, including the Virgin Islands. In the Pacific, it occurs in Australia, Cook Islands, Fiji, Hawaii (the United States of America), French Polynesia, the Marshall Islands, New Caledonia, the Philippines, Samoa, Tonga, and Vanuatu. In the Indian Ocean, it appears in Comoros, Chagos Islands (British Indian Ocean Territory), India, Madagascar, Maldives, Mauritius, Réunion, Seychelles and Sri Lanka (Gestal-Otero, 2014).

In the Pacific region there are about 3 400–4 700 ciguateric intoxications per year, which most probably represent only 10–20 percent of the real number of cases (Gestal-Otero, 2014). It has been reported that a classical CP case presented to

36 clinicians in southern Florida (the United States of America) (an endemic area), and 68 percent identified the syndrome, but only half of them were aware of the obligation to report the case to health authorities (Friedman *et al.*, 2017). Tables 12 and 13 show incidences in certain locations with high values.

TABLE 12 **HIGHEST INCIDENCE RATES PER 10 000 POPULATION IN SELECTED LOCATIONS**

LOCATION	REPORTED NUMBER OF CASES PER 10 000 POPULATION	REFERENCE
United States Virgin Islands (Saint Thomas ER records)	180	Radke *et al.*, 2013
United States Virgin Islands (Saint Thomas)	120	Radke *et al.*, 2013
Puerto Rico (Culebra)	75	Tester *et al.*, 2010
Tokelau	65.3	Lewis, 1986
Montserrat	58.6	Tester *et al.*, 2010
French Polynesia	54.5	Lewis, 1986
Tuvalu	43.9	Lewis, 1986
French Polynesia	36	Chateau-Degat *et al.*, 2007
Antigua and Barbuda	34.4	Tester *et al.*, 2010
Kiribati	32.4	Lewis, 1986
Iles Santes (Guadeloupe)	30	Czernichow *et al.*, 1984
Marshall Islands	28.2	Lewis, 1986
Hawaii (United States of America)	20.3	Anderson *et al.*, 1983
New Caledonia	20	Lewis, 1986
British Virgin Islands	19.9	Tester *et al.*, 2010
Niue	13	Lewis, 1986
American Samoa	8.7	Lewis, 1986
Florida (the United States of America) (Monroe)	8.4	Radke *et al.*, 2013

TABLE 13 **POISONING CASES WITH CAUSATIVE FISH AND TOXINS**

LOCATION	YEAR	No.	FISH SPECIES	TOXIN RANGE EQUIVALENT IN FISH	SYMPTOM ONSET TIME	LENGTH OF SYMPTOMS (in some cases)	REFERENCE
New South Wales (Australia)	2014–17	37	Spanish mackerel, redthroat emperor, purple rockcod, grouper, green jobfish	0.023–1 µg/kg (CTX1B)	1–6 h	1–7 months	Farrell *et al.*, 2017
Queensland (Australia)	1995	4	Coral trout	1.3 ng/g		More than a week with mannitol treatment	Fenner *et al.*, 1997
Hamburg (Germany)	2009	14	Bigeye trevally, red grouper		6 h	2 weeks	Schlaich *et al.*, 2012
Philippines	2001 2006 2010	50, 33, 22	Barracuda red snapper				Mendoza *et al.*, 2013
Mangalore (India)	2016	> 200	Red snapper	1.1–2.6 µg/kg (CTX3C)	4–5 h	Several days	Karunasagar *et al.*, 2018
Guadeloupe	2010–12	41	See Hossen *et al.*, 2015	0.02–0.47 CTX1B eq/kg (individual intakes of 4.2 to 70.6 eq/kg)	2–9.5 h		Hossen *et al.*, 2015
Guadeloupe	1992	3	Grey snapper, grouper, black jack	0.24–13.8 ng/g C-CTX1			Pottier, Vernoux and Lewis, 2001
Canary Islands (Spain)	2008–17	25	See Epidemiología, 2018				Epidemiología, 2018
Anaho Bay (French Polynesia)	2015	3	*Tripneustes gratilla* (echinoid)	µg/g CTX3B	< 1 h	More than 1 month	Darius *et al.*, 2018a
Anaho Bay (French Polynesia)	2014	9	*Tectus niloticus* (gastropod)	0.9–14.81 µg/g CTX3C (not from causative samples [Darius *et al.*, 2017])			Gatti *et al.*, 2015
Miami (United States of America)	1972–76	129	See Lawrence *et al.*, 1980		Few hours		Lawrence, *et al.*, 1980
Caribbean countries	1980–10	4 952					Celis and Mancera Pineda, 2015
French Polynesia	2013–17		See Chinain *et al.*, 2018	0.24–8.38 ng/g CTX3C	2–48 h	More than 3 months in 20 percent of hospitalized cases; death in some cases	Chinain *et al.*, 2018
Madagascar	2013	124	Shark (*Carcharhinus leucas*)	6.54–16.28 CTX1B	2–12 h		Diogene *et al.*, 2017
Kiribati	1947	7	Moray eel			3 weeks; death in some cases	Cited in Chan, 2017
Kiribati	1961	2	Moray eel			death	Cited in Chan, 2017

(continues)

TABLE 13 (continued)

LOCATION	YEAR	No.	FISH SPECIES	TOXIN RANGE EQUIVALENT IN FISH	SYMPTOM ONSET TIME	LENGTH OF SYMPTOMS (in some cases)	REFERENCE
Marshall Islands	1953	6	Moray eel			1 death after 25 days	Cited in Chan, 2017
Mariana Islands	1949	57	Moray eel			Several deaths after 18 days	Cited in Chan, 2017
Guangzhou, Shenzhen, Dongguan, China, Hong Kong SAR	1999 2004 2004 2005	9 18 5 5	Moray eel		1–4 h	From 13–14 days to 3 weeks	Cited in Chan, 2017
Taiwan Province of China	2004	1	Moray eel				Cited in Chan, 2017
Japan	1930–68	95	Moray eel				Cited in Chan, 2017
New Zealand	1999 2003 2016	2 2 3	Moray eel		1 h	Several weeks	Cited in Chan, 2017
London (United Kingdom of Great Britain and Northern Ireland)	1979	1	Moray eel		0.5 h	More than 7 weeks	Cited in Chan, 2017
Madeira (Portugal)	2008	11	Amberjack	53.76 mg/g CTX3C	4 h		Otero *et al.*, 2010
Canary Islands (Spain)	2004 2008 2012	5 (Perez-Arellano *et al.*, 2005), 30 (Boada *et al.*, 2010), 4 (Nunez *et al.*, 2012)	Amberjack	1 ng/g (Perez-Arellano *et al.*, 2005), 0.17 ng/g C-CTX1B (Boada *et al.*, 2010)	30 min	48 h	Boada *et al.*, 2010; Perez-Arellano *et al.*, 2005; Nunez *et al.*, 2012
China	2004 1994–08	200 (see (Chan, 2015b)	Tiger grouper (see Chan, 2015b)				Chan, 2015b
Guadeloupe	2013–16	234	See Boucaud-Maitre *et al.*, 2018				Boucaud-Maitre *et al.*, 2018
Germany	2000–13	61	Red snapper, (see Mattei *et al.*, 2014)				Mattei *et al.*, 2014
China, Hong Kong SAR	1989–08	(see Chan, 2014)					Chan, 2014

