

Marine biotoxins

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Foreword

FAO is publishing this Food and Nutrition Paper on Marine Biotoxins in an effort to support the exchange of scientific information on an important subject of concern for food safety worldwide. Marine biotoxins represent a significant and expanding threat to human health in many parts of the world. The impact is visible in terms of human poisoning or even death following the consumption of contaminated shellfish or fish, as well as mass killings of fish and shellfish, and the death of marine animals and birds.

This paper provides an extensive review of different aspects of five shellfish poisoning syndromes (paralytic shellfish poisoning, diarrhoeic shellfish poisoning, amnesic shellfish poisoning, neurologic shellfish poisoning, azaspiracid shellfish poisoning), as well as one fish poisoning syndrome (ciguatera fish poisoning). Various aspects of these poisoning syndromes are discussed in detail including the causative toxins produced by marine organisms, chemical structures and analytical methods of the toxins, habitat and occurrence of the toxin producing organisms, case studies and existing regulations. Based on this analysis, risk assessments are carried out for each of these different toxins, and recommendations elaborated to better manage these risks in order to reduce the harmful effect of these toxins on public health.

Work undertaken during this study has underlined the difficulties of performing a scientific-based risk assessment given the lack of data on the toxicology and exposure of diverse marine toxins. The allowance levels currently valid for phycotoxins are generally based on data derived from poisoning incidents in people. However, these data are seldom accurate and complete, and usually restricted to acute toxicity. Therefore, increased attention must be paid to expanding and improving initiatives to monitor, detect and share information on marine biotoxins in the future in order to reduce the public health risks associated with the consumption of contaminated shellfish and fish.

Abbreviations

AOAC	Association of Official Analytical Chemists
ASP	Amnesic shellfish poisoning
AZA	Azaspiracid
AZP	Azaspiracid shellfish poisoning
BRC	Bureau Communautaire de Référence
BTX	Brevetoxin analogues, metabolites formed in fish
bw	Body weight
CE	Capillary electrophoresis
CEN	European Committee for Standardization
CFP	Ciguatera fish poisoning
CID (= CAD)	Collision induced dissociation (= collision activated decomposition)
CRL	Community Reference Laboratory
CTX	Ciguatoxin
C-CTX	Caribbean ciguatoxin
P-CTX	Pacific ciguatoxin
CZE	Capillary zone electrophoresis
DA	Domoic acid
DAP	Domoic acid poisoning
DSP	Diarrhoeic shellfish poisoning
DTX	Dinophysistoxin
EC	European Commission
EIA	Enzyme immuno assay
ELISA	Enzyme-linked immunosorbent assay
ENSO	El Niño-Southern Oscillation
ESI	Electrospray ionisation
EU	European Union
FAB	Fast atom bombardment
FIA	Flow injection analysis
GC	Gas chromatography

GNTX	Gonyautoxin
GTX	Gambiertoxin
HILIC	Hydrophylic interaction liquid chromatography
HP	Hepatopancreas
IAC	Immuno affinity columns
ICES	International Council for the Exploration of the Sea
KB cells	A human cell line derived from epidermoid carcinoma
LC	Liquid chromatography
LC-FD	Liquid chromatography with fluorescence detection
LC-ISP-MS	Ion Spray LC-MS
LC-MS	Liquid chromatography with mass spectrometric detection
LC-UV	Liquid chromatography with ultra violet detection
LOAEL	Lowest observed adverse effect level
LOD	Limit of detection
MAB	Monoclonal antibodies
MEKC	Micellar electrokinetic capillary chromatography
MIA	Membrane immunobead assay
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MS ⁿ	Multiple tandem MS
MTX	Maitotoxin
MU	Mouse units
NMR	Nuclear magnetic resonance
NOAEL	No observed adverse effect level
N : P ratio	Nitrogen : phosphorus ratio
NRL	National Reference Laboratories
NSP	Neurologic shellfish poisoning
OA	Okadaic acid
PbTx	Brevetoxin
PSP	Paralytic shellfish poisoning

