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Ioannis S. Arvanitoyannis [a], Konstantinos V. Kotsanopoulos [a] & Anna Papadopoulou [a]
[a] Department of Ichthyology and Aquatic Environment, School of Agricultural Sciences, University of Thessaly, Fytokou Str, 38446, 38446 Nea Ionia Magnessias, Volos, Hellas, Greece Phone: +30 24210 93104 Fax: +30 24210 93104
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Ioannis S. Arvanitoyannis*, Konstantinos V. Kotsanopoulos and Anna Papadopoulou

Department of Ichthyology and Aquatic Environment

School of Agricultural Sciences

University of Thessaly

Fytokou Str, 38446

38446 Nea Ionia Magnessias

Volos, Hellas (Greece)

Tel.: +30 24210 93104

Fax: +30 24210 93137

e-mail: parmenion@uth.gr

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*whom all correspondence should be addressed to

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Abstract

Among the various hazards occurring in fish and seafood chemical hazards and in particular toxins [ciguatera, scombroid fish poisoning (SFP), paralytic shellfish poisoning (PSP), neurotoxic (brevetoxic) shellfish poisoning (NSP), puffer fish poisoning (PFP), diarrhetic shellfish poisoning (DSP)] have an important place in food poisoning cases. On the other hand,

some of the chemical hazards are often due to the pollution of the environment [heavy metals, dioxins, polychlorinated biphenyls (PCBs) and Halogenated aromatic hydrocarbons (HAHs)] and their detection is neither rapid nor facile. As a result there was a great need for developing new rapid and effective methods towards the chemical hazards determination mainly because of their high toxicity. The aim of this review is to provide the information about the new up-to-date detection techniques (Immunological, Chemical & Biochemical, Molecular assays) in conjunction with detection limits. The latter is made possible by means of inclusion of seven comprehensive and, in most case cases, much extended tables. A reference is also made on risk characterization of toxins in view of their importance with regard to food contamination or poisoning.

Keywords: toxins, chemical hazards, detection methods, detection limits, immunochemical, molecular assays

1. Introduction

Fish and shellfish are among the most important foods for the human dietary balance. However, accumulation of toxins in these foods is a major problem because it can easily lead to food-poisoning. (Garthwaite, 2000). Seafood is a very important food because of its high proteinic level as well as its rich content in beneficial lipids. Specifically, the long chain omega-3 fatty acids are widely known for their nutritional value (Moon & Choi, 2008). It has been shown that fishes contain a large amount of nutrients such as proteins, vitamins, traces, decreased concentrations of saturated fats and increased levels of omega-3

polyunsaturated fatty acids. It is thought that among other nutritional advantages, the latter contribute significantly to the prevention of coronary heart disease (Domingo & Bocio, 2007).

For hundreds of years people have been aware that consumption of fish and shellfish can cause food-poisoning and can even lead to death. The nature of these food-borne incidents has been chemically and biologically examined only over the last 70 years (Luckas, 1992). Filter-feeding mollusks such as clams, oysters and mussels as well as several fishes can produce toxic substances for humans during the presence of "red tides". The latter are formed due to the rapid development of specific algae species, which are frequently harmless. However, some of these species can form dangerous toxic substances that, transmitted throughout the food chain, can negatively affect or even cause the death of zooplankton, shellfish or humans that consume these products either directly or indirectly (Palleschi et al., 2000).

The most important diseases caused by finfish and shellfish in the US are among others the ciguatera disease, scombroid fish poisoning (SFP), paralytic shellfish poisoning (PSP), neurotoxic (brevetoxin) shellfish poisoning (NSP), and puffer fish poisoning (PFP), diarrhetic shellfish poisoning (DSP), and amnesic shellfish poisoning (ASP). Symptoms appear after consumption of tissues contaminated with toxic substances capable of maintaining their chemical structure at normal cooking temperatures. The presence of these substances can not be identified by organoleptic tests (Ahmed, 1991).

Toxicity level is usually assessed with the mouse bioassay (MBA). During this procedure, a sample of the shellfish is inserted by injection into mice. Death of mice indicates the presence of toxins (Garthwaite, 2000). MBA is widely used in the seafood industrial field, but several issues of ethical background remain unsolved (e.g. experiments on animals) (Humpage *et*

al, 2007). Innovative analytical techniques frequently applied include High Performance Liquid Chromatography (HPLC), Liquid Chromatography - Mass Pectrometry (LC-MS), immunoassay, cellular bioassays and molecular probes. These methods significantly contribute to the control of the procedure and the detection of the environmental conditions and fishes related to toxins' formation. These innovative techniques can be achieved in shorter periods of time. They are also more accurate and are not distinguished by any ethical constraints (Garthwaite, 2000).

Several experiments and epidemiological data indicate that marine pollutants can be responsible for the cause of toxicity in exposed organisms such as neurotoxicity and immune dysfunction. A few substances (e.g. algal toxins) are mainly characterized by high levels of toxicity, while others, such as dioxins and polychlorinated biphenyls (PCBs), are also very harmful because they lead to chronic effects derived from exposure at low levels of the toxin for long periods of time (Hahn, 2002). The hazards arising from the exposure to seafood that contain numerous microbial factors, parasites and natural toxic substances are common in all countries and should be assessed at a global level.

2. SEAFOOD TOXINS

2.1.1. Natural toxins

Unicellular algae particles (mainly 20 to 200 mm size) consist a significant part of the plankton consumed by mussels, oysters and scallops. The prevalence of appropriate environmental conditions and the maintenance of several parameters such as light, temperature, salinity, water column stability and nutrients, at suitable levels can lead to excessive growth of only a few

algae species that can reach to millions of cells / litre causing discolourness of the seawater. It is believed that only a small percentage of dinoflagellate species (about 30 from 2000) form toxic substances leading to human toxicosis after consumption of fish or shellfish (http://www.foodstandards.gov.au/_srcfiles/TR14.pdf).

Numerous neurogical gastrointestinal, and cardiovascular syndromes can occur due to exposure to toxins. Many of these syndromes can lead to death or long-term morbidity (Sobel & Painter, 2005). One of the basic shellfish poisonings is PSP which can rarely be mortal. It seems to cause respiratory paralysis. Other significant poisonings include DSP which leads to acute gastrointestinal symptoms and can result in the formation of stomach tumors, NSP which leads to respiratory problems, and ASP which can even cause irreversible brain damage accompanied with short-term memory loss (http://www.foodstandards.gov.au/_srcfiles/TR14.pdf). HPLC techniques can be applied to detect and measure PSP toxins in shellfish. PSP toxins are characterized by a weak naturally derived chromophore and modification is essential before the application of the method. After oxidation in alkaline solution, a purine is developed, which behaves as fluorescent in acidic solution. The purines are assessed by fluorescence detection (Ben-Gigirey & Villar-Gonzalez, 2008).

2.1.2. NSP

NSP causes incoordination, paralysis and convulsions and appears after exposure to the lipid soluble brevetoxins (BpTX) (Garthwaite, 2000). BpTX is a group of neurotoxins formed by marine algae such as *Karenia brevis* and act

cumulatively throughout the marine food chain, leading to toxicosis of marine animals and people exposed to seafood (Selwood et al., 2008). BpTX belong to polyether ladder toxins. Numerous of these lipid-soluble cyclic polyether substances have been fully examined, but due to their chemically unstable nature, their analysis is still quite incomplete (http://www.foodstandards.gov.au/_srcfiles/TR14.pdf). *Gymnodiniuim breve* (=*Ptychodiscus brevis*) is responsible for the formation of these toxic substances. It is proved that the latter are subject to modification by shellfish. National regulations define the maximum permitted level (MPL) of these compounds at 80μg brevetoxin/ 100 g flesh (or 20 standard mouse units/ 100g flesh) (Garthwaite, 2000). The excessive growth of *Karenia brevis* (*Gymnodiniuim breve*) leads to the formation of increased levels of BpTX in seawater, which can have direct effects on fish, birds and marine mammals, resulting in generalized epizootic incidents. BpTX' consumption causes toxicity even at significantly decreased levels. As a result, it is obvious that the levels of these substances should be analytically assessed with sensitive and accurate techniques in order to find the substances at sub symptomatic stages (Naar, 2002). NSP results in a variety of gastrointestinal and neurogical problems such as nausea and vomiting, paresthesias of the mouth, lips and tongue as well as distal paresthesias, ataxia, slurred speech and dizziness. Among the most important neurogical symptoms, partial paralysis and respiratory distress have been repeatedly observed (Watkins et al., 2008).

2.1.3. DSP

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DSP occurs after exposure to okadaic acid (OA) and analogues, such as the lipid-soluble dinophysis toxins DTX-(1-3) (Garthwaite, 2000). Consumption of mussels, scallops, or clams fed mainly with *Dinophysis fortii* or *D. acuminata* and other species of *Dinophysis* and possibly *Prorocentrum*, can result in DSP (Ahmed, 1991).

The basic indications of exposure are diarrhea incidents, and the disease can be characterized as self-limiting. In contrast with diarrhea caused by bacteria, indications frequently start within 30 min to a few hours after consuming shellfish that contain the substances (Garthwaite, 2000). DSP toxins can be categorized into three different groups depending on their carbon skeleton (Fremy et al., 1999). According to several studies, these groups are: okadaic acid groups and DX-toxins (dinophysistoxins), pectenotoxins group (PTXs) (e.g. PTX 1-7), and yessotoxins (YTXs) such as yessotoxin and homoyessotoxin (Alfonso *et al*, 2004, Ciminiello *et al*, 2007, Fremy *et al*, 1999, Rossini, 2005) and azaspiracids (AZAs) (Krock *et al*, 2008, Stobo *et al*, 2005). Furey *et al* (2002) and James *et al* (2002) reported that Azaspiracis (AZA), a toxic substance, which has recently detected leads to azaspiracid poisoning (AZP) after human exposure to contaminated shellfish. OA toxins and PTXs are formed by dinoflagellates such as *Dinophysis* and *Prorocentrum* (Fremy et al., 1999), while *Protoceratium reticulatum* and *Lingulodinium polyedrum* have been connected to the formation of YTXs (http://www.foodstandards.gov.au/_srcfiles/TR14.pdf).

2.1.4. PSP

PSP can cause severe health problems basically characterized by neurological disorders (Lehane, 2001). It basically occurs after exposure to PSP toxins through ingestion of bivalves fed with toxin-producing dinoflagellate. (Okumura *et al*, 2005).

Shellfish and specifically mussels, clams, oysters, and scallops feed on dinoflagellate microorganisms and their consumption can cause the appearance of PSP syndrome. A toxic substance defined as saxitoxin (STX) is the causal agent of this disease (Watters, 1995). PSP is a very severe intoxication, which can easily be mortal.

Numerous PSPs such as STXs, gonyautoxins (GTXs) C-toxins (Humpage *et al*, 2007) and connected substances (neosaxitoxin - NeoSTX) and decarbamoylsaxitoxin (dcSTX) were analyzed and named (Louzao *et al*, 2001).

STX is formed by many Gonyaulax species such as *Gonyaulax catenella*, *G. tanrarensis*, *G. excavata*, *G. polyedra*, *G. monilata*, *Gymnodinium breve*, *G. veneficum*, *G. splendens* (Bates *et al*, 1978). *Aphanizomenon* sp. is a group of cyanobacteria living in fresh water and considered responsible for the formation of PSP (Humpage *et al*, 2007).