If, as suspected, only 10–20 percent of actual intoxications are formally reported to the authorities, the problem of CP poisoning is much larger than official figures show (Friedman *et al.*, 2017). In Florida (the United States of America), an outbreak is considered one single case (Klekamp, Bodager and Matthews, 2015), while CDC requires at least two cases (CDC, 2000). The number of reported cases is also related to the awareness of clinicians to report it (for example, in Florida [the United States of America], in many cases doctors are not aware of the need to report it [Friedman *et al.*, 2017]), and with the actual identification of the poisoning, as the symptoms

are in many cases misguiding and lead to incorrect diagnosis (Friedman *et al*., 2017). It is estimated that 50 000–500 000 poisonings per year may be occurring (Fleming *et al*., 1998). In Puerto Rico and United States Virgin Islands alone, it has been estimated that 20 000–40 000 illnesses per year may occur (Tosteson, 1995). It has been estimated that the annual incidence is about 10 percent of local Pacific island populations (Lewis and Ruff, 1993). The prevalence of the poisoning in endemic areas covers a range from 0.1 percent of the population in continental lands (Queensland [Australia] or Florida [the United States of America]) to more than 50 percent in small South Pacific or Caribbean islands (Dickey and Plakas, 2010; Skinner *et al*., 2011). One of the most affected countries is Tuvalu, with a prevalence of 240 cases per 10 000 population, while in the United States of America it is 50–70 cases per 10 000 population (Gestal-Otero, 2014). There were 39 677 reported cases in 17 Pacific Island Countries and Territories (PICTs), with an annual incidence of 194 cases per 100 000 population from 1998 to 2008. This, compared with the 104 cases per 100 000 population from 1973 to 1983, means a 60 percent increase (Skinner *et al*., 2011). Using the conservative estimate that the official reported CP accounts for 20 percent of actual incidence, the real overall incidence for the region would be 970 cases per 100 000 population for 1998–2008.

In the United States of America, from 1998 to 2015, out of the 260 000 people intoxicated by fish in 857 outbreaks, 227 outbreaks were caused by CTX, especially linked to grouper fish (Barrett *et al*., 2017). In Florida (the United States of America) alone, there were 137 cases from 2012 to 2014 (Klekamp, Bodager and Matthews, 2015). In China, Hong Kong SAR, the annual incidence of CP of 10.2 cases per million people from 1989 to 2008 was caused by groupers (replacing snappers as the main cause) (Chan, 2015a, 2014). In French Polynesia, the prevalence between 1992 and 2001 was of 35.6 cases per 10 000 population, but the incidence decreased to 14.5 cases per 10 000 in 2008 by improving the public health system (Château-Degat *et al*., 2009). A risk assessment evolution in French Polynesia concluded that the locations known by local people as safe would actually be safe, with low *Gambierdiscus* populations (Darius *et al*., 2007), but those fish with sizes thought to be safe were actually toxic (Gaboriau *et al*., 2014). In Cook Islands, annual CP incidence varied from 204 cases per 10 000 population to 1 058 cases per 10 000 population between 1994 and 2010 (Rongo and van Woesik, 2013, 2011). The prevalence of poisoning in New Caledonia is in general low, 10 cases per 10 000 population, and doctors rarely report food-borne intoxications. It is a common situation that CP is often underdiagnosed and under-reported, with only 2–10 percent of cases reported to health authorities (Friedman *et al*., 2017). Estimates of the annual incidence of CP in Oceania have ranged from 0.5 cases per 10 000 population in Hawaii (the United states of America) to 5 850 cases per 10 000 population in French Polynesia.

Ciguatera poisoning had never been reported on the coast of West Africa until a 2004 outbreak in the Canary Islands (Spain), which was followed by two additional outbreaks in 2008-2009. These cases were linked to consumed lesser amberjack (*Seriola rivoliana*) captured in local waters. The Canary Islands had been considered

a non-endemic region for CP (Perez-Arellano *et al*., 2005). After these incidents several species of *Gambierdiscus* were identified in the zone (Rodriguez *et al*., 2017).

The patient showed all the typical symptoms of CP. In 2008, public health authorities of the Canary Islands (Spain) investigated a second outbreak of CP involving 20–30 patients that showed symptoms a few hours after eating lesser amberjack, similar to the 2004 outbreak. The amberjack involved in this event were captured off the north coast of the Canary Islands (Spain), near the Selvagens Islands (Portugal), another area reported to have caused intoxications (Otero *et al*., 2010). In 2009, a third outbreak occurred in the same location, affecting 10–40 individuals who had eaten lesser amberjack bought from a supermarket, and as in former incidents, the fish had been captured north of the Canary Islands (Spain), near the Selvagens Islands (Portugal).

In other supposedly non-endemic areas, such as the eastern Mediterranean, there have been confirmed reports of intoxications from rabbit fish (*Siganus sp.*) caught in Haifa Bay (Israel) (Raikhlin-Eisenkraft and Bentur, 2002). Barracuda (*Sphyraena barracuda*) and snapper (*Lutjanus sp.*) from Cameroon have also caused intoxications (Bienfang, Oben and DeFelice, 2008).

In recent years, there has also been an increase in inquiries for CP patients who have spent their holidays in tropical regions, particularly in the Pacific islands and the northern regions of Australia, and in the Caribbean.

Outside the Canary Islands (Spain), the frequency of intoxications in Spain is very low, mostly from tourists returning from Cuba or the Dominican Republic (Herrero-Martínez *et al*., 2011). In 2011, a case was described (Herrero-Martínez *et al*., 2011) of a 44-year-old woman after returning from Santo Domingo (the Dominican Republic) after consumption of boiled silk snapper (*L. vivanus*).

In Europe, intoxications are a consequence of international trade. The episode in Hamburg (Germany) in 2009 was caused by fish (bigeye trevally [*Caranx sexfasciatus*] and red grouper [*Cephalopholis miniata*]) caught in the Caribbean (Schlaich *et al*., 2012). This poisoning affected 14 sailors who had consumed frozen fish, and symptoms persisted for 14 days. Further outbreaks occurred in Germany between 2012 and 2017 caused by snappers imported from India, Indonesia and Viet Nam (Friedemann 2019).

A recent report of a large single poisoning was reported in Mangalore, India, in 2016. This case affected more than 200 people working in a seafood processing unit, with typical symptoms of abdominal pain, vomiting, weakness and tingling sensations (Karunasagar *et al*., 2018). Each individual has consumed 1–6 pieces of cooked heads of large red snapper (*Lutjanus bohar*). Gastrointestinal symptoms started after 4–5 h, and after 12 h all were sick, with neurological symptoms. While 25 percent of these cases were mild, the rest were severe, and 10 percent experienced cardiovascular symptoms (hypotension, and sinus bradycardia) that were responsible for a longer hospitalization. Analysis suggested Caribbean and Indian Ocean CTXs, in amounts equivalent to CTX3C of 1.1–2.6 ng/g.