PTX	Pectenotoxin
PTX2SA	Pectenotoxin-2 seco acid
RIA	Radioimmunoassay
SAX-SPE	Strong anion exchange-solid phase extraction
SEC	Size exclusion chromatography
SIM (= SIR)	Selected ion monitoring
SIR (= SIM)	Selected ion recording
SMT	Standards, Measurements and Testing Programme
SPIA	Solid-phase immunobead assay
SRM	Selected reaction monitoring (an MS/MS technique that is similar to selected ion monitoring (SIM))
STX	Saxitoxin
STXOL	Saxitoxinol
TDI	Tolerable daily intake
TLC	Thin layer chromatography
TTX	Tetrodotoxin
UV	Ultraviolet (Detection)
Wideband activation	A type of resonance excitation (in ion trap MS) in which the RF voltage is applied to a mass window substantially wider than the parent ion window.
YTX	Yessotoxin

1. Introduction

Microscopic planktonic algae of the world's oceans are critical food for filter-feeding bivalve shellfish (oysters, mussels, scallops, clams) as well as for the larvae of commercially important crustaceans and finfish. Among the 5 000 existing marine algal species, approximately 300 can sometimes occur in such high numbers (blooming) that they obviously discolour the surface of the sea, the so-called "red tides" (Hallegraeff *et al.*, 1995; Lindahl, 1998). The word "bloom" is used to indicate the explosive growth of any of these organisms, which may vary in colour from the commonly cited red (so called "red tides") to different shades of yellow, green, brown or blue depending on the type of protista and their depth and concentration. The commonly used term "red tide" comes from the fact that a massive number of organisms often appear as red streaks across the surface of the water (Bower *et al.*, 1981). The conditions for an algal bloom are not yet fully elucidated but the phenomenon is probably influenced by climatic and hydrographic circumstances (Van Egmond and Speijers, 1999). The explosive growths sometimes appear during changes in weather conditions but important contributing causes may be variations in upwellings, temperature, transparency, turbulence or salinity of the water, the concentration of dissolved nutrients, wind or surface illumination (Bower *et al.*, 1981).

There are no reasons to assume that shellfish intoxication can be predicted by the properties of the regional area. In general, red tides often occur when heating or freshwater runoff creates a stratified surface layer above colder, nutrient-rich waters. Fast-growing algae quickly strip away nutrients in the upper layer, leaving nitrogen and phosphorus only below the interface of the layers, called the pycnocline. Non-motile algae cannot easily get to this layer whereas motile algae, such as the dinoflagellates, can thrive. Many swim at speeds in excess of 10 metres a day, and some undergo daily vertical migration; they reside in surface water like sunbathers and then swim down to the pycnocline to take up nutrients at night. As a result, blooms can suddenly appear in surface waters that are devoid of nutrients and seem incapable of supporting such prolific growth (Anderson, 1994).

Evidence is increasing from diverse areas (such as the Hong Kong Harbour, the Seto Inland Sea in Japan and North European coastal waters) that "cultural eutrophication" from domestic, industrial and agricultural wastes can stimulate harmful algal blooms. It is even possible that algal species which are normally not toxic may be rendered toxic when exposed to atypical nutrient regimes (e.g. phosphate deficiencies) resulting from cultural eutrophication. Changed patterns of land use, such as deforestation, can also cause shifts in phytoplankton species composition by increasing the concentrations of humic substances in land runoff. Acid precipitation can further increase the mobility of humic substances and trace metals in soils (Hallegraeff, 1993).