The hazards related to this kind of toxins have led to the requirement for new analytical methods such as several biological, physicochemical, and immunochemical tests (Usleber *et al*, 2001). The disease can only affect the nervous system (Sobel & Painter, 2005) and causes numbness, tingling and burning of the lips and skin, giddiness, ataxia and fever. Due to the lack of particular antidote, only symptomatic therapy can be followed (Garthwaite, 2000).

2.1.5. ASP

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The domoic acid (DA) is a water-soluble toxic substance that leads to ASP (Garthwaite, 2000). It has been detected in several varieties of the diatom *Nitzschia pungena* and concentrated in mussels and clams in Atlantic Canada mainly during the excessive growth of the microorganism population (Ahmed, 1991). Furthermore, *Chondriu aurmutund* was firstly identified as producer organism of DA. DA can cause very acute neurotoxicosis and can be categorized to a group of excitatory neurotransmitting toxins (Wright, 1995).

The symptomatology of the disease can be summarized to loss of balance, nausea, headache, disorientation, and vomiting incidents. It can finally lead to irreversible degradation of short-term memory (Garthwaite, 2000). Figure 1 represents the effects of depuration time on domoic acid contents of mussels (*Mytilus galloprovincialis*) and accumulation of the same toxin in tissues of mussels (*Mytilus edulis*) exposed for 4 days to toxic *Pseudo-nitzschia multiseries*.

2.1.6. SFP or HFP

Scombroid intoxication can be caused by consumption of fish contaminated with increased quantities of free histamine. The illness can be defined as histamine fish poisoning (HFP) (or SFP). It is proved that SFP was connected with ingestion of scombroids such as tuna, mackerel, bonito, and saury. Also different groups of fishes have been detected to contain the responsible toxin. Some of them are: mahi-mahi (dolphin fish), bluefish, jack mackerel, amberjack, skipjack, herring, sardine,

and anchovy. SFP is highly related to symptomatology of gastrointestinal, neurological, hemodynamic, and coetaneous nature (Ahmed, 1991).

Histamine is a toxic substance that belongs to biogenic amines. Putrescine and cadaverine are also included in this group of chemical substances. Histamine toxin is formed in the fish flesh by decarboxylating free histidine by bacteria that have the enzyme histidine decarboxylase. Histamine formation can be optimally performed at 25°C (Kerr *et al*, 2002). Free histidine, which is necessary for the formation of the toxin, can be detected in increased quantities in groups of fishes connected to SFP (Ahmed, 1991).

Histidine decarboxylase enzyme is formed by particular species of bacteria naturally found on fishes. According to Taylor et al. (1979), a histamine-producing strain of *Klebsiella pneumonia* was detected in tuna sashimi associated with an incident of SFP.

2.1.7. PFP

Tetrodoxin (TTX) is known for its increased neurotoxicity. It has been detected in puffer fish, and its ingestion is quite dangerous for people who do not take preventive measures before consumption of these fishes. It is also very interesting from a scientific point of view due to its particular chemical structure (Nakamura et al., 1984). The accumulation of the toxin takes place mainly in the ovaries and liver of numerous species of Tetraodontidae fish. It has a direct effect on the nervous system, leading to paralysis if inserted in the organism through the oral cavity, or by subcutaneous route. Several articles deal with the

origin of the toxin in marine organisms (Simidu et al., 1987). The same toxin has been also detected in specific species of octopus (Hokoma, 1988). PSP can occur after consumption of bivalve mussels (mussels, clams, oysters and scallops) fed with dinoflagellates that form toxins. Due to its high toxicity the toxin can lead to death and as a result is considered as extremely poisonous.

Symptomatology includes neurological disorders that can mainly occur after consuming contaminated shellfish. In nonlethal incidents, symptoms last for several days. Among symptoms, tingling, numbness and burning of the lips and fingertips; ataxia; giddiness; staggering; drowsiness; dry throat and skin; incoherence; dysphasia; aphasia; rash and fever are the most frequently observed (Ahmed, 1991).

2.1.8. CFP

Ciguaterra poisoning (CFP) is caused by ingestion of a phycotoxin formed by the benthic algae *Gambierdiscus toxicus*. The toxin can generally be ingested by humans through consumption of fin-fish, clams and marine snails that contain the substance. The lipophilic ciguatoxins (CTX) are characterized by high toxicity and present symptoms related to NSP toxins, even though CFP incidents are considered more acute, causing intense vomiting and diarrhea (Garthwaite, 2000).

The chemical formula of CTX can be described as $C_{60}H_{88}O_{19}$ and is a polyether that belongs to PbTX. Its toxicity is estimated to be 100 times higher than that of tetrodotoxin (TTX) (Ahmed, 1991). CFP is most frequently detected in tropics and subtropics and consists a major hazard for humans. It is related to the ingestion of CTX, which is a low Dalton lipid

polyesther (Hokama et al., 1987). It usually causes alternating feeling of hot and cold, muscular aches, tingling and numbness of lips, tongue and perioral region. Symptomatology intensity can be very high leading to inability of performing basic movements for long periods of time (Ahmed, 1991).

2.1.9. HFP

Hallucinogenic fish poisoning (HFP) or "dreamfish poisoning" is among the most well-examined diseases in Hawaii. It can be the result of the ingestion of specific mullet, goatfish, surmullet, manini tangs, and nenuerudder fish and can only be detected at specific periods during the year (e.g. during summer). Hallucinations, insomnia, intense nightmares, weakness, circumferential chest pain, and burning of the throat are quickly observed after consumption of contaminated fishes (Ahmed, 1991). It is important to mention that the abbreviation HFP can be referred to both "histamine fish poisoning" and "hallucinogenic fish poisoning" which should not be correlated. **Table 1.1** summarizes the natural toxins, their description and their occurrence.

2.2. Chemical toxins

It is proved that numerous toxins can be found in oceans worldwide. Both natural existent compounds and substances derived from human processes can present toxic properties. Among the most well known toxins, inorganic and organic metals, petroleum and combustion derived hydrocarbons, toxic substances formed by marine animals, chlorinated pesticides and

halogenated aromatic hydrocarbons (HAHs) are the most important. These substances can be detected inside sediments, dissolved in water, in the sea surface microlayer or isolated from numerous fishes and shellfishes such as different organisms consumed by humans or animals (Hahn, 2002).

As regards chemical substances (e.g. methyl mercury and PCBs), consumption of marine species is the main cause of human intoxication (Bordajandi et al., 2006; Lana et al., 2008). HAHs are of high scientific concern because of their massive production, their capability of remaining in the environment for long periods of time, and the high toxicity level of several of their compounds (congeners) (Hahn, 2002). The most important HAHs are the polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polyhalogenated biphenyls (PCBs and PBBs), polyhalogenated biphenyl ethers (PCDEs and PBDEs) and Hexachlorobenzene (HCB) derived from chlorine gas and pesticide formation (Opheim, 2004). Mercury (Hg) is categorized among the most toxic substances found in the environment (Reyes et al., 2008). It can be detected in several chemical forms. Organically derived sources of Hg (e.g. methylmercury) are frequently detected in fishes and shellfishes and their toxicity is much more potent than that of the inorganic sources (Kuballa et al., 2008). After ingestion, Hg ban be rapidly inserted in the circulatory system of humans and animals (Moore, 2000).

The main heavy metals derived from industrial procedures and released to the environment are Mercury, Lead, Chromium, and Arsenic. Quantities of heavy metals also exist naturally in the environment (Opheim, 2004). Furthermore, a large variety of HAH isomers and congeners of various numbers and points of their halogen substituents' have been detected and analyzed for their toxicity. These numerous substances and their numerous levels of toxicity as well as their effect on

microorganisms makes the assessment of the existent hazards very difficult (Hahn, 2002). The European Food Safety Authority (EFSA) has recently developed a hazard evaluation procedure for Hg contamination in marine animals (Kuballa et al., 2008).

PBDEs are usually applied for the production of polymers used to electronic devices. According to Wu et al. (2008), PCBs were historically used as coolants and lubricants in transformers and capacitors and as hydraulic and heat exchange fluids in electrical/ electronic equipment (EEEs)". It has been repeatedly proved that organisms can accumulate increased amounts of particular organic toxins, directly related to the amounts of these toxins in each region. Animals feeding in water can usually accumulate high concentrations of these substances in their bodies through the consumption of contaminated food and their exposure to toxins. The above fact is usually defined as bioconcentration, bioaccumulation or biomagnification. The examination of these bioconcentration compounds is very important especially when their concentration increases over time (Mackay & Fraser, 2000).

Aquatic Biocide Tributyltin (TBT) is also included among the most usual toxic substances related to the environment and there are several articles that cite a vast number of negative impacts on production, development and longevity of many marine animals (Burton *et al*, 2002).

Numerous methods have been developed for the measurement of toxic contents in the marine environment. Among them, gas chromatography using electron capture or mass spectrometry is frequently applied giving very accurate and reliable results (Hahn, 2002).

Many pesticides such as DDT, toxaphene, chlordane, lindane and HCH (Opheim, 2004) as well as several resins and additives, anti-oxidants, and other chemical substances were applied by industries during the last decades. Many of these compounds were included in the National Priorities List (NPL) site in 1984 after lindane detection in groundwater and DDT detection in soil. It was proved that DDT and its primary metabolites, dichlorodiphenyl-dichloroethane (DDD) and dichlorodiphenyl-chloroethane (DDE) were extremely harmful for marine animals (Hinck *et al*, 2008).

Many alterations of biochemical nature or particular "biomarkers" have widely used during the last 20 years as methods for detecting toxins. This trend was the result of growing knowledge about toxins. One of the most well evaluated biomarker used to detect dioxins and related chemical substances, is the inducted cytochrome P450 1A (CYP1A). Furthermore, numerous in vitro bioassays can be used to detect the existence of dioxin-like substances (Hahn, 2002).

Several methods such as gas chromatography (GC), HPLC including atomic fluorescence spectrometry, atomic emission spectrometry, atomic absorption spectrometry and inductively coupled plasma mass spectrometry (ICP-MS), are frequently used to analytically determine different mercury types (Reyes *et al*, 2008). Many articles indicate the negative results on humans derived from insertion of toxins into the human body. These negative results can be basically summarized to weakness of immune system, damages on reproductive organs and the presence of different incidents of cancer. The toxicity degree is highly related to several agents such as toxin quantity, exposure parameters (period of exposure, frequency of ingestion) as well as age and health (Opheim, 2004).

It is proved that many toxic substances can be found in several marine animals used as human food. The assessment of toxins' concentrations and their distribution over time and different regions is essential for ensuring the safety of consumers. Although chemical techniques are very accurate, they can be characterized by high cost of implementation and cannot be used to elicit large bulks of information on biological activity of each substance. Further investigation on seafood toxins is required for the minimization of the related hazards (Hahn, 2002).

A brief description of chemicals and their occurrence in seafood is given in **Tables 2.1** and **2.2**.

3. Rapid detection method of seafood toxins

3.1. Bioassays methods

Biological methodology is related with the examination of the potential effects of toxic substances on animals and cells. The MBA was firstly employed for sanitary assessment of shellfish. The method is applicable to acetone abruption of mussel digestive gland (Fremy et al., 1999). Bates et al. (1978) counted the toxic effects of several toxin-producing dinoflagellates by applying mouse and fish bioassays and a chemically based test for STX. The dinoflagellates, derived from natural blooms. Detection of STX was not possible in any sample of dinoflagellates except *Gonyaulax catenella* and *Gonyaulax excavata*. More toxic substances were detected in *Gyrnrwdinium brave*, *Anrphidlniunr rnrrnl*, *Gonyaulax excavata* and *GonyaWax*

catenella by applying fish and mouse bioassays. The majority of dinoflagellates were found to contain no toxins. Finally, a *Mytilus edulis* (blue mussels) sample caught in Spain was detected to be rich in saxitoxin.