6.4 TREATMENTS

No therapy has demonstrated efficacy for treating acute or chronic manifestations of CP. Supportive medical management of CP relies on symptom-managing medications, such as antispasmodic, antiemetic, antidiarrheic, cardioactive and antidepressant drugs, as well as chronotropic pressors, rehydration, calcium gluconate, antihistaminics, analgesics, calcium channel blockers, and/or B1, B6, B12 vitamins (Berlin, King and Blythe, 1992; Lewis, 2001; Kumar-Roiné *et al*., 2010; Friedman *et al*., 2017).

The use of mannitol at an early stage of the poisoning has been suggested to reduce Schwann cell oedema and to act as a scavenger of free radicals (Birinyi-Strachan *et al*., 2005a, Palafox, 1992; Palafox *et al*., 1988). Infusion of 10 ml/kg of 20 percent solution, infused slowly for up to 45 min, within 48 h post-poisoning, has been shown to treat neurological symptoms in well-rehydrated patients (Pearn, 2001; Bagnis *et al*., 1992; Mullins and Hoffman, 2017).

The effectiveness of pregabalin and gabapentin for the alleviation of pain and allodynia has not been demonstrated in randomized trials (Friedman *et al*., 2017).

In the Indo-Pacific and West Indies endemic regions, the use of 90 different traditional herbal remedies such as *Heliotropium foertherianum*, *Euphorbia hirta*, *Rosmarinus* spp., *Vitex* spp. as well as castor oil and carapat oil have been reported (Kumar-Roiné *et al*., 2011), but none of them has been clinically evaluated (Pottier, Vernoux and Lewis, 2001; Friedman, *et al*., 2017).

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CHAPTER 7
EXPOSURE ASSESSMENT

7.1 INTRODUCTION

While there is evidence to suggest that individuals may experience multiple instances of CP over time and may exhibit chronic adverse health effects, there is little evidence that chronic exposure to CTXs at subacute poisoning levels is associated with distinct adverse health effects. Consequently, assessment of dietary exposure to CTXs is primarily concerned with acute (single event or single day) exposures.

7.2 DETECTION FREQUENCY OF CTXs AND LEVELS OF CONTAMINATION

Table 14 summarizes information on concentrations of CTXs in samples of fish associated with CP cases (suspect) and from monitoring studies (random). These concentrations were derived using a range of activity- and structure-based analytical methods, with concentrations expressed as equivalents of a range of different CTX. Consequently, concentration values are not necessarily comparable from study to study. Only recently, with the advance of analytical methods, has this information become more complete. Nevertheless, the lack of CTX standards is still a major limitation to understand the nature of the intoxications.

While some of the studies in Table 14 are classified as random, all information currently available on CTXs or ciguatoxicity in fish are from studies that are targeted to some extent. A number of studies relate to samples of fish implicated as the cause of cases of CP. A smaller number of prospective monitoring studies are available, but often focus on particular risk fish species or risk environments.

Table 14 contains summary information from studies that were considered to contain sufficient detail to be of potential use for exposure assessment.

TABLE 14 **SUMMARY OF STUDIES ON THE CIGUATOXIN CONTENT OF MARINE FISH**

COUNTRY	SPECIES	YEAR	SAMPLING[1]	METHOD	RESULTS EXPRESSED AS	LOD	LOQ	SAMPLE TYPE	N	N > LOD	CONCENTRATION (µg/kg)				REFERENCE
											Mean LB	Mean UB	P95	Max	
Australia	Spanish mackerel	2015	Random	LC-MS/MS	CTX1B	0.1	0.3	Flesh	88	4	0.024	0.12		1.0	Kohli *et al.*, 2017
Australia	Spanish mackerel	2015	Random	LC-MS/MS	CTX1B	0.1	0.3	Liver	84	1				1.39	Kohli *et al.*, 2017
Australia	Shark species	NS	Random	LC-MS/MS	CTX1B, CTX2, CTX3	0.05		Flesh (also liver for some specimens)	22	0					Meyer, *et al.*, 2016
Kiribati	Various	NS	Random	LC-MS/MS	Total (sum of: CTX1B, CTX2, CTX3)	0.0005		Flesh > herbivore > omnivore > carnivore	20 19 113		0.20 0.41 2.47	0.20 0.41 2.47		1.67 1.81 69.5	Mak *et al.*, 2013
Kiribati	Various	2009	Random	N2a	CTX1B	0.002		Flesh > herbivore > omnivore > carnivore	41 13 117		0.36 0.69 2.57			81.8	Chan *et al.*, 2011
French Polynesia (Australes)	Various	NS	Random	N2a	CTX3C			Flesh > Raivavae > Rapa	7 13	0 5	0.020			0.12	Pawlowiez *et al.*, 2013
Caribbean	Lionfish	NS	Random	N2a	CTX1B		0.004–0.01	Flesh	120	27	0.018	0.025[2]	0.11	0.33	Solino *et al.*, 2015
Gulf of Mexico	Great barracuda	2003	Random	N2a	C-CTX1	NS	NS	Flesh	20	10			0.06	0.14[3]	Villareal *et al.*, 2007
Bahamas	Great barracuda	2008–09	Random	N2a	C-CTX1	0.0003		Flesh	12	8	0.013	0.013		0.099	O'Toole *et al.*, 2012
Bahamas	Great barracuda	2008–09	Random	N2a	C-CTX1	0.0003		Liver	12	11	0.029	0.029		0.17	O'Toole *et al.*, 2012

(continues)

TABLE 14 (continued)

COUNTRY	SPECIES	YEAR	SAMPLING[1]	METHOD	RESULTS EXPRESSED AS	LOD	LOQ	SAMPLE TYPE	N	N > LOD	CONCENTRATION (µg/kg)				REFERENCE
											Mean LB	Mean UB	P95	Max	
Australia	Various	NS	Suspect	LC-MS/MS	CTX1B	0.03			46	27				11.4	Stewart *et al.*, 2010
Australia	Various	2013–18	Suspect	NS	Total (sum of: CTX1B, CTX2, CTX3)	0.15		Flesh	59	35	1.02	1.08	3.48	6.6	QFSS, meeting submission
Australia	Various	NS	Suspect	NS	CTX1B	0.1	0.3	Flesh	13	11	0.274	0.29		1.0	Farrell *et al.*, 2017
United States of America	Barracuda, grouper	2010–11	Suspect	LC-MS/MS	C-CTX1			Flesh	3	3		1.2		1.9	Graber, *et al.*, 2013
French Polynesia	Various	NS	Suspect	RBA	CTX3C			Flesh	13	13	2.48	2.48	7.08	8.38	Meeting submission
French Polynesia	Various	NS	Suspect	RBA	CTX1B			Flesh	13	13	1.05	1.05	2.99	3.51	Meeting submission
Canary Islands (Spain)	Ciguatera suspect species	NS	Suspect	N2a	CTX1B			Flesh	13	4	0.84	0.85		6.23	Caillaud *et al.*, 2012
Guadeloupe	Various	2010–12	Suspect	N2a	CTX1B			Flesh	10	10		0.12	0.34	0.47	Hossen, *et al.*, 2015

Notes:
NS: not stated;
LOD: limit of detection;
LOQ: limit of quantification;
LC-MS/MS: liquid chromatography-tandem mass spectrometry;
N2a: mouse neuroblastoma assay;
RBA: receptor binding assay;
P95: 95th percentile.
LB: lower bound estimate of mean concentration, calculated by substituting zero for analytical results below the LOD.
UB: upper bound estimate of mean concentration, calculated by substituting a concentration equal to the LOD for analytical results below the LOD.