Some species produce basically harmless water discolorations. On the other hand, some species can bloom so densely, under exceptional conditions in sheltered bays, that they indiscriminately kill fish and invertebrates due to oxygen depletion. Other algal species can be harmful to fish and invertebrates (especially in intensive aquaculture systems) by damaging or clogging their gills. Furthermore, there are micro-algal species (about 75) which have the capacity to produce potent toxins (called phycotoxins) that can find their way through levels of the food chain (e.g. molluscs, crustaceans and finfish) and are ultimately consumed by humans causing a variety of gastrointestinal and neurological illnesses. Some algal species already produce toxins at low abundances of some hundreds of cells per litre, while other algal species must occur in some millions of cells per litre in order to cause any harm. Most of the harmful species have a restricted distribution pattern but some harmful species have a worldwide distribution (Hallegraeff *et al.*, 1995; Lindahl, 1998).

It is not clear why some micro-algal species produce toxins. These toxins are secondary metabolites with no explicit role in the internal economy of the organisms that produce them and with very specific activities in mammals. They are probably used by their producers as a way to compete for space, fight predation or as a defence against the overgrowth of other organisms (Botana *et al.*, 1996).

During the past two decades, the frequency, intensity and geographic distribution of harmful algal blooms has increased, along with the number of toxic compounds found in the marine food chain. Different explanations for this trend have been given such as increased scientific awareness of toxic algal species, increased utilization of coastal waters for aquaculture, transfer of shellfish stocks from one area to another, cultural eutrophication from domestic, industrial and agricultural wastes, increased mobility of humic substances and trace metals from soil due to deforestation and/or by acid precipitation (acid rain), and unusual climatic conditions (Hallegraeff *et al.*, 1995). In addition, monitoring for toxic algae and/or (shell)fish is now carried out in several coastal areas of the world. Figures 1.1 and 1.2 illustrate monitoring in coastal waters of European and North American countries in the International Council for the Exploration of the Sea (ICES).¹ The transportation of dinoflagellate resting cysts, especially from paralytic shellfish poisoning toxin producers (McMinn *et al.*, 1997), either in a ship's ballast water or through the movement of shellfish stocks from one area to another provides another possible explanation for the increasing trend of harmful algal blooms (Hallegraeff *et al.*, 1995).

The resting cyst or hypnozygote is the immobile form of some dinoflagellates. These cysts sink to the bottom of the sea and accumulate at the borderline of water and sediment where they overwinter. When favourable growth conditions return, the cysts may germinate and reinoculate the water with swimming cells that can subsequently bloom. In this way the survival of certain dinoflagellates from one season to the other season is assured (Mons *et al.*, 1998).

Exchanges in mid-ocean of a ship's ballast water that is derived from the open harbour, with ballast water from the open ocean can be partly effective in controlling not only cysts but also the harmful dinoflagellates and diatoms themselves. Incomplete elimination of harmful organisms is caused by the incomplete discharge of water and sediments in the ballast tank during reballasting (Zhang and Dickman, 1999). However, mid-water exchange within regional seas (for example the North Sea, Irish Sea or English Channel) is less efficient than within oceanic waters. Mid-water exchange in regional seas may reduce the risk from polluted European harbour waters but may result in the transportation of potentially harmful phytoplankton species from the regional seas (Macdonald and Davidson, 1998)