According to Nicholson et al. (2002), the mouse synaptoneurosome assay is capable of measuring toxic substances that interact with site 5 on the voltage-gated sodium channel (e.g. brevetoxins and ciguatera toxic substances), which lead to NSP. Standard STX was shown to be a very effective inhibitor of the membrane depolarization of the sodium channel activator veratridine. Furthermore, inhibition of veratridine-induced depolarization was evident in samples contaminated with PSP toxins. It was finally demonstrated that the mouse synaptoneurosome assay is as sensitive as the official CD1 mouse assay for measuring toxicity.

Nowadays, bioassays are widely used to evaluate the toxicity of DSP and PSP on cultivated cells that can be used instead of rodent bioassays. According to Manger et al. (2003), a PSP cell bioassay was formed after modification. The method was proved to be as quick as in vitro techniques for sodium-channel-enhancing toxins and was developed in the basis of a STX-dependent antagonism of the quick in vitro impacts of two different toxins (PbTX or CTX). After examining both normally formed PSP residues using antagonism cell bioassay and MBA, it was proved that they were highly correlated.

Okumura et al. (2005) developed a new technique for detecting PSP by applying neuroblastoma cell culture with MTX as a Ca^{2+} agonist to the cell membrane. Maitotoxin (toxin connected with the reduction of incubation time to 6 h) and WST-8 (a dehydrogenase which is applied to determine the existence of tetrazolium salt) were used. After the HPLC analysis, it was determined that the amounts (μM) of GTX_1, GTX_2, GTX_3 and GTX_4 were 47.8, 31.5, 6.75 and 8.70, respectively. The mean

total toxicity was estimated at 172.4 MU equiv./ml, while the corresponding toxicity calculated after mouse bioassay was about 175.3 MU equiv./ml. It is therefore obvious that the two results ranged at similar levels.

According to Kerr et al. (1999,) the in vitro rat hippocampal slice can be used in order to effectively determine marine algal toxic substances such as saxitoxin, brevetoxin, and domoic acid. A particular electrophysiological signature was also recorded for each toxic substance. PbTX (PbTX3, 50-200 nM) reduced significantly the orthodromic population spike amplitude, while antidromic population spikes and field EPSPs were slightly limited. DA (100 nM) presented a huge, enhancement in amplitude mic spikes that could be reversed. Field EPSP and fiber spike examination proved that the method is suitable for determining toxin levels that range among 25-200 nM. It was concluded that the in vitro prepared hippocampal slice can be effectively used for determining and analyzing STX, PbTX, and DA.

Humpage et al. (2007) examined a neuro-2A cell-related assay for the assessment of the toxic effects of PSPs derived from freshwater cyanobacteria. To better evaluate the method, the neuroblastoma assay was achieved in different experiments in Paris (France) and Adelaide (Australia). Similar results were extracted. It was demonstrated that the method was effective on determining STX neurotoxins in freshwater cyanobacteria, and could be also effectively used for the identification of particular toxic substances that cannot be assessed through chromatographic methods.

Shimojo & Iwaoka (2000) examined a quick hemolysis assay aiming at reporting sodium channel-particular toxic substances presented in the marine environment. The technique was highly connected to the mouse neuroblastoma sample culture assay for sodium channel particular toxins using red blood cells (RBCs) from the red tilapia (*Sarotherodon*

mossambicus). The above technique has the advantage of optimizing the implementation of live animal bioassay testing for toxic compounds. Veratridine, which is a sodium channel activator and ouabain used to inhibit Na^+/K^+ ATPase, can react with the tilapia RBCs by influencing on the permeable characteristics of the cell membrane. In this method, RBCs were applied for the detection of particular sodium toxins of biological origin. Pure concentrations of a sodium channel-specific toxic substance could be used to reduce hemolysis at a level of about 0.3 mg/ml STX, 3.5 mg/ml for neo-STX, 3.0 mg/ml for GTX, and 5.0 mg/ml for TTX (by using ouabain and veratridine). CTX content was estimated at 50 mg/ml.

Croci et al. (1997) examined the development of a technique mainly connected to direct microscopy of alterations in the morphology of BGM cell cultures. It was demonstrated that the MBA and the above assay were highly correlated when carried out on mussels (*M. galloprovincialis*), which contained OA toxin. OA content of about 100, 50 and 25ng/100µl led to irreversible damages of the monolayer of the cells after the first hour of exposure. Lower concentrations caused moderate alterations (cell rounding, vacuolization and death of 35-40% of the cells) after passing 2 and 3 hours, leading to extended cell damage (death of the 90-95% of the cells) within 4-5 hr after exposure. Hahn (2002) evaluated a number of biomarkers and bioassay techniques for the detection of dioxin-like substances in the sea. Several biomarkers assessed in fish and shellfish are defined, in terms of toxin concentration, in vivo. In the same way, in vitro biomarker data, recorded in cell culture bioassays, can be effectively applied to evaluate the level of ‘dioxin equivalents’ in samples of environmental matrices. As a result, all degrees of effectiveness of numerous in vitro bioassays for toxic substances, such as receptor-binding techniques, DNA-binding and transcriptional activation of native (CYP1A) or reporter (luciferase) genes, can be easily evaluated and recorded.

For the achievement of a microtiter filter-based receptor assay (*in vitro* assay) developed with the aim of detecting toxic substances, the necessary equipment is essential for the implementation of the process and is based on conventionally formed liquid scintillation counting for evaluating the resulting data. The development of a very effective assay for the PSP toxins based on the enhanced specific interaction of these substances with their biological receptor was achieved by Doucette et al (1997). It was proved that the receptor binding assay can accurately estimate toxicity determined by mouse bioassay, and that it is a quick and low-cost method that can be used instead of live animal testing for determining PSP toxicity values in fish, shellfish and algae samples (Doucette *et al*, 1997).

3.2. Chemical methods and Biochemical techniques

Chromatographic processes were among the principal techniques applied in an effort to substitute conventional toxin-detection methods. All groups of toxic substances require the implementation of very specific assays and the absence of chromophores on most toxins makes derivatisation reaction essential for toxin detection. Chromatographic techniques using UV or fluorescence applications can now be used for most shellfish toxic compounds (Garthwaite, 2000). The detection of a few types of natural-existent toxins (e.g. DSP toxins) can be achieved by applying HPLC, capillary electrophoresis (CE) and capillary electrochromatography (CEC) with UV, fluorescence detection (FLD), MS detection and immunoaffinity chromatography (IAC) (Gago-Martinez *et al*, 2003). The recent development of HPLC-MS analytic technique and its application on shellfish toxins has resulted in immediate advance of production, accumulation, depuration, and chemical

knowledge of toxins. LC-MS is based on an interface method to transfer a decreased quantity of the HPLC flow into the mass spectrometer. Two interface techniques can be used: 1) ion-spray/electrospray (ESI) and 2) atmospheric pressure chemical ionization (APCI) (Garthwaite, 2000). Liquid and gas chromatography (LG & GC) and MS consist the common guide for the usual toxicological examinations in the seafood industries (Palleschi et al., 2000). It was recently proved that the most adequate techniques are those relied on LC/MS (Garthwaite, 2000).

Tandem mass spectrometers, and mainly hybrid equipment such as triple-quadrupole linear ion-trap methods, are considered to have increased sensitivity and effectiveness, but the need for application in a laboratory environment and trained staff is vital to draw safe conclusions. New methods combining liquid chromatographs coupled with tandem mass spectrometers (LC-MS-MS) have extended orifices by demanding increased pumping capacities. As a result, LC-MS-MS technology could be effectively used in field and specifically in oceanography (e.g. examination of toxic bloom behavior and toxin transfer through food webs) (Krock *et al*, 2008).

According to Sullivan et al. (1985), the HPLC is basically applied to detect and assess the quantity of OA, DTX1 and DTX2. Moreover, HPLC is effectively used to determine PSP toxins in shellfish. The application of HPLC compared with MBA techniques was examined by Sullivan et al. (1983). Toxic substances of PSP in shellfish were examined and measured by applying a HPLC and a common AOAC mouse bioassay. The two methods were highly correlated at levels equal or lower than 80μg toxin/100g, Increased quantities of toxins led to small deviations from the actual values.

The first study of pectenotoxin-2 (PTX-2) in algae (*Dinophysis fortii*), connected to poisoning due to consumption of seafood in Europe, was accomplished by Draisci et al (1996). The substance was detected both in crude methanolic phytoplankton samples and in the neutral fraction. The methods applied included reversed phase HPLC combined with UV diode-array detection (LC-UV-DAD) or mass spectrometry (LC-MS) and LC-MS-MS. Furthermore, OA was detected in *D. fortii* samples and measured at 15 pg/cell. Determination of the quantities of PTX-2 was impossible due to the fact that there was no available pure toxin. Nevertheless, the increased PTX-2:OA levels indicated important levels of PTX-2 in the *D. fortii* samples.

Yin et al. (2008) studied the formation of an interface to connect HPLC with atomic fluorescence spectrometry (AFS) in order to separate and detect inorganic mercury, methylmercury (MeHg), ethylmercury (EtHg) and phenylmercury (PhHg). In hyphenated applications, formic acid in mobile phase was effectively applied as reaction reagent for UV-CVG. Optimization of detection limits was defined at 0.085 μg/l for inorganic mercury, 0.033 μg/l for MeHg, 0.029 μg/l for EtHg and 0.038 μg/l for PhHg. The technique was evaluated by determining certified reference material DORM-2. The UV-CVG with the use of formic acid, made the procedure easier limiting at the same time the required cost.

Hess et al. (2000) evaluated the information extracted from an inter-laboratory examination carried out by 4 laboratories. The examination compared the HPLC-UV and LC-MS techniques applied with the aim of detecting and measuring the quantity of DA in standard solutions and in shellfish extracts. Firstly, 3 of the laboratories applied HPLC only or combined with ultraviolent technique. The quantities of DA found in samples were all among accepted levels. Then, a gonad homogenate

that included higher levels (one order of magnitude) of DA also range among accepted levels. Similar quantities of DA were found by applying LC-UV or LC-MS method.

James et al. (2000) developed a quick and effective, isocratic fluorimetric LC technique, by applying 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBDF) in order to derivatise and implement the technique for analyzing DA in marine phytoplankton and shellfish tissues. DA from shellfish samples was recovered at a level of >95%. After application of a strong anion exchange SPE cartridge for cleaning-up the extracts, determination reached at 6 ng DA/g mussel tissue. It was proved that this innovative technique was very effective in measuring DA in shellfish and phytoplankton (*Pseudonitzschia* sp).

Anderson *et al* (1989) implemented HPLC techniques to determine toxicity level of the dinoflagellate *Gymnodinitun catenatum* in northwest Spain region. Increased levels of the low potency sulfocarbamoyl toxins (*ca*. 90-95 mole %) were found in dinoflagellate samples, but toxicity value was mostly influenced by STX measured at 5-10% of the total concentration.

Furthermore, Wu et al. (2005) evaluated the extracted data with experiments on toxin-contaminated shellfish-foods collected from Shanghai seafood markets. PSP and DSP toxins in shellfish tissues were firstly assessed by a MBA and further examination took place by applying HPLC to chemically define each toxic substance. As regards MBA, it was proved that 8 extracts contained PSP toxins and 7 extracts contained DSP toxins. HPLC implementation demonstrated PSP levels of about 0.2 to 1.9 μg/100 g tissues mainly including gonyautoxins 2/3 (GTX2/3). As concerns DSP, detection of OA was achieved in 3 samples, and its levels were among 3.2 and 17.5 μg/100 g of sample. Dinophysistoxins (DTX1), was also detected in one

sample. It was proved that, higher percentages of gastropod (*Neverita didyma*) and scallop (*Argopecten irradians*) contained PSP and DSP toxins, and the majority of the toxic samples was found in Tongchuan and Fuxi.