[1] Suspect refers to fish samples believed to have caused ciguatera poisoning, while random refers to samples not specifically associated with ciguatera poisoning

[2] The upper bound estimate of the mean was calculated by substituting a highest LOQ value of 0.01 µg/kg CTX1 equivalent for samples without detectable ciguatoxicity

[3] Eight of the 20 samples were reported to contain trace amounts of C-CTX1 equivalents. However, the LOD and LOQ were not reported.

7.3 CONSUMPTION

Information on fish consumption, particularly in countries with high rates of CP, is fragmentary.

7.3.1 MEAN ESTIMATES OF FISH CONSUMPTION

Analysis of food balance sheets confirms that the countries with the highest per capita pelagic fish availability for consumption levels are mainly island nations. The highest per capita pelagic fish availability was for the Maldives (163 kg/year or 446 g/day), followed by Kiribati (142 g/day), Iceland (120 g/day) and Samoa (92 g/day). It should be noted that these are population mean availability figures and are not generally useful for assessing acute dietary exposure.

An analysis of CP cases in Guadeloupe included information on the portion size consumed for 25 cases of people who consumed fish fillet and two cases of people who consumed fish head (Hossen *et al.*, 2015). The mean fillet serving size was 204 g (range 100–400 g), while for the two cases who had consumed fish head the serving size was 50 g in both cases. On a body weight basis, the mean fillet serving size was 3.2 g/kg bw (range 1.1–7.7 g/kg bw) for cases where both serving size and body weight information was available (n = 21) and 0.59 g/kg bw for the two cases who consumed fish head. The minimum serving size for fish fillet associated with CP was 100 g.

The FAO/WHO Chronic Individual Food Consumption database (CIFOCOss) is based on studies that surveyed at least two consumption days. While these data are not strictly appropriate for acute dietary exposure assessment, the highest individual mean consumption is 202 g/day (3.1 g/kg bw).

The EFSA Comprehensive European Food Consumption Database (EFSA, 2018) contained food consumption information from 60 surveys carried out in 25 European countries. Mean single-day estimates for fish (meat) consumption by adults are in the range of from 45 g/day (Denmark) to 220 g/day (Hungary). However, as Hungary is a land-locked country, the higher estimate is likely to include a substantial proportion of freshwater fish species. The highest single-day mean fish consumptions for maritime countries were 215 g/day (Romania) and 212 g/day (Croatia). The mean of the various European mean estimates was 139 g/day. Estimated single-day mean fish consumption by children was lower than for adults; in the range of from 33 g/day (Denmark) to 130 g/day (Austria). The mean of all mean estimates was 81 g/day.

7.3.2 HIGH PERCENTILE ESTIMATES OF FISH CONSUMPTION

Data submitted to the current consultation by Food Standards Australia New Zealand, for the Australian population, include large portion sizes (97.5th percentile of consumers only) for a range of seafood species. Large portion sizes range from 15 g (anchovies) to 490 g (snapper).

The current model for the International Estimate of Short-term Intake (IESTI) includes an even higher large-portion size for marine fish of 1 040 g (WHO). The large portion database, used by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for the Global Estimate of Acute Dietary Exposure (GEADE) for veterinary drugs includes a 97.5th 1-day fish consumption figure of 2 000 g, based on data from Slovakia. However, little information is available to assess the reliability of this very high consumption figure.

The EFSA Comprehensive European Food Consumption Database reported 97.5th percentile single-day estimates for fish consumption by adults as being in the range of from 183 g/day (Denmark) to 717 g/day (Poland). However, due to the number of respondents in the latter survey, the 97.5th percentile was not considered to be a reliable high percentile. The highest reliable 97.5th percentile single-day fish consumption estimate was 558 g/day (Croatia). Estimated single-day 97.5th percentile fish consumption by children was in the range of from 120 g/day (Germany) to 340 g/day (Italy). The highest reliable 97.5th percentile was 296 g/day (Spain).

As mentioned above, CIFOCOss is based on studies that surveyed at least two consumption days. While these data are not strictly appropriate for acute dietary exposure assessment, the highest consumer 97.5th percentile consumption figure for marine fish is 655 g (9.98 g/kg bw) for the Brazilian population.

Based on these data, four "reference" fish consumption levels were selected: 100 g/day (mean; non-major fish-consuming nations); 200 g/day (mean; major fish-consuming nations); 500 g/day (97.5th percentile; major fish-consuming nations); and 1 000 g/day (highest reliable single-day consumption estimate), for scenario assessment.