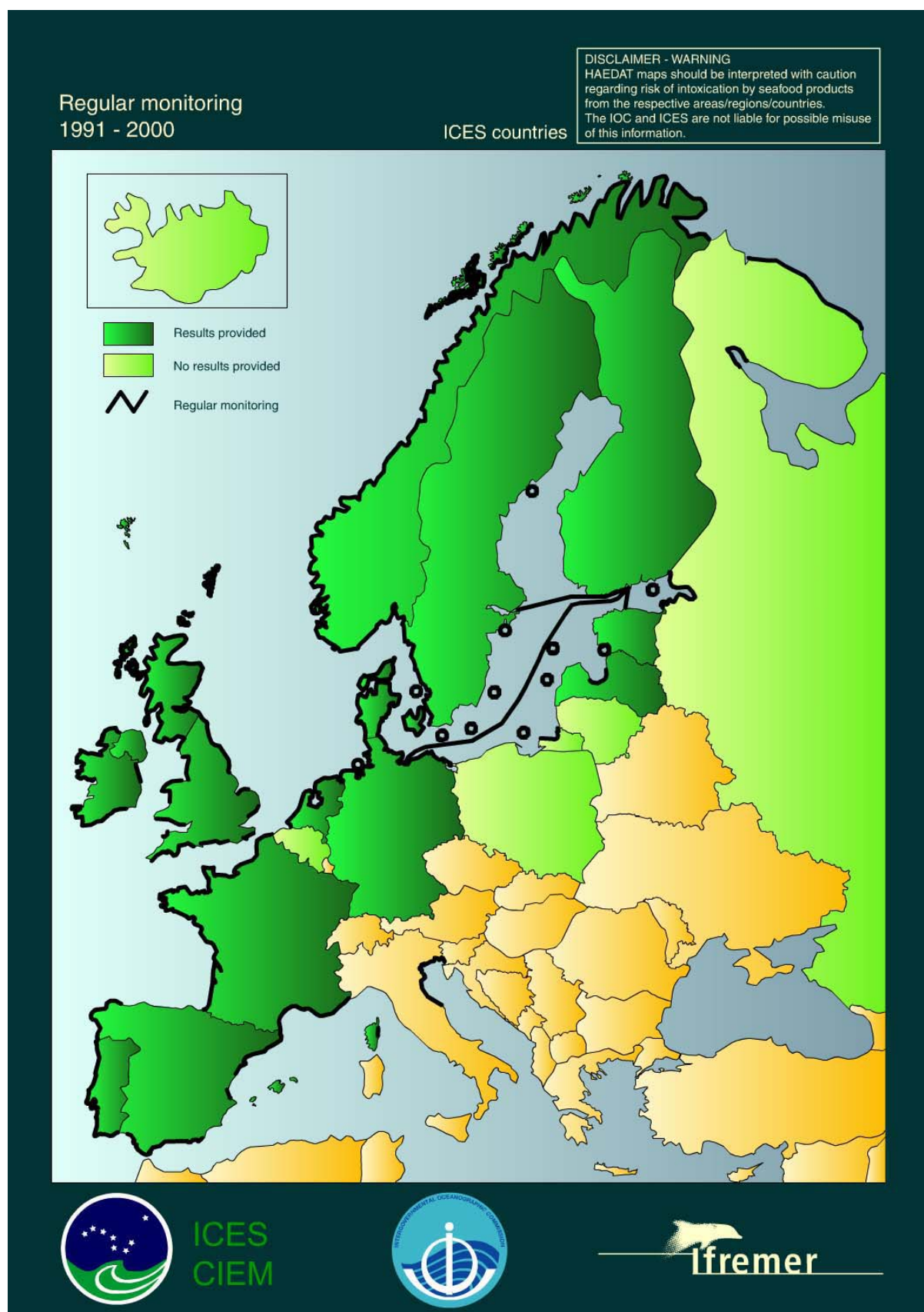
The most important marine phycotoxins are shellfish toxins and ciguatoxins. Until now, five groups of shellfish toxins have been distinguished, namely:

- i. paralytic shellfish toxins causing paralytic shellfish poisoning (PSP);
- ii. diarrhoeic shellfish toxins causing diarrhoeic shellfish poisoning (DSP);
- iii. amnesic shellfish toxins causing amnesic shellfish poisoning (ASP);
- iv. neurotoxic shellfish toxins causing neurotoxic shellfish poisoning (NSP); and
- v. azaspiracid shellfish toxins causing azaspiracid shellfish poisoning (AZP) (Hallegraeff *et al.*, 1995; Lindahl, 1998).