According to Bouaïcha et al. (1997), micellar electrokinetic chromatography (MEKC) was developed and used to effectively and quickly determine the existence of OA in phytoplankton and shellfish samples. A detection limit of 40 pg was defined. The UV intensities of of the substance evaluated at 200 nm presented optimum linearity at values among 40-640 pg. Detection of OA was achieved in mussels spiked with 10 ng/g whole tissue. Existence of OA and dinophysistoxin-2 was detected in the crude extract of all dinoflagellate *Prorocentrum lima* samples.

Unialgal samples of the *Protogonyaulax (=Gonyaulax) tamarensis/cateneta* species complex, which belongs to dinoflagellates and is considered responsible for causing PSP, were toxically examined by HPLC. The study was carried out by Cembella et al. (1987). *Protogonyaulax* samples from different geographical regions such as northeastern Pacific (British Columbia), eastern Canada, Portugal, the United Kingdom and New Zealand were assessed. Concentrated quantities of toxic substances were significantly different between regions, but the toxin ratios of individual samples were maintained at relatively stable levels. Samples collected in the Plymouth (U.K.) did not contain toxins while in two samples from Vancouver Island, small amounts of toxins were found after application of HPLC. For the same samples, mouse bioassay detected no toxins. It was proved that no correlation could take place between toxicity or toxic substances and morphology.

According to Dickey et al. (1990), OA was detected in samples of the Caribbean dinoflagellate *Prorocentrum concave.* Purification of the toxic substance was achieved through normal and reversed-phase column chromatography and

determination was accomplished using 1H NMR and MS. OA has been also repeatedly determined as a toxic substance contained *P. lima* (Ehrenberg) Dodge. Both *P. concavum* and *P. lima*, are responsible for causing ciguatera and as a result this substance may be highly connected with this poisoning.

Powell & Doucette (1999) compared an effective receptor binding assay for determining PSP toxic substances in shellfish and algal samples, with the usually applied HPLC technique. Increased correlation was observed among all receptor-based STX levels except from zooplankton extracts and the result were similar with those extracted through HPLC. Although PSP receptor binding technique cannot be used to describe toxin composition, it is a very effective and quick method for evaluating PSP-like toxic levels in both laboratory and field extracts.

Except from the useful data extracted through the use of LC-MS, information can also be obtained by applying LC/MS/MS. Jorgensen & Jensen (2004) published an article, which examines and analyzes the distribution route of DSP toxins in 13 consignments of Danish blue mussels, during 2002 and 2003. The concentration of DSP toxic substances was examined with the use of LC combined with tandem MS technique, and the average detected rates in the 13 consignments ranged between 58-243 μg/ kg. Distribution pattern of DSP toxic substances was highly homogenized (relative standard deviation≈ 7-19%).

Analysis of clams and blue mussels (Mytilus edulis) from 1999-2004 was achieved with the use of LC combined with LC/MS/MS. The purpose of the study was the detection of DSP toxin esters. Concentration of total okadaic acid equivalents was recorded between 224 to 2516 μg/ kg in surf clams. The existing concentration of OA esters of the total OA and

derivatives was estimated to be between 83 and 98% (mean 95%). The corresponding concentration of total OA and derivatives was detected between 43 and 1631 μg/ kg in blue mussels. The rate of OA esters was 21 to 86%, mean 59% of all OA derivatives (Jorgensen *et al*, 2005).

Application of HPLC technique on extracted samples of shellfish and phytoplankton derived from the Gulf of Mexico proved the existence of toxic substances (0.162n μg/g shellfish) and DA (2.1 pg/cell phytoplankton). The existence of DSP and ASP toxins in a place where no other incidents were ever reported may demonstrate that human poisoning can be caused when accumulation of toxins is feasible Dickey et al, 1992).

The development of a quick and effective HPLC-UV technique was achieved with the aim of analyzing DA and analogues in shellfish without the requirement of SPE application. Isocratic chromatographic separation of DA and its isomers from shellfish samples and from the prevalent aminoacid, tryptophan was carried out giving special attention to the mobile phase pH. The most appropriate pH was detected at 2.5. The DA assay was very accurate; %RSD=1.63 and %RSD=3.7, and was effectively used for the examination of numerous samples from different shellfish species (López-Rivera *et al*, 2005).

According to Stobo et al. (2005) an innovative technique based on LC/MS was successfully applied to examine the presence of OA dinophysistoxin-1 (DTX-1), DTX-2, YTX, homoYTX, 45-hydroxy-YTX, 45-hydroxyhomo-YTX, pectenotoxin-1 (PTX-1), PTX-2, azaspiracid-1 (AZA-1), AZA-2, and AZA-3. Methanol-water (80%, v/v) was used for the extraction of toxins while C8 reversed-phase column was used for the analysis of samples. The technique was evaluated for its ability of detecting OA, YTX, PTX-2, and AZA-1 in 4 species of shellfish (mussels, *Mytilus edulis*; cockles, *Cerastoderma*

edule; oysters, *Crassostrea gigas*; king scallop, *Pecten maximus*) selected from United Kingdom (UK). The validated linear range was estimated at 13-250 μg/kg for OA, PTX-2, and AZA-1 and 100-400 μg/kg for YTX. Recovery was about 72-120 and precision was about 1-22%. It was proved that the method was effective enough, providing reliable results.

The HPLC technique gives the advantage of detecting a variety of amines by examining a single sample while the examination takes about 40 min to be applied (Wills *et al*, 1987). Draisci *et al* (1999) applied a LC-MS technique for determining DSP toxins and examined the potential use of a new method that combines LC-MS with ionspray ionization to directly determine different DSP toxic substances in mussels and phytoplankton. The examined substances were: YTX, OA and four of its analogues, dinophysistoxins (i.e. DTX-1, DTX-2, DTX-2B, DTX-2C), and pectenotoxins (PTXs), involving PTX-2, two PTX-2 secoacids (PTX-2SAs), PTX-2SA, 7-epi-PTX-2SA, and AC1, the three isomers highly connected to PTX-2. Analysis of extracts that contain toxic substances can be carried out through reversed-phase, positive ion mode SIM LC-MS. PTX-2SAs and AC1 were detected in phytoplankton and shellfish from Ireland and shellfish samples from Italy. Furtherome, PTX-2 was detected in Irish phytoplankton. YTX was detected in shellfish extracts from Italy. Four isomers of OA were found in Irish extracts (OA, DTX-2 and DTX-2B were detected in shellfish, and OA, DTX-2 and DTX-2C were found in phytoplankton). Puente *et al* (2004) studied the development of a very sensitive and quick technique for the detection of OA, DTXs and PTXs in shellfish and phytoplankton by applying LC-MS/MS with minimally prepared extracts. Five DSP toxic substances were separated on a C18 column (Luna-2, 150 mm x 2.1 mm, 5 micron). For the formation of a multiple reaction monitoring (MRM) technique, the optimum combined precursor-product ions were defined: OA (803/255), DTX2 (803/255),

DTX1 (817/255), PTX2SAs (875/137) and PTX2 (857/137). The method was proved to be very accurate, even detecting quantities of 1 pg (on-column). The method could easily assess the quantity of DSP toxins in phytoplankton even when only a few cells were available (N = 12-40). In *D. acuta* quantities of OA (7.0 pg), DTX2 (11 pg) and PTX2 (7.2 pg) were found.

LC–MS/MS is an extremely sensitive technique used for the detection of toxic substances when only minimal quantities of extracts are available for examination. Through this technique, identification and quantification of DSP toxins in shellfish and phytoplankton can be effectively carried out (Draisci et al., 1996). Krock *et al* (2008) applied a triple-quadrupole linear ion-trap hybrid LC-MS-MS method to determine the existence and quantity of toxins such as DA, gymnodimine, spirolides, dinophysistoxins, OA, PTXs, YTSs, and azaspiracids (AZAs). Numerous phycotoxins were found in North Sea plankton extracts. Domoic acid and 20-methylspirolide G were the substances found in the largest quantities. A lot of crude cultures (>100) were diluted and examined for the existence of AZAs growth for a long period of time. One of the cultures that were found contaminated with AZA, was used for the isolation of a dinoflagellate. It was demonstrated that any mass spectrometer can be used in ship laboratories without presenting any problems due to on-ship conditions. LC–MS–MS is a very significant technique for examining phycotoxins in plankton.

Wu et al (2008), evaluated the concentrations of PBDEs and PCBs in water and numerous aquatic animals from a reservoir near to e-waste recycling industries in China, and examined the degree of bioaccumulation of these substances in the animals. Increased quantities of PBDEs [52.7 to 1702 ng/g wet weight (ww)] and PCBs (20.2-25958 ng/g ww) were detected many biota species in comparison to reference samples (13.0-20.5 ng/g ww for PBDEs and 75.4-82.8 ng/g ww for PCBs). log BAF

(bioaccumulation factor) was estimated at 2.9 to 5.3 for PBDEs and at 1.2 to 8.4 for PCBs, and was highly related to congeners and species.

The examination aiming at determining the levels of MeHg in a cod-sample was carried out by Inagaki *et al* (2008). A cod fish was caught close to Japan. The sample was powdered and put into 600 bottles (10 g). Sterilization was achieved with gamma-ray irradiation. The samples were certified by applying isotope dilution gas chromatography combined with pled plasma mass spectrometry (SSID-GC-ICPMS). The samples were extracted according to two different methodologies: KOH/methanol and HCl/methanol extractions using ethylation or phenylation. Moreover, the total mercury was assessed by ID-ICPMS and the contained Hg was determined by GC-ICPMS. The estimated MeHg level was about 0.58 ± 0.02 mg/kg. According to Kuballa et al. (2008), the contents of mercury and methylmercury in marine animals (tilapia, sword fish, mackerel) can be evaluated using GC-AED technique. Several alkaline and acidic digestion processes were assessed. The optimum choice was the digestion of 0.5 g sample in methanolic potassium hydroxide solution. Gas chromatography and atomic emission detection were applied for quantifying the sample. It was demonstrated that the process was very effective with a detection limit of about 6.1 μg/kg for methylmercury. A 91 ± 19% of the substance was finally recovered. In the study of Bayen et al. (2005), the concentrations of numerous heavy metals (i.e. As, Cd, Cu, Pb, Hg) and POPs (PCBs, PBDEs, as well as organochlorine pesticides) were determined in 20 species of marine animals using ICP-MS and GC/MS. The average heavy metal contents were determined from minimum to 14.2 μg/g wet weight (ww) for As (shark), to 0.50 μg/g ww for Cd (kunning), to 25.5 μg/g ww for Cu (gray prawn), to 0.58 μg/g ww for Hg (eel), and to 1.21 μg/g ww for Pb (salmon).

Chlordane, PCBs, and p,p'-DDT [2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane] as well as other connected chemical substances were the basic POPs detected in seafood, and mainly in salmon fillets and green mussels. PBDE levels in salmon (2.75 ng/g ww) were significantly decreased in comparison to the increased content of PCBs (28.5 ng/g ww). According to the same author "daily intake of As, DDTs, heptachlor, and PCBs in seafood exceeded the conservative cancer benchmark concentrations set by the U.S. Environmental Protection Agency (EPA), indicating that a lot of people can be in danger in Singapore over a lifetime from seafood consumption". Hermanussen et al. (2008) determined increased contents of PCDD/Fs in marine turtles, mammals and seafood from Queensland in Australia. The HRGC–HRMS technique was used for carrying out the analysis**.** The PBDE concentration estimated was decreased in comparison to marine biota from the northern hemisphere, presenting decreased input into Queensland region. However, it was previously proved that increased quantity of PBDE was contained in human milk and sera in Australia. As a result, it was demonstrated that transfer of PBDEs can take place in in Australian seawater.