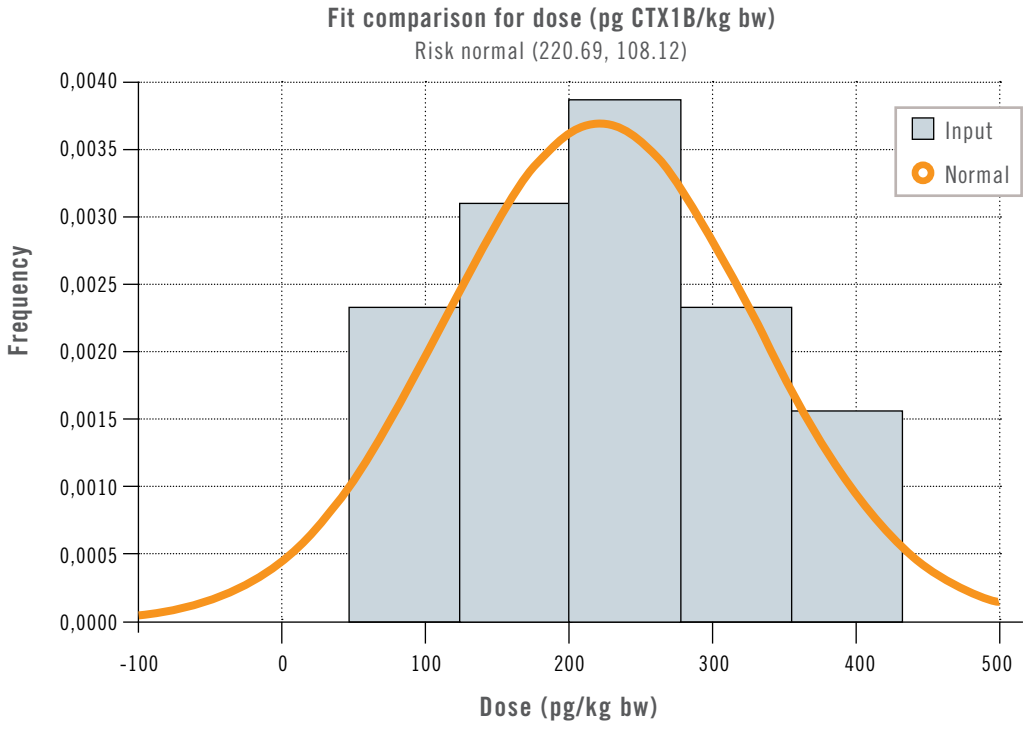
7.4 CIGUATOXIN DOSES CAUSING CIGUATERA POISONING

The study by Hossen *et al.* (Hossen, *et al.*, 2015) appears to be unique in the literature in reporting estimated doses of CTX associated with cases of CP, rather than only the concentration of toxins or toxicity in the implicated fish (Figure 11). Data were available for 17 cases for which the consumption amount, the CTX concentration (determined by N2a, as CTX1B equivalents) and the consumer body weight were available. The estimated doses eliciting CP were in the range 48.4–429 pg/kg bw CTX1B equivalents, with a mean of 221 pg/kg bw CTX1B equivalents and a median of 220 pg/kg bw CTX1B equivalents. The distribution of doses can be well represented by a normal distribution (Figure 11, mean 220 pg/kg bw CTX1B equivalents, standard deviation 108 pg/kg bw CTX1B equivalents). The minimum eliciting dose of 48.4 pg/kg bw CTX1B equivalents provides an estimate of a LOAEL for CP in humans.

It should be noted that, although results were expressed in terms of CTX1B, this was due to availability of that CTX and the actual CTXs present were Caribbean CTXs, which was confirmed by LC-MS/MS.

This study was carried out in a CP endemic area, and it is possible that the cases analysed were chronically exposed to CTX in addition to the exposure quantified from the suspected fish meal.

FIGURE 11 **DISTRIBUTION OF DOSES OF CTX1B EQUIVALENTS ASSOCIATED WITH CP CASES IN GUADELOUPE**



Source: Hossen *et al.*, 2015.

Lehane and Lewis (Lehane and Lewis, 2000) estimated a minimum dose likely to cause mild CP from the minimum concentration of toxin detected in fish associated with CP outbreaks (0.1 µg/kg CTX1B) and assuming a 500 g consumption level and a 50 kg body weight. However, the estimated exposure dose (1 ng/kg bw CTX1B equivalents or 1 000 pg/kg bw CTX1B equivalents) is higher than any of the doses estimated in the study by Hossen *et al.* (Hossen *et al.*, 2015). A number of factors could contribute to this apparent large difference in estimates of LOAEL, including the sensitivity of the analytical method used.

Yasumoto *et al.* (Yasumoto, Raj and Bagnis, 1984) reported that illness in adults could result from oral intake of as little as 0.1 µg of CTX. Assuming a 60 kg body weight, this would equate to an exposure dose of 1.7 ng/kg bw or 1 700 pg/kg bw.

Both the FDA and EFSA have proposed a fish CTX concentration of 0.01 µg/kg CTX1B as being unlikely to elicit symptoms of CP. This concentration is just below the lowest concentrations seen in fish samples associated with CP cases (0.02 µg/kg CTX1B equivalents) (Farrell *et al.*, 2017; Hossen *et al.*, 2015).

7.5 DIETARY EXPOSURE TO CIGUATOXINS

Given the targeted nature of all of the studies summarized in Table 13 and the diversity of analytical methods and bases for analytical results, the available concentration data do not provide a suitable basis for nationally or internationally applicable acute dietary exposure assessment. Instead, a scenario-driven approach to dietary CTX exposure has been adopted. Table 15 provides estimates of acute dietary exposure based on four fish-serving sizes (100 g, 200 g, 500 g and 1 000 g) and four concentrations of CTX in fish. The particular CTX has not been specified; this is so that these scenarios can be fitted to a variety of situation.

TABLE 15 **SCENARIO-BASED ESTIMATION OF ACUTE DIETARY CTX EXPOSURE**

CTX1B CONCENTRATION (µg/kg)	HUMAN LOAEL[1] (pg/kg)	ESTIMATED DIETARY EXPOSURE (pg/kg bw) FOR SERVING SIZE (g)[2]			
		Mean consumer (g)		High consumer (g)	
		100	200	500	1 000
0.001	48.4	1.6	3.3	8.3	17
0.01		16	33	83	170
0.1		160	330	830	1 700
1.0		1 600	3 300	8 300	17 000

[1] LOAEL from the study of Hossen *et al.* (Hossen *et al.*, 2015).

[2] Based on a nominal 60 kg body weight.

Concentrations of ciguatoxicity in fish suspected to have caused CP as low as 0.02 µg/kg CTX1B equivalents have been reported (Farrell *et al.*, 2017; Hossen *et al.*, 2015). The minimum doses associated with CP cases from these two studies were determined using different analytical methods (LC-MS/MS and N2a, respectively). Depending on the rate of consumption of the fish, this level of toxin may equate to a dietary exposure of 33–340 pg/kg bw CTX1B equivalents for serving sizes of 100–1 000 g.

There is no evidence to suggest that the susceptibility of children to the effects of CTX is any different from that of adults. However, children consume food at a higher rate than adults in relation to their body weight, and this may increase their risk of CP from consumption of CTX-contaminated fish, compared with adults consuming flesh from the same fish.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CHAPTER 8
CONCLUSIONS

Ciguatera poisoning can be caused by consumption of several marine organisms, mainly fish, which have accumulated CTXs via the food chain in tropical waters. Abundance and distribution of CTX-producing organisms (*Gambierdiscus* and *Fukuyoa* species) can be modulated by multiple factors. These include eutrophication of water through excess nutrient input, damage to coral reefs or water temperature change, which may result in specific favouring environmental conditions in regions that had no prior history of *Gambierdiscus* growth. Taking into consideration the circumtropical distribution of ciguatera, international trade and export of potentially toxic fish, and tourist travel to endemic areas, ciguatera poisoning has become a global problem.

While, due to the existing data gaps, it was not possible to carry out and complete a full risk assessment, the Expert Meeting did outline the following considerations.

CTX classification

Taking into account the current knowledge of structural features as well as existing nomenclature of CTXs, the Expert Meeting suggested and used a classification of five groups: CTX3C, CTX4A, C-CTX, I-CTX (the existence of this group is still speculative as the structure elucidation is pending), and other *Gambierdiscus* metabolites.