Ciguatoxins cause ciguatera fish poisoning (CFP). PSP, DSP, ASP, NSP and AZP are caused by human consumption of contaminated shellfish products whereas CFP is caused by the consumption of subtropical and tropical marine carnivorous fish that have accumulated ciguatera toxins through the marine food chain. Various aspects of these toxins will be reviewed in this publication.

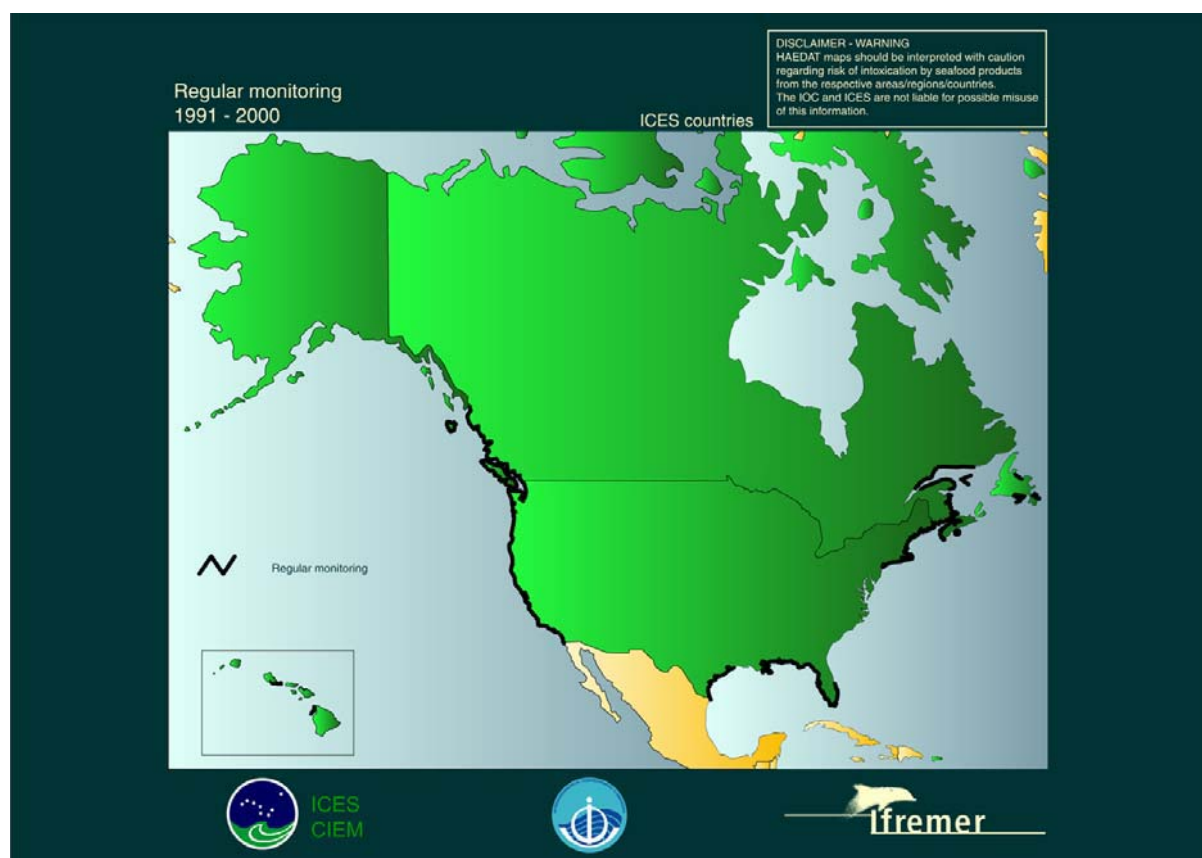
¹ Source: www.ifremer.fr/envlit/documentation/dossiers/ciem/aindex.htm

Figure 1.1 Monitoring of coastal waters in European ICES countries for toxic algae and/or shellfish from 1991 to 2000



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

Figure 1.2 Monitoring of coastal waters in North American ICES countries for toxic algae and/or shellfish from 1991 to 2000



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