A large number of different species of fish and shellfish were bought from several fish markets of Dalian, Tianjin and Shanghai in China and examined for PCBs and OCPs as well as DDTs, HCHs, HCB, and CHLs, by applying GC and GC-ECD. The levels PCBs found in seafood from Dalian, Tianjin and Shanghai were about 1.11 to 8.04 ng/g, 1.26 to 5.60 ng/g and 0.83 to 11.4 ng/g on as regards wet weight, respectively. The contents were decreased in comparison with samples from developed countries (e.g. Japan and Italy). Mean contents of HCB, HCHs, CHLs and DDTs were estimated at 0.38, 0.92, 0.47 and 28.9 ng/g, respectively.

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Several biochemical techniques are mainly relied to the natural inhibition of OA, DTX1 and DTX2 by protein phosphatases type 1 (PP1) and 2A (PP2A) *in vitro*. Honkanen *et al* (1996) examined the application of an effective rapid phosphatase assay (PP2A) for detecting OA in oyster samples. The method was proved capable of determining toxic levels of OA ≥0.2 μg/ g.

3.3. Immunological assays/ immunochemical methods

Immunoassays can be widely used for the determination of shellfish toxins (Garthwaite, 2000). Antibodies are biochemical molecules characterized by high complexity. Their production by an organism is the result of the insertion of a foreign material known as antigen. Polyclonal antibodies have been applied for decades. Both monoclonal and polyclonal antibodies are very effective tools for diagnostic kits (Kalamaki *et al*, 1997).

One of the most applied processes is the enzyme linked immunosorbent assay (ELISA). ELISAs are characterized by low cost of application and require short period of time for their conduction. As a result, they can be effectively applied for examining large bulks of extracts. Immunoassays are carried out by applying antibodies, which lead to appropriate structures of toxins. The selection of the proper antibody is essential for the determination of the correct toxic levels (Garthwaite, 2000).

Radioimmunoassays and enzyme immunoassays are very sensitive techniques used to quantify several biologically active small molecular structures (Naar *et al*, 2002). In Sandwich ELISA**,** antibodies are fixed to a solid phase (e.g. the inside walls of test cubes). In a Gold Labeled Immunosorbent Assay test, antibodies used for the detection of a bacterium are connected

tocolloidal gold. Then, a sample can be put into the sample port of the test kit. In the Enzyme-Linked Fluorescent Immunoassay (ELFA), the inner place of a device used as a pipette, comprises a mixture of antibodies against a single antigen of a bacterium (Kalamaki *et al*, 1997).

The bacterium *Vibrio cholerae* is responsible for the appearance of cholera, an illness directly connected to water and fisheries' contamination. The formation of the bacterium colonies leads to the production of cholera toxin (CT) causing diarrhea in humans. An effective flow injected liposome immunoanalysis (FILIA) method was formed for the detection of CT. CT levels were determined with the development of a sandwich complex between the immobilized antibody and GM1 liposomes. According to the procedure, the extract was initially inserted into the column, and then injection of liposomes followed. The use of the octyl glucopyranoside led to the release of the molecules from the liposomes. The molecules were then quantified. The detection limit of the method was estimated at 6.6 x 10^{-17} g/ml in 200 μL of extracts (Ho *et al*, 2008). Latex Agglutination techniques are relied on integration of antibody-coated colored latex compounds when special antigens exist. The Immunodiffusion/Motility Enrichment Test integrates both accurate enrichment and antibody antigen reactions. The Immunoblot ELISA technique implementation is based on populations of bacteria that grown on an agar plate (Kalamaki *et al*, 1997).

Immunosensors (or immunochemical sensors) are specific devices that use an antibody-relied biorecognition molecular structure, for recognizing toxins. The Immunomagnetic Separation (IMS) method can be alternatively applied instead of selective enrichment. Most of immunosensors used today for one-shot sensors can indirectly detect substances by applying

enzyme and fluorescent labels. Electrochemical Immunosensors affinity sensors are mostly based on the application of electroactive label, and especially labeling and amplification methods (Palleschi et al, 2000).

Several methods for the preparation of ganglioside-incorporated liposomes have also widely used for the development of toxin-detection assays. It is proved that liposomes can be more effectively applied in diagnostics than enzyme-relied assays. Liposomes can contain sites for ligands. Also, increased amounts of dye or different markers can be found in their cavity, effectively producing intense signals. Moreover, ganglioside-liposomes are characterized by easier preparation processes before implementation, in comparison to immunoliposomes, which were previously used in diagnostic tests (Ahn & Durst, 2008).

Naar et al (2002) studied the implementation of a rapid, effective and accurate ELISA technique used for the quantification of brevetoxins in fisheries. The study also demonstrated that brevetoxins produced by *Karenia brevis* in Seawater, Shellfish, and Mammalian Body Fluid can be effectively found even when very decreased quantities are disposable and in slightly prepared samples. The process is based on secondary biotinylated antibodies, streptavidine-horseradish peroxidase conjugate, and chromogenic enzyme substrate. The detection limit for this type of toxic substances in spiked oysters was about 2.5μg/100 g sample.

Implementations of immunochemically based methods for PSP toxic substances are relied on microtiter plate enzyme immunoasays and enzyme-linked immunofiltration assays for detecting the toxic compounds and immunoaffinity

chromatography (IAC) for extract cleanup. Enzyme immunoassay (EIA) is preferred mainly due to the simple and quick integration of the process, and the very accurate results, in comparison to other techniques (Usleber *et al*, 2001).

Hokama et al (1987) examined the development of a quick enzyme immunoassay stick test, characterized high simplicity, for detecting ciguatoxin and connected polyether substances in fish extracts. The method can be applied for the examination of seafood responsible for causing ciguatera poisonings, fishes caught together with contaminated samples, fishes that do not contain toxic substances, and near shore reef fishes. The catches collected from regions connected to ciguatera incidents gave toxicity levels of 45%, 80.6% and 42.5% as regards Hawai, Kauai and Oahu, respectively, for *Ctenochaetus stigosus*. The fishes that contained toxins and led to poisonings were proved positive with the stick examination, demonstrating the effectiveness of the method.

The formation of the radioimmunoassay (RIA) process was achieved by applying ciguatoxin after purification. Antibodies were firstly prepared in sheep and rabbits by injecting the toxin in conjunction with human serum albumin. The detection of ciguatoxin and connected polyethers in fishes can be achieved by applying monoclonal antibodies in the stick enzyme immunoassay.

Ahn & Durst (2008) examined the formation of a very acute bioassay for detecting CT in specific seafood extracts, with the use of ganglioside-conjugated liposomes. For the conduction of the experiment, samples were spiked with different levels of CT. The limit of detection (LOD) was significantly enhanced in the majority of cases, in comparison to the LOD in the buffer system. It was revealed that bioassays with ganglioside-liposomes can be used for the direct detection of the toxic

substances in the field, by testing seafood quickly (≈20 min) and effectively, without requiring complicated procedures and devices.

According to the study of Wong (1996), food samples that contained different levels of CT were used for carrying out a quick technique based on the implementation of monoclonal antibody (Mab) enzyme immunoassay, with the aim of detecting *Clostridium botulinum* type E. Hybridomas secreting specific Mab against the type E epitope were formed. A number of these hybridomas (5) were prepared and maintained in liquid N_2. Immunoglobulin subisotyping demonstrated that these Mabs were related to the IgG. Production of increased amounts of Mabs was achieved in ascites dilution. After purification of Mabs, a biotin-avidin double sandwich enzyme-linked immunosorbent assay was used to determine the toxic levels of the bacterium in seafoods at concentrations of about 1-10 MLDs/ml (5-10 pg/ml).

Kania et al. (2003) examined the use of of polyclonal antibodies (pAbs) for the detection of DA. ELISA method was used to evaluate the process. The optimized horseradish peroxidase (HRP) ELISA was characterized by a detection limit of about 0.6ng/ml (ppb) and a efficiency of application at 0.8–300 ppb DA. A screen-printed electrode (SPE) technique was applied in order to quantify DA. It was proved that DA detection tests for ELISA had a deviation level of ±12%, while SPE was estimated to have a deviation level of about ±25%.

According to Kawatsu & Hamano (2000), an indirect competitive enzyme immunoassay (idc-EIA) was used for the examination of 10 samples of blue mussels (Mytilus edulis) from Japan in order to evaluate their concentration in DA. The

method was relied on an anti-domoic acid monoclonal antibody. It was proved that the toxin existed in all extracts at concentrations of about 0.11-1.81 ng/g mussel flesh.

A reversed-phase liquid chromatographic technique using UV radiation at (242 nm) for detecting toxic substances was assessed in comparison to a radioimmunoassay method used for the detection of domoic acid. After the examination of numerous seafood extracts (razor clams, anchovies, crabs) its was proved that both methods presented similar results as regards spiked samples of mussels and rat serum. The latter were effectively detected at concentrations level of about 0.15-7.3μg/g of DA (Lawrence et al., 1994).

A new electrochemical enzyme-linked immunosorbent assay used for detecting DA was formed by applying a screen-printed electrode device with a monoclonal antibody. The latter was used for determining the detected molecules. It was demonstrated that competitive immunoassays for the detection of DA can be effectively applied and that the immunosensor used for detecting DA presents a common range of detection and a detection limit appropriate for "on-site" regulation (Micheli et al., 2004).

The study of Kreuzer et al. (2002) examines the application of a screen-printed electrode (SPE) method for determining numerous seafood toxic substances (e.g. okadaic acid, brevetoxin, domoic acid and tetrodotoxin). A screen-printed carbon electrode in conjunction with amperometric determination of p-aminophenol at +300 mV vs. Ag/AgCl, were applied for signal assessment. ELISA was firstly applied for the development of all toxins, prior to applying SPE. The SPE technique is characterized by high simplicity and it is relatively cheap while integrating an examination at about 30 min. Moreover,

examinations can be effectively carried out in the field. Recovery tests on specific toxic substances were proved very accurate presenting a ±10% deviation for the exact value.

Two new techniques, a competitive direct enzyme-linked immunosorbent assay (cdELISA) and a competitive indirect ELISA (ciELISA), were applied for the determination of antibodies and the detection of DA in blue mussels and clams. When blue mussel matrix was used, the detection limit was estimated at <25 ng/g, while the overall recovery level was measured at 25-500 ng/g. The effectiveness of cdELISA was also revealed by using HPLC. Determination of DA in shellfish extracts demonstrated that 10 of the 15 samples contained DA at concentrations lower than 50 ng/g (Yu *et al*, 2004).

Two test kits: the DSP-Check™ kit from UBE Industries and the OA ELISA kit from Rougier Bio-tech were applied for the detection of toxins. MIST Alert™ was assessed for determination of PSP toxins in shellfish. Numerous types of shellfish collected from the UK shellfish toxin-monitoring program, were examined. All extracts were found to be contaminated with toxins at concentrations of about 80 μg STX equivalents/100 g shellfish flesh. Detection of toxins was effectively achieved in almost all extracts with STX concentrations of >40 microg equivalents/100 g. It was demonstrated that MIST Alert can be used as an initial detection method for PSPs. It is also important to mention that these kits can be easily used by humans with no technical or scientific knowledge providing very accurate results (Mackintosh *et al*, 2002).