Analytical challenges

Regarding the analytical determination of CTXs, the Expert Meeting identified the following major constraints: (i) there is no certified CTX standard; (ii) there is no reference material; (iii) there is no reference chemical protocol for CTX extraction in a biological matrix validated internationally; and (iv) there is no single- or multi- interlaboratory validation for any method, either LC-MS/MS, screening methods or bioassay. The current lack of reference materials makes it impossible to completely validate the analytical methods and, therefore, extremely difficult to compare analytical results between methods and/or laboratories. Moreover, all current screening methods/bioassays have been only incompletely validated. To date, LC-MS/MS is the only known confirmatory method, but it has also been only incompletely validated. According to current best analytical practices, the recommendation is to use screening methods prior to the use of LC-MS/MS. Among all the methods examined (LC-MS/MS, N2a, RBA, MBA and ELISA), the detection

methodology with the lowest limit of detection (LOD) is the neuroblastoma assay (N2a). The LOD of this assay is 0.01 µg/kg fish flesh when optimized; however, this LOD is not always achieved.

While researchers have made some progress using purified toxins that are being made available, investment of significant resources is required in order to develop commercial standards and ultimately certified reference materials for the major toxin forms. For this reason, criteria for such purified compounds should include sufficiently high purity, characterization of impurities, and broad and continued availability.

Purified toxin standards are required for at least the following: (i) of the group of CTX4A analogues, CTX4A, CTX4B, CTX1B (these three are critical), 52-*epi*-54-deoxyCTX1B and 54-deoxyCTX1B, M-seco-CTX4A/B; (ii) of the group of CTX3C analogues, CTX3C (critical), 49-epiCTX3C, 51-hydroxyCTX3C, 2-hydroxyCTX3C, 2,3-dihydroCTX3C, M-seco-CTX3C; and (iii) of the group of C-CTX analogues, C-CTX1 (critical), C-CTX2. Moreover, Indian Ocean CTXs are poorly understood, and research is required in order to determine their algal source, chemical structures, and toxicity. The high fatality rate associated with shark poisoning in the Indian Ocean region makes further investigation of the structure, toxicity and prevalence of these toxins a high priority.

The Expert Meeting also noted that, due to the lack of information on the genes responsible for CTX production by *Gambierdiscus*, it is not possible at present to use targeted molecular approaches to identify toxigenic species. Research on this topic is needed as it remains an important step to elucidate the specific genetic pathways responsible for toxin production by *Gambierdiscus* spp.

Hazard identification and ciguatoxin toxicity

Animal data show that CTXs are: (i) efficiently absorbed and distributed in the body after ingestion in laboratory animal models; and (ii) eliminated mainly through faeces in animals. Most pure CTX ADME animal studies involve Pacific CTXs and CTX1B in particular, and all have used activity-based detection methods to quantify toxins in tissues. On the other hand, the available human data show that: (i) ingested CTX is rapidly systemically distributed and mucosal absorption appears possible; (ii) CTX4A and B are metabolized *in vitro* by CYP3A4; and (iii) CTXs may cross the placenta and enter breast milk.

The toxicological studies performed to date show that, with regard to acute toxicity: (i) all tested CTX analogues from all regions are acutely toxic and induce common effects in mice; (ii) with the accepted extraction procedures, CTX extracts induced similar effects in mice to those from purified and synthesized substances; (iii) the limited data suggest that the effects observed are similar following ip and oral administration, and based on limited ip LD50 data the potencies of the different analogues within and between the different classes appears to vary; and (iv) the method used for extraction of the CTX (i.e. the first solvent) has to be taken into consideration. The acetone method is designed to eliminate interference with MTX, which is acetone-insoluble, and should be used.

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The results obtained with repeated dose studies show that there are very few oral studies in mice or rats, only using purified CTX1B (and in one study, purified CTX4C). Respective information is lacking for the other CTX compounds. The available studies include two short-term studies in mice, one medium-term study in rats (focused on neurological effects), and one long-term study in mice (focused on heart toxicity). None of these studies is suitable to establish a health-based guidance value (acute or chronic).

With regard to the mode of action, it is well accepted that the shared primary molecular mechanism of CTXs is the binding and the activation of the VGSC. This leads to an increase in membrane excitability, as well as to secondary cellular effects that are the basis of most human symptoms. In addition, CTXs block the VGPC, and this effect also contributes to increased membrane excitability. The potencies of CTXs in affecting these two channels vary according to CTX congeners.

The available information about TEFs suggests that the toxicity of all CTXs may not be covered by the *in vitro* bioassays and that *in vitro* bioassays have not yet been sufficiently validated for use in risk assessment. Based on the existing data, MBA is the only current *in vivo* assay that allows relative effect potencies to be determined; however, due to limited data from oral *in vivo* studies, it has not been possible to derive TEFs.

Clinical features and epidemiology of Ciguatera poisoning

The clinical features of CP can be classified into acute and chronic symptoms. Acute symptoms are observed in almost all organ systems and are qualitatively observed in all geographical CP regions. While it is unclear whether the frequency of all symptoms between various CP regions differs, fatalities have been reported in all regions. With regard to chronic symptoms, they are characterized by the presence of neurological, neuropsychiatric and systemic symptoms beyond three months after the initial poisoning. At least 20 percent of affected persons show chronic symptoms (and the prevalence can reach 100 percent after a single marine invertebrate CTX exposure), and a transient recurrence or exacerbation of neuromuscular or other systemic symptoms may appear due to certain food-based, behavioural or other external triggering factors. This phenomenon can be present from the early days of the individual poisoning and can last for months or years, and decrease in intensity and frequency over time.

The epidemiological data suggest that CP is vastly under-reported, limiting the understanding of its epidemiology. Considering the scale of affected countries, consolidated data on the global burden of CP are needed. It should be noted that there are no specific treatments for CP; hence, symptomatic treatments are being used. Mannitol is commonly administered quickly after the onset of neurological symptoms to manage CP. However, its efficacy in treating CP remains controversial. Traditional remedies have been reported; however, their efficacy remains to be demonstrated in clinical trials.

Exposure assessment

Available data do not provide a suitable basis for nationally or internationally applicable dietary exposure assessment. To date, none of the available CTX monitoring studies is suitable for exposure assessment. There is only one publication available providing CTX dose information using N2a assay, allowing the estimation of the exposure dose in cases and identification of a LOAEL in humans. For risk assessment, the acute exposure to CTX is the most relevant. However, the data on which the assessment is based are from endemic areas where multiple exposures cannot be excluded. It is important to highlight the fact that the animal and human data available are insufficient to derive an acute reference dose. Moreover, while human data allowed identification of a LOAEL of 50 pg/kg bw CTX1B (other studies report a higher LOAEL), the observed uncertainties do not allow the derivation of an acute reference dose. The lowest concentration measured in fish associated with symptoms in humans was determined at about 0.02 µg/kg CTX1B-equiv in fish flesh from the Caribbean. Depending on the rate of consumption of the fish, this level of toxin may equate to a dietary exposure of 33–340 pg/kg bw CTX1B equivalents for serving sizes of 100–1 000g. Children consume food at a higher rate than adults in relation to their body weight and this may increase their risk of CP from consumption of CTX-contaminated fish, compared with adults consuming flesh from the same fish.