Numerous extracts of mussels (*Mytilus sp*.) were examined for OA and connected substances by applying four different techniques (HPLC procedure, solid-phase immunobead assay, enzyme-linked immunosorbent assay and idiotypic-anti-idiotypic competitive immunoassay). The experiments were conducted with animals collected form *Dinophysis sp*. blooms or

processed seafood implicated in human DSP incidents. Homogenized hepatopancreas gland tissue was used for the conduction of the tests. All samples presented the same results, but it was demonstrated that quantitative immunochemical techniques with microplate readers were characterized by higher sensitivity in comparison to HPLC (lowest detection limits of 0.02 mu g OA/g hepato-pancreas and 0.4 mu g as regards immunoassays and HPLC, respectively) (Fremy *et al*, 1994).

3.4. Biochemistry and molecular techniques (DNA-based methods)

Molecular methods are relied on the determination of a small piece of the microorganisms' genome. The selection of the sequence is essential for the conduction of the procedure and is highly connected to the target of each process (detection or typing).

PCR is used for amplification of viral or bacterial nucleic acid without the requirement of previous culture or use of isolated strains from the extract, which is very significant for determining non-culturable viruses or stressed bacteria. The PCR technique is very selective and can detect the substances even when just a few cells are available in the extract (Pommepuy & Guyader, 1998).

Kumar et al. (2001) examined the existence of Shiga-toxigenic *Escherichia coli* (STEC) in Indian fish and fisheries. According to this study, after isolation of *E. coli* from numerous seafoods (e.g. fresh fish, clams) and water, the samples were tested for the detection of STX, hlyA and rfbO157 genes by applying PCR. After the conduction of the experiment it was proved that 5% of clams and 3% of fish extracts contained non-O157 STEC, indicating that STEC can be transmitted through

these foods. It was finally demonstrated that STEC is contained in many seafood products in India, and non-O157 serotype is the most usually detected.

A TaqMan PCR assay was used for the determination *V. cholerae* in pure cultures, oysters, and synthetic seawater. Probe formed from the nonclassical hemolysin (*hly*A) sequence of the bacterium was used. Sixty bacterial strains were examined. The method was very sensitive in a range of values between 6 to 8 cfu/g as regards spiked raw oyster and 10 cfu/g as regards synthetic seawater. The required time for the complete conduction of the method was about 3 h. It was proved that the TaqMan probe and primer formed can be applied to rapidly detect the existence of *V. cholerae* in oysters and seawater without requiring any preparation of the samples (Lyon, 2001).

An effective PCR assay (TaqMan assay) used for the quantification of the bacterium *Clostridium botulinum* type E was examined by Kimura *et al* (2001). The method included amplification of a 280-bp sequence from the botulinum neurotoxin type E (BoNT/E) gene, hydrolysis of an internal fluoregenic probe and control over intensity enhancement of fluorescence during PCR. It was proved that the technique was very sensitive and accurate. During the examination of contaminated jack mackerel, the range of detection was estimated at 10^2 to 10^8 cfu/ml or g indicating the sensitivity of the method. much earlier than toxin could be detected by mouse assay. It was finally demonstrated that the assessment of BoNT/E DNA using this technique was very effective in estimating the potential hazards of this type.

According to Jinneman et al. (2003), a multiplex real-time PCR technique for the determination of the *stx1* and *stx2* genes of Shiga toxin-forming *E. coli* and a specific single-nucleotide polymorphism in the *E. coli* O157:H7/H$^-$ *uidA* gene was

evaluated. It was proved that the technique provided 98.6% sensitivity and 100% specificity after examination of 138 samples. It was also very efficient (≥1.89), with a detection limit of 6 cfu/reaction. Furthermore, Bowers et al (2000) evaluated the development of a real-time PCR-relied assay that could rapidly and accurately detect *Pfiesteria piscicida* and *P. shumwayae* in different cultures and water samples. It was demonstrated that the method could be used to quantify the detected microorganisms. The same method couldbe otentially applied for field-relied experiments.

V. cholerae O1 and *V. cholerae* non-O1 strains detected in different samples in São Paulo, Brazil, during cholera epidemics were evaluated for the existence of toxic substances. Furthermore, other *V. cholerae* O1 strains detected in extracts in Peru and Mexico, and *V. cholerae* O139 strains detected in India were assessed for the existence of CTX and ZOT *(zonula occludens* toxin gene) by applying PCR. A modified DNA-extraction technique was used to effectively recover genomic DNA from vibrios. It was demonstrated that the toxins can be detected by PCR and the same method can also be used for the evaluation of the epidemiological route of *V. cholerae* (Rivera *et al*, 1995).

Rapid methods (Biological, Chemical, Biochemistry, Molecular and Immunochemical) for toxin detection is given in **Table 3.1** and rapid detection methods detections of seafood toxins is given in **Table 3.2**.

4. The limit of detection of methods (LOD)

Determination of dietary exposure to shellfish toxins cannot be achieved by following the methodology for detection of different substances, mainly due to the sporadic cases and incidents of contamination, and the important fluctuations of time

and spaces as regards poisonings and detection of toxins. `Most of extracts examined presented minimal concentrations of toxins or absence of toxic substances. Exception is the existence of an algal bloom. In this case toxic levels are increased significantly. As a result, the determination of an overall mean or median contaminant degree of toxin to evaluate the medium degree of consumption cannot be successfully used (http://www.foodstandards.gov.au/_srcfiles/TR14.pdf).

Several rapid techniques can indirectly evaluate the toxicity by measuring the potential hazards and as a result they can be applied for the establishment of limits (Kalamaki *et al*, 1997). The limit of detection of rapid methods (LOD) is given in **Table 4.1**.

5. Risk characterization

Nowadays, different toxic substances found in the aquatic environment lead to contamination of fish and fisheries causing several problems to the aquaculture industry. Distribution of toxins is global and can rapidly cause death to large populations of fishes or can make these foods hazardous requiring their removal from the market and resulting in substantial financial losses (Wright, 1995).

The severity and duration of many intoxication incidents related to ingestion of seafood toxins makes these substances a very significant public health subject. Nevertheless, the knowledge on the organs affected by the different tya commonly pes of toxins and the critical toxic concentrations are very incomplete making it difficult to determine safe exposure limits. Moreover,

acceptable daily intake (ADI) has not been defined for any kind of aquatic toxic substances (http://www.foodstandards.gov.au/_srcfiles/TR14.pdf).

The degree of the potential hazard from seafood consumption is highly related to the species of finfish and shellfish and the region of harvesting. Generally speaking, the preventive measures are based on minimization of harvesting from hazardous regions making known that some species and regions are of high risk and on the implementation of different other methods (e.g., freezing foods before raw consumption, informing consumers for the advantages of proper cooking etc.) (Ahmed, 1992).

Marine toxins are characterized by high heat stability and therefore are almost unaffected by cooking processes. These toxins can lead to different neurological, gastrointestinal, and cardiovascular diseases that can even cause death or long-term morbidity. Common and rapid clinical diagnostic examinations have not been establishes for these substances and diagnosis is relied on symptoms and any possible ingestion of seafood in the proceeding 24 h. When humans affected by aquatic toxins are in immediate danger of dying, the possibility of toxin contamination and hospitalization in an intensive care unit as well as providing artificial ventilation (when required) are essential measures for the recovery of the patients (Sobel & Painter, 2005).

Incidents of seafood poisonings due to ingestion of toxins are frequently presented in numerous regions all over the world. Also, it is proved that more and more toxic substances, and especially phycotoxins, are determined every year (Rossini, 2005).

Table 5.1. displays various regional outbreaks of seafood toxins, the assessment of toxins in seafood, clinical symptoms of this toxicity and the specific, symptomatic and supportive medical treatments that were given in incidents.

Finally, the diagnosis of seafood poisoning are based on the clinical scenario and the patient's recent fish-eating history. Total outbreaks are associated with gastrointestinal (GI), cardiovascular, neurological and neuropsychiatric symptoms and signs.

6. Conclusions

Among the different types of hazards found in fish and seafood, chemical hazards and especially toxins such as BpTX, OA, PSP toxins, DA, etc. and PCBs, are considered especially hazardous for the health of consumers and are responsible for a high number of food-borne poisonings worldwide. Natural toxins are usually derived from several toxic algae species, which constitute the food of different molluscs. A large number of neurogical, cardiovascular and gastrointestinal diseases (NSP, DSP, PSP, ASP, SFP, PFP, etc.) occur due to consumption of these substances. The main chemical toxins occurring in fish and seafood are methyl mercury and PCBs. Their high toxicity and potential accumulation in ecosystems make them very severe hazards. Therefore, many methods such as GC, MS, biomarkers as well as several bioassays and immunochemical/ immunological techniques, have been developed and used for the detection of these compounds, targeting to ensuring the safety of consumers. Moreover, biochemical and molecular techniques like PCR can be effectively applied for the rapid detection of toxic substances.

As a concluding remark, it can be said that despite the tremendous amount of research on the substances concerned, the limitations of the methods demonstrate the urgent need to improve the currently available techniques and develop even more effective methods with the aim to providing safe fish and seafood products.

APPENDIX 1

A. **Natural toxins** - abbreviations

Disease	Full name	Natural toxins	Explication
CFP	Ciguatera fish poisoning	CTXs	Ciguatoxins
		MTX	Maitotoxin
SFP or HFP	Scombroid fish poisoning or histamine fish poisoning	Hist	Histidine
PSP	Paralytic shellfish poisoning	STX	Saxitoxin
		NEO	1-hydroxysaxito

			xin
		dcNEO	decarbamoylneosaxitoxin
		dc-STX	decarbamoylsaxitoxin
		Cl-4	N-Sulphocarbamoyl toxins
		GTX	Gonyautoxin
NSP or BSP	Neurotoxic shellfish poisoning or Brevetoxic shellfish poisoning	PbTx	Brevetoxins
		Cl, C2, Bl	Sulf

			ocarbamoyltoxins
PFP	Puffer fish poisoning	TTX	Tetrodotoxin
DSP	Diarrhetic shellfish poisoning	OA	Okadaic acid
		DTXs	Dinophysistoxins
		DTX-l	Dinophysistoxin-1 or 35methylokadaic acid
		DTX-2	Dino

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			phys istox in-2
		DTX-3	Dino phys istox in-3
		PTXs	Pect enot oxin
		PTX-2	Pect enot oxin-2
		YTX	Yess otoxi n
		AZAs	Azas pirac ids
ASP	Amnesic shellfish poisoning	DA	Dom oic acid
HFP	Hallucinogenic fish poisoning or dreamfish poisoning	DOM	Dom oic acid

B. **Chemical toxins** - abbreviations			
HAHs	Halogenated aromatic hydrocarbons	PCDDs	Polychlorinated dibenzo-p-dioxins
		PCDFs	Polychlorinated dibenzofurans
		PCDEs	Polyhalogenated diphenyl ethers

		PCDD/Fs	Polychlorinated dibenzo-p-dioxins and dibenzofurans
		DLPCBs	Dioxin-like polychlorinated biphenyls
		PCBs	Polychlorinated

			Biphenyls
		PAH	Polycyclic aromatic hydrocarbons
		DL-PCBs	Dioxin-like polychlorinated biphenyls
		TCDD	3,3’, 4,4’, 5 pentachlorobiphen

			yl PCB -126
		TBT	Aquatic biocide tributyltin
		OCPs	Organochlorine pesticides
		HCB	Hexachlorobenzene
		HCHs	Hexachlorocyclohexane isomers

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		CHLs	Chlordane compounds
POPs	Persistent organic pollutants	PBDEs	Organochlorine pesticides, polybrominated diphenyl ethers
MeHg	Methylmercury	EtHg	Ethylmercury
		PhHg	Phenylme

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			rcury
DDT	Dichloro-Diphenyl-Trichloroethane	DDE	Dichlorodiphenyldichloroethylene
		DDD	Dichlorodiphenyldichloroethane
Γ. Chemical toxins of microorganisms - abbreviations			
cfx	Choleratoxin		
zot	Zonula occludens toxin		
STEC	Shiga toxin *E. coli*		
BT	Botulinum neurotoxin		
LPS	Lipoppollysaccharides		
DT	Diphtheria toxin		
TT	Tetanus toxin		

Attention: The definition of HFP has two interpretations like HFP (Histamine fish poisoning) and HFP (hallucinogenic fish poisoning) and they should not be confused.