8.1 RISK MANAGEMENT CONSIDERATIONS

Considering the currently existing scientific gaps, it was not possible to complete a full risk assessment. However, the Expert Meeting noted that, by drawing on existing knowledge as well as traditional practices from CP endemic areas, some risk management considerations could be given to inform regional interventions.

The Expert Meeting noted that effective and integrated risk management options would require definition of toxin profiles in each region, both in algal strains and in seafood to define risk evaluation protocols. Recognizing the strong influence of the regional and local circumstances on the occurrence of the organisms as well as the production of the toxins, any conclusions should be considered as of local or regional significance only, and care must be taken when transferring these to other areas. While the link between algal CTXs and those observed in fish has been established in the Pacific, evidence from the Caribbean and the Indian Ocean is still needed and warrants ongoing investigation.

The following considerations can provide assistance in the identification and management of CP.

Establishing and/or strengthening surveillance programmes

Causative organisms should be defined, if possible, in the areas where more-toxic species have been identified. It might also be useful to develop hazard maps for each region. The Expert Meeting identified the following combination of causative organisms and regions that may be useful in informing the establishment of surveillance programmes:

- *G. polynesiensis* (currently only known from the Pacific);
- *G. scabrosus* (currently found in Japan and Okinawa [Japan]);
- *G. excentricus* (currently known from the Atlantic and Caribbean);
- *G. silvae* (currently known from the Atlantic and Caribbean);
- *G. australes* (currently known from the Pacific, Atlantic and Mediterranean);

Currently, it cannot be excluded that other species may be significant contributors to the occurrence of CTXs. It would also be important to consider long-term studies for a comprehensive description of population dynamics of *Gambierdiscus* and *Fukuyoa*, in order to help address seasonality variations.

Sampling

It is necessary to establish a careful sampling design for *Gambierdiscus* and *Fukuyoa* to address identification of species, quantification of populations and eventually identification and quantification of toxins in natural populations. The sampling of the causative organism and sentinel fish species needs to consider the relatively low density of benthic organisms in the water and the temporal and spatial dispersions of *Gambierdiscus* and *Fukuyoa* as well as of fish species. The sampling of migratory

fish species may benefit from a better understanding of migratory patterns, and sampling plans may also be informed by the traditional knowledge of indigenous fishers. During the sampling step, it should be endured that enough samples are collected to make further analysis using SEM and qPCR techniques, which require different preservation methods.

The use of passive sampling should be considered for an extensive overview of toxins in water, including those toxins not directly involved in CP. This allows for further differential diagnosis in poisoning events – in particular, the co-exposure to multiple toxins is to be suspected in the presence of atypical poisoning cases.

Monitoring ciguatera transmission in the food web

Depending on the region of interest and habitat, sentinel invertebrate or fish species can be selected for biomonitoring. Because fishing habits differ based on sociocultural, historical, biodiversity and regional factors, a first step would include a survey of fish across multiple trophic levels (herbivore, mesopredator [including invertivores] and predator). The Expert Meeting identified a combination of marine species in specific regions that are known to exhibit high site fidelity (i.e. resident species that stay within a small area for their whole life) (Table 16).

TABLE 16 **MARINE SPECIES IN SPECIFIC REGIONS THAT ARE KNOWN TO EXHIBIT HIGH SITE FIDELITY**

COMMON NAME	LATIN NAME	REGION	TROPHIC LEVEL
Striated surgeonfish	*Ctenochaetus striatus*	French Polynesia	Herbivore
Ocean surgeon	*Acanthurus bahianus*	Caribbean	Herbivore
Spotted surgeonfish	*Ctenochaetus strigosus*	Hawaiian Islands	Herbivore
Moray eel	*Gymnothorax javanicus*	French Polynesia	Predator
Red-hind grouper or red hind	*Epinephelus guttatus*	Caribbean	Predator

Note: In some areas, these fish may be protected. Therefore, to avoid species depletion, advice should be sought from local fisheries ecologists regarding the selection and feasibility of collecting these fish for long-term sentinel monitoring.

Definition of key target species

There is large regional variation in the occurrence of CTXs in fish in general, including within the same species. There is inconclusive evidence that size or weight of fish is an indicator for the risk of CP in every region. If possible, the marine species found to contain CTXs and commonly consumed by humans should be defined by region. Epidemiology surveys can also aid in selection of target species that have been associated with illness, although caution needs to be taken to ensure correct species identification. Selection of species identified in such surveys should start with species that have high site fidelity. Moreover, there should be a set of audit/inspection schemes that provide suitably protective measures to allow trade from endemic areas (imported fish). In addition, it should also be noted that, while changes in the occurrence of CTX in migratory species have been observed, it is unclear to what extent they can be traced back to anthropogenic factors.

Therefore, it is extremely difficult to predict the risk of CP associated to migratory fish species. Traceability is an important issue in the identification of fish species, and, therefore, accurate seafood product labelling should also be improved.

Good practices

There is evidence that removing the viscera, liver and head of the fish prior to consumption will lower the risk of CP. In areas with a known high prevalence of CTXs in seafood, managers should consider the development of guidance that includes avoidance of visceral organs, roe (fish eggs), and carcasses (e.g. fish heads, eyes and bones), as these tissues can contain high levels of CTXs and other bioactive compounds that may pose a significant health risk. Moreover, good practices should provide guidance on the disposal of ciguatoxic fish, fish head and viscera.

Awareness raising and communication

Awareness raising is an important part of the risk management options, including the need for risk communication to be tailored to local situations with regard to the possible limitation of consumption and trade of specific marine species. Public outreach programmes need to inform consumers about the potential risks posed by consuming fish (including head and viscera) as well as invertebrates.

The increase in occurrence of ciguatera poisoning in certain regions suggests that surveillance, public outreach/education and further research are required also in areas where this is becoming an emerging issue.