APPENDIX 2	
Rapid methods - abbreviations	
Methods- abbreviations	Explication
AAS	Atomic absorption spectrometry
AES	Atomic emission spectrometry
AFS	Atomic fluorescence spectrometry
APCI	Atmospheric pressure chemical ionisation
API	Atmospheric pressure ionization
BGM	Buffalo green monkey kidney cell cultures
bead-ELISA	Bead-enzyme-linked immunosorbent assay
CE	Capillary electrophoresis
CEC	Capillary electrochromatography

cELISA	Competitive enzyme-linked immunosorbent assay
CID in LC-MS-MS	Collision-induced dissociation in multiple tandem mass spectrometry
CRF	Chromatographic response function
CZE	Capillary zone electrophoresis
ECD	Capillary electrophoresis detection
EI	Electron impact mass spectrum
EIA	Microtiter plate enzyme immunoassay
ELIFA	Enzyme-linked immunofiltration assay
ELISA	Enzyme-linked immunosorbent assay
cdELISA	Competitive direct enzyme-linked immunosorbent assay
ciELISA	Competitive indirect ELISA
ERICs-PCR	Enterobacterial repetitive intergenic consensus sequences- Polymerase chain reaction-based

ESI	Ion-spray/ electrospray
ESI	Electrospray ionisation
FAB	Fast atom bombardment
FD	Fluorescence detection
FI-CV-AFS	Flow injection-cold vapor-atomic fluorescence spectroscopy
FIA	Flow injection analysis
FIA–MS	Flow injection analysis- Mass spectrometry
FILIA	Flow injection liposome immunoanalysis
FLD	Fluorescence detection
FRET	Fluorescence resonance energy transfer
GC	Gas chromatography
GC-AED	Gas chromatography with atomic emission detection
GC-ECD	Gas chromatographic system with electron capture detection
GC-MS	Gas chromatography-mass spectrometry
HPCE	High-performance capillary electrophoresis
HPLC	High performance liquid

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	chromatography
HPLC–AFS	HPLC with atomic fluorescence spectrometry
HPLC-ICP-MS	High performance liquid chromatography and inductively coupled plasma mass spectrometry
HPLC-MS	High performance liquid chromatography-mass spectrometry
HPTLC	High performance thin layer chromatography
HRGC–HRMS	High-resolution gas chromatography/ high-resolution mass spectrometry
HR-MS	High resolution mass spectrometry
IAC	Immunoaffinity chromatography
ICP-MS	Inductively coupled plasma-mass spectrometry
IMS	Immunomagnetic separation technique
IP	Immunoprecipitation tests
ISP	Ion spray liquid

	chromatography
ISP	Ionspray
ISP	Onspray interface
ISP	Ion-pair chromatography
LC	Liquid chromatography
LC–FLD	Liquid chromatog raphy–fluorimetric detection
LC-FL	Liquid Chromatography - fluorescence detection
LC/MS	Liquid chromatography-mass spectrometry
LC with fluorimetric, SRM μLC-MS-MS	Liquid chromatography with fluorimetric, mass spectrometric and tandem mass spectrometric
LC ISP-MS	Ionspray liquid chromatography-mass spectrometry
LC-MS/MS	Liquid chromatography–multiple tandem mass spectrometry
LC–MS–MS	Highly sensitive triple-quadrupole linear ion-trap.
LC–MS, LC–MS–MS	Mass spec trometry and tandem mass spectrometry
LC MS and HPLC/fluorescence	Liquid chromatography with

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	mass spectrometry and high performance liquid chromatography/ fluorescence
LC-UV	Liquid Chromatography with UltraViolet absorption detection
LC-u.v.-DAD	High-performance liquid chromatography either by U.V. diode-array detection
LC-ESI-MS	Liquid chromatography-electrospray ionization mass spectrometry
LFIC	Lateral Flow Immuno-Chromatography
LIF	Laser-induced fluorescence
LM	Light microscopy
Mab	Monoclonal antibody (enzyme immunoassay)
MBA	Mouse bioassay
MEKC	Micellar electrokinetic chromatography
MEKC with UV	Micellar electrokinetic chromatography with ultraviolet detection

MEKC-LIF	Micellar electrokinetic capillary chromatography (MEKC) in conjunction with laser-induced fluorescence (LIF) detection
MGB	Minor-groove binder
MIST AlertTM	Recently a commercial antibody-based rapid qualitative test
MRM	Multiple reaction monitoring mass spectrometry
MS	Mass spectrometry
MS-MS	Tandem mass spectrometry
MT	Mouse lethality test
NMR	Nuclear Magnetic Resonance
OR	Orifice potential
PCR	Polymerase chain reaction-based assays
PF	Permeability factor
PFU	Plaque-forming unit
PSPase-based assay	Protein phosphatase-based assay
RBA	Receptor Binding Assay
Real-time PCR STEC method	Real-time polymerase chain reaction-based assays-Shiga toxin-producing *E.coli*

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RI	Radioimmunoassay
RIA	Radioimmuno-assays
RIAs	Radioimmunoassays
RIC	Reconstructed ion current profile
SIM	Selected-ion-monitoring mode chromatogram
SPE	Solid phase extraction
RT	Reversed-phase fluorimetric
RT-PCR	Reverse transcriptase-Polymerase chain reaction-based assays
SEC	Size exclusion chromatography
SEM	Scanning electron microscopy
SIDMS	Speciated isotope dilution mass spectrometry
SIM	Selected ion monitoring mode
SPE	Electrochemical, screen-printed electrode system, immunosensors
SRM	Selected reaction monitoring
SSID–GC–ICPMS	Species-specific isotope dilution gas chromatography

	inductively coupled plasma mass spectrometry
TaqMan assay	*Thermus aquaticus* DNA polymerase
TC-CC	Tissue culture assays- cell culture
TCID	Tissue culture infectious dose
TLC	Thin layer chromatography
The real-time PCR- MGB	The real-time polymerase chain reaction-based assays and minor-groove binder
UV	Ultraviolet radiation
UVD	Ultraviolet absorbance detection

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Table 1.1. Natural toxins and seafood involved

Shellfish toxins / Disease	Natural toxins	Seafoods involved	References
Ciguatera fish poisoning (CFP)	Ciguatoxin-1, (phycotoxin produced by *Gambierdiscus toxicus*, *Prorocentrum concavum*, *P. lima*, *Ostreopsis siamensis*, *O. lenticularis* and *O. ouata*)	Shellfish (clams, mussels, and oysters)	Pierce & Kirkratrick, 2001
		Sponges: *Halichondria okadai* and *H. melanodocia*	Dickey *et al*, 1990
		Ctenochaetus strigosus and *Thunnus thynnus*	Hokama, 1988
	Ciguatoxin-1 (phycotoxin produced by *Gambierdiscus toxicus*), maitotoxin, scaritoxin	Clam *Ruditapes philippinarum*	Okumura *et al*, 2005
		Red tilapia *Sarotherodon mossambicus*, *A. triostegus*, *M. cephalus*, and *K. sandsicensis*)	Shimojo & Iwaoka, 2000
		Finfish (barracuda, amberjack, horseye jack, black jack, other large species of jack, king mackerel, large groupers and snappers) and *Ctenochaetus striatus*	Kalamaki *et al*, 1997
		Cigua, Reef fish (grouper, snapper, amberjack, barracuda, ulua, surgeonfish)	Watters, 1995

		Ctenochaetus strigosus and *Thunnus thynnus*	Hokama, 1987
	CTX3C and 51-hydroxyCTX3C	100 species of fishes	Inoue & Hirama, 2004
	CTX3C	Butter clams (*Saxidomas giganteus*)	Manger *et al*, 2003
		Reef fish*	Oguri *et al*, 2003
	CTX1B and CTX4B	Moray eel	Takai *et al*, 2003
	CTX (phycotoxin produced by *Gambierdiscus toxicus*)	Subtropical and tropical fin-fish, clams and marine snails	Garthwaite, 2000
	CTX (phycotoxin produced by *Prorocentrum concavum*)	Amberjack, snapper, grouper, barracuda, goatfish, and reef fish belonging to the Carangidae	Ahmed, 1991
	Maitotoxin (MTX) producd by *Gambierdiscus toxicus*	Clam *Ruditapes philippinarum*	Okumura *et al*, 2005 Verhoef *et al*, 2004
Scombroid fish poisoning (SFP) or histamine fish poisoning (HFP)	Histamine, putrescine and cadaverine	Mahi mahi, tuna, mackerel, bonito, skipjack and bluefish	Kerr *et al*, 2002
		Scombridae (tuna and mackerel) and S*comberesocidae* (saury) as well as non-scombroid species (mahi-mahi, sardines, pilchards, anchovies, herring, marlin and bluefish) can also be involved	Lehane & Olley, 2000
		Scombridae (tuna, mackerel, skipjack and	Watters, 1995

		mahimahi (bonito) or marlin)	
		Raw molluscan shellfish, finfish, fresh/frozen mahimahi (dolphin fish), tuna, and bluefish, amberjack, snappers, groupers, barracuda, goatfish and other carnivorous fishes	Ahmed, 1992
		Tuna, bonito, mackerel and eel	Yen & Hsieh, 1991
		Puffer fish	Wills *et al*, 1987
	Histamine	*Istiophorus platypterus*	Hwang *et al*, 1995
		Mahi mahi, tuna, mackerel, bonito and skipjack	Kalamaki *et al*, 1997
Paralytic shellfish poisoning (PSP)	Carbamate toxins: Saxitoxin (STX)	Mussels, oysters, clams and scallops	Garthwaite, 2000
		Mussels, clams (Alaskan butter clam *Saxidomus giganteus*), oysters and scallops	Watters, 1995
		Scallop or mussels	Yasumoto *et al*, 1985
	Saxitoxin (produced by different species of dinoflagellate *Prorogonyaula sp*.)	All filter-feeding molluscs	Kalamaki *et al*, 1997
	Saxitoxins produced by *Alexandrium sp.*	Commercially harvested shellfish	Report of the Joint FAO/IOC/WHO,