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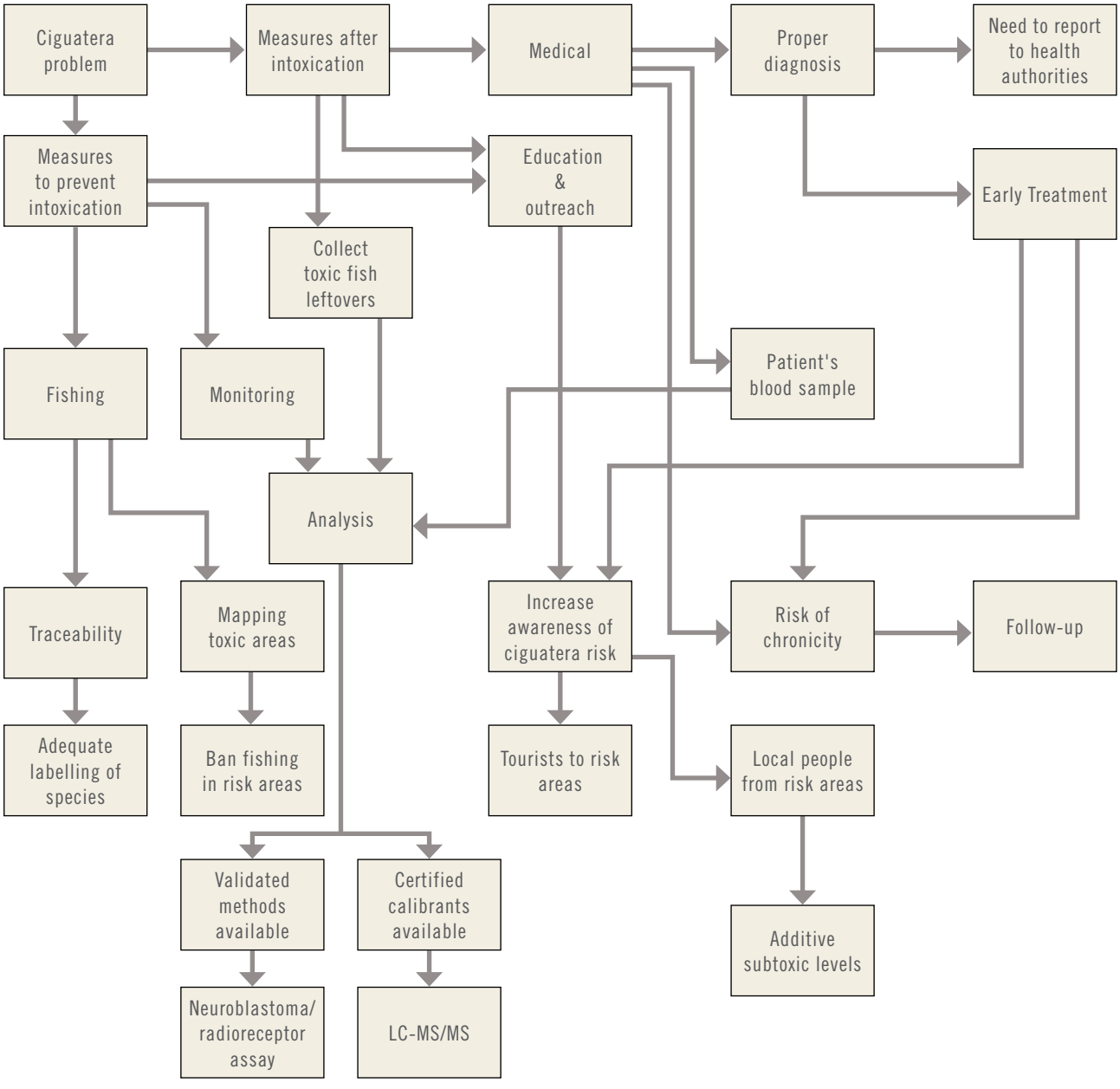
ANNEX

CP FLOW DIAGRAM, AND RESEARCH PROJECTS

FLOW OF CP RESPONSES AND NEEDS



RESEARCH PROJECTS

CIGUATOOLS

This project, already finished, was funded by the Research Executive Agency (FP7/2007-2013) under Grant Agreement 311765 between three academic partners and Cifga, a European ISO 17034 company for the production of certified marine toxins, to develop immunoassay-based methods and obtain certified standards. The result of the project was the availability of gambierone (Rodriguez *et al*., 2015) as potential certified calibration for ciguatoxin (CTX) analysis by LC-MS/MS. The antibody research part was not successful. A high-resolution mass spectrometry (HRMS) method was developed (Silva *et al*., 2015).

EUROCIGUA

The European Food Safety Authority (EFSA) is partner with several Member States' organizations in a multiannual project Risk Characterization of Ciguatera Food Poisoning (CP) in Europe (http://www.aecosan.msssi.gob.es/AECOSAN/web/ciguatera/home/aecosan_home_ciguatera.htm).

The project is cofunded by and 14 European organizations. The main objectives of the project are:

> The estimation of the incidence of ciguatera in Europe and the determination of the epidemiological characteristics of cases.

> The assessment of the presence of ciguatoxin in *Gambierdiscus* and in fish in Europe.

> The development and validation of methods for the detection, quantification and confirmation of the presence of ciguatoxin-contaminated specimens.

To accomplish the first objective, a surveillance protocol for CP in the European Union was created. The protocol includes: the definitions for ciguatera cases and outbreaks; the recommended public health measures for CP; and two specific questionnaires for ciguatera cases and outbreaks. Based on these questionnaires, a database for collecting the data was created. A list of possible data sources (at country and European level) for ciguatera cases and outbreaks was elaborated (http://www.aecosan.msssi.gob.es/AECOSAN/web/ciguatera/subseccion/documents_and_publications.htm).

Sampling and culturing of *Gambierdiscus* and sampling of fish in Europe is ongoing, together with the evaluation of the presence of CTX. The development, optimization and validation of LC-MS/MS and HRMS for identification and confirmation of CTX is also ongoing.

ALERTOXNET

This European Interreg project is designed to implement a network of emerging toxin warnings and characterization. The network includes 14 partners and, starting in October 2017, will run for three years.

In Spain, at the national level, there is a project with Cifga specifically devoted to the development of reference standards for early, reliable and specific detection of CTXs (Reference EMP-TU-2016-4878). The project started in September and will run for three years.

The GlobalHAB programme, funded by the Scientific Committee on Oceanic Research (NSF) and UNESCO Intergovernmental Oceanographic Commission, aims to promote coordinated research on harmful algal blooms (HABs), including CP. GlobalHAB focuses on the human health impacts of marine microalgae-produced toxins, ecophysiology and oceanic processes that modulate HAB dynamics, and ecology and epidemiology of HAB illnesses (Berdalet *et al*., 2017). As another example, a Global Burden of Disease CP study (e.g. http://www.healthdata.org/gbd) would allow for a better understanding of the extent and economic implications of CP, and changes in international CP distribution. The One Health approach (http://onehealthinitiative.com/) seeks cooperation among environmental and health disciplines in order to improve CP diagnosis, treatment and reporting worldwide.



REPORT OF THE EXPERT MEETING ON CIGUATERA POISONING

ROME, 19–23 NOVEMBER 2018

Ciguatera poisoning (CP) is one of the most common food-borne illnesses related to seafood consumption. While in some regions it has been known for centuries, its true incidence is not fully understood, with an estimation of 10 000–50 000 people affected every year. CP is caused by the consumption of marine species that have become toxic from feeding on toxic benthic dinoflagellates (*Gambierdicus toxicus*) or from the consumption of carnivorous marine species that have consumed other toxic species that have fed on the dinoflagellate. *Gambierdicus toxicus* is found primarily in the tropics and more than 400 aquatic species are known to be vectors of ciguatera. CP is predicted to become one of the increasing food safety threats due to climate change and globalization of trade, which might further contribute to its spread. This publication reports on the deliberations of an FAO/WHO expert group and provides a risk assessment of known ciguatoxins as well as guidance on the development of risk management options.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO)

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