			2004
	1-hydroxysaxitoxin (NEO) and the epimers of 11-hydroxysaxitoxin sulphate and 11 - hydroxyneosaxitoxin, decarbamoylsaxitoxin (dc-STX), Bl-2 and Cl-4 (N-Sulphocarbamoyl toxins)	Shellfish (e.g., mussels, clams, scallops)	Luckas, 1992
	Gonyautoxins II, III, I and IV (GTX), NEO (neosaxitoxin), dcNEO decarbamoylneosaxitoxin produced by *Gonyaulax catenella*. Cyanotoxin, conotoxin, N-sulphocarbamoyl, carbamate and decarbamoyl toxins	Shell clams (*Mya arenaria*), blue mussels (*Mytilus edulis*), and scallop viscera (*Placopecten magellanicus*)	Indrasena & Gill, 1998
		Pyrodinium bahamense var. *compressa*	Wright, 1995
		Bivalve molluscs (mussels, clams, oysters, scallops)	Ahmed, 1991
		Shellfish	Cembella *et al*, 1987
	Gonyautoxins (*Alexandrium lusitanicum*) and saxitoxin (STX)		Rossini, 2005
	PSP produced by *Alexandrium* (*tamarense, minutum, catenella*) *Pyrodinium bahamense* and *Gymnodinium sp.*	Shellfish (clams, mussels, and oysters)	Pierce & Kirkratrick, 2001
Neurotoxic shellfish poisoning or brevetoxic shellfish poisoning (NSP or BSP)	Brevetoxins - PbTx-(1-6) (neurotoxins)	Filter-feeding shellfish	Garthwaite, 2000
		Molluscs	Ahmed, 1991
		Shellfish	Kalamaki *et al*, 1997
	Brevetoxin PbTx-2 and PbTx-3 produced by	Shellfish: green-shell mussels	Melinek *et al*,

	Ptychodiscus brevis		1994
			Kerr *et al*, 1999
	PbTx-2, PbTx-3, PbTx-9 or a mixture of the three toxins	Oysters	Naar *et al*, 2002
	Semisynthesis of *S*-Desoxybrevetoxin-B2 and Brevetoxin-B2 (toxic marine dinoflagellate alga *Karenia brevis*)	Mussels (*Perna canaliculus*)	Selwood *et al*, 2008
Puffer fish poisoning (PFP)	Tetrodotoxin (TTX) and chiriquitoxin	Puffer or globe fish	Rossini, 2005
		Seafood and mussels	Kreuzer *et al*, 2002
		Red tilapia *Sarotherodon mossambicus* and fishes *A. triostegus*, *M. cephalus*, and *K. sandsicensis*	Shimojo & Iwaoka, 2000
		Puffer fish (family *Tetraodontidae*), salamanders and colored frogs Japanese ivory and trumpet shellfish, the California newt, and the Australian blue-ringed octopus	Watters, 1995
		Buffer fish belonging to the genus *Sphoeroides*	Ahmed, 1992
		Puffer or globe fish	Ahmed, 1991
	Palytoxin	Puffer fish	Wright, 1995
			Ahmed, 1991
	Purified T_1 and T_2 with TTX	Three species of puffer fish, *Takifugu poecilonotus*, *T. vermicularis* and *T.*	Nakamura *et al*, 1984

		radiates. Responsible dinoflagellate: *Protogonyaulax tamarensis*	
Diarrhetic shellfish poisoning (DSP)	Okadaic acid (OA)	Shellfish: clams, mussels and oysters	Garthwaite, 2000
			Pierce & Kirkratrick, 2001
		Mussels	Bouaicha *et al*, 1997
	OA derivatives named dinophysistoxins (DTXs) dinophysistoxin-1 or 35methylokadaic acid (DTX-l), dinophysistoxin-2 (DTX-2), and dinophysistoxin-3 (DTX-3) (diatoms *Dinophysis acuta*/ *acuminate*/ *sacculus*/ *fortii*/ *caudate*, *Phalachroma rotundatum*, *Prorocentrum lima*)	Mussels: *Mytilus edulis*	Puente *et al*, 2004
		Mussels: *Mytilus edulis* and *M. galloprovincialis*	Croci *et al*, 1997
		Mussels: *Mytilus galloprovincialis*	Draisci *et al*, 1995
		Sponge *Halichondria okadai*, Irish mussels and Japanese group	Wright, 1995
		Blue mussel *Mytilus edulis*, scallops *Putinopecten yessoensis*, European mussels and Japanese scallops	Luckas, 1992
		Mussels, clams and scallops	Ahmed, 1991
		Bivalve molluscs globally	Report of the Joint FAO/IOC/WHO, 2004
	35(S) -Methylokadaic acid	Oysters	Honkanen *et al*,

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			1996
		Scallops and Japanese mussels	Yasumoto *et al*, 1985
	Neutral toxins: consists polyether-lactones of the pectenotoxin group (PTXs) such as pectenotoxin-2 (PTX-2 lipid soluble toxin)	Mussels: *Mytilus edulis*	Rossini, 2005
		Mollusks	Draisci *et al*, 1996
		Mussels	Terao *et al*, 1986
		Bivalve molluscs globally	Report of the Joint FAO/IOC/WHO, 2004
	Yessotoxin (YTX) (responsible diatoms *Protoceratium reticulatum* and *Lingulodinium polyedrum*), a brevetoxin-type polyether and its analogue 45hydroxyyessotoxin (45-OH YTX), Desulfoyessotoxins (1 - DesulfocarboxyhomoYTX and 4 DesulfocarboxyhomoYTX) and azaspiracids (AZAs) formed by algae *Dinophysis fortii* or *D. acuminata* and other species of *Dinophysis*, possibly Prorocentrum *ilma* and *Protoperidinium* species (*P. crassipes*)	Adriatic mussels	Ciminiello *et al*, 2007
		Mussels	Krock *et al*, 2008
		Mussels: *Mytilus edulic*, cockles: *Cerastoderma edule*, oysters: *Crassostrea gigas*, king scallop: *Pecten maximus*	Stobo *et al*, 2005
		Scallop *Patinopecten yessoensis* and mussels *Mytilus galloprovincialis*	Alfonso *et al*, 2004
		Cultivated Irish mussels and Portuguese mussels	Fremy *et al*, 1999
		Bivalve molluscs	Report of the Joint FAO/IOC/WHO, 2004

Amnesic shellfish poisoning (ASP)	Domoic acid (DA or DOM) (neurotoxic amino acid)	Blue mussel (*Mytilus edulis*), clam (*Meretrix lusoria*) and oyster (*Crassostrea gigas*)	Rossini, 2005
		Shellfish	Hesp *et al*, 2005
			Kania *et al*, 2003
		Mussel (*Mytilus edulis*) and clams *Siliqua patula*	James *et al*, 2000
	DA formed by some varieties of the diatom *Nitzschia pungena*, *N. pseudodelicatissima*, *Pseudonitzschia australis*/ *seriata*/ *pungens*/ *multiseries*	Shellfish: clams, mussels and oysters	Garthwaite, 2000
		Blue mussels (*Mytilus edulis* L.)	Quilliam *et al*, 1998
			Nijjar *et al*, 1992
		Mussels and clams	Ahmed, 1991
		Blue mussels	Report of the Joint FAO/IOC/WHO, 2004
	Domoic acid produced by red macroalgae *Chondria armata* (algae of the family *Rhodomelaceae*)	Mussels	Micheli *et al*, 2004
		Razor clams and blue mussels (*Mytilus edulis*)	Wright, 1995
		Blue mussels (*Mytilus edulis* L.)	Quilliam *et al*, 1989

* Reef fish: Snapper, Mullet, Grouper, Surgeonfish, Jack, Moray eel, Amberjack, Parrot fish, Barracuda, Sea bass (Noone, 1996).

Table 2.1. Chemical toxins

Chemical fish toxins	Source of Environmental Pollution	Fishes and shellfishes most likely to be contaminated	Reference
	▪ Willamette River and lower Santiam River, Headwater Reservoirs or Upper River	▪ Largescale sucker (*Catastomus macrocheilus*), ▪ Northern pikeminnow (*Ptychocheilus oregonensis* ▪ Mountain whitefish (*Prosopium williamsoni*) ▪ Smallmouth bass (*Micropterus dolomieui*) ▪ Largemouth bass (*Micropterus salmoides*) ▪ Black crappie (*Pomoxis nigromaculatus*) ▪ Bluegill (*Lepomis macrochirus*) ▪ Brown bullhead (*Ameiurus nebulosus*) ▪ Rainbow trout (*Oncorhynchus mykiss*) ▪ Coastal cutthroat trout (*Oncorhynchus clarki clarki*) ▪ Carp (*Cyprinus carpio*) ▪ Bullheads (*Ameiurus sp*.) ▪ White crappie (*Pomoxis annularis*)	Henny *et al*, 2008
	▪ Marine species from bays and coastal waters from Queensland, Australia	▪ Green turtle (*Chelonia mydas*) ▪ Hawksbill turtle (*Eretmochelys imbricata*) ▪ Flatback turtle ▪ Dugong (*Dugong dugon*) ▪ Banana prawn (*Penaeus merguiensis*) ▪ Mudcrab (*Scylla serrata*) ▪ Mullet (*Mugil cephalus*) ▪ Yellow-fin bream (*Acanthopagrus australis*) ▪ Flathead (*Platycephalus fuscus*) ▪ Longtom (*Tylosurus gavialoides*) ▪ Tailor (*Pomatomus saltrix*) ▪ Jelly-fish	Hermanussen *et al*, 2008

		▪ Carnivorous loggerhead turtles ▪ Hawksbill	
	▪ Spanish Atlantic Southwest Coast	▪ Wegde sole (*Dicologoglossa cuneata*) ▪ Common sole (*Solea vulgaris*) ▪ White seabream (*Diplodus sargus*) ▪ Sardine (*Sardina pilchardus*) ▪ Angler fish (*Lophius piscatorius*) ▪ Two shellfish species (*Donax trunculus* and *Chamelea gallina*) ▪ Common cuttlefish (*Sepia officinalis*) ▪ Prawns (*Parapenaeus longirostris*)	Bordajandi *et al*, 2006
	▪ Korea	▪ Squid (*Todarodes pacificus*) ▪ Alaska pollack (*Theragra chalcogramma*) ▪ Mackerel (*Scomber japonicus*) ▪ Yellow croaker (*Pseudosciaena manchurica*) ▪ Anchovy (*Engraulis japonica*) ▪ Tuna (*Thunnus thynnus*) ▪ Hairtail (*Trichiurus lepturus*) ▪ Clam (*Tapes philippinarum*) ▪ Crab (*Portunus trituberculatus*) ▪ Shrimp (*Solenocera melantho*) ▪ Olive flounder (*Paralichthys olivaceus*) ▪ Octopus (*Octopus minor)* ▪ Spanish mackerel (*Scomberomorus niphoniu)* ▪ Oyster (*Crassostrea gigas*) ▪ Saury (*Cololabis saira*) ▪ Cod (*Gadus macrocephalus)* ▪ Rockfish (*Sebastes schlegeli*)	Moon & Ok, 2006

		▪ Eel (*Conger myriaster*) ▪ Flounder (*Eopsetta grigorjewi*) ▪ Catfish (*Parasilurus asotus*) ▪ Ark shell (*Scapharca subcrenata*) ▪ Angler fish (*Lophiomus setigerus*) ▪ Filefish (*Thamnaconus modestus*) ▪ Ray (*Raja kenojei)* ▪ Mussel (*Mytilus edulis*) ▪ Herring (*Clupea pallasii)*	
	▪ European, North American, Asian and African areas	▪ *Scomber japonicus, Clupea pallasii, Conger myriaster, Thunnus thynnus, Trichiurus lepturus, Scomberomorus niphoniu*	Domingo & Bocio, 2007
Polychlorinated Biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAH) - Dioxin-like polychlorinated biphenyls (DL-PCBs) - TCDD (3,3',4,4',5 pentachlorobiphenyl PCB-126) - Aquatic biocide tributyltin			
	▪ The North sea in the Netherlands	▪ Flatfish sole (*Solea solea*)	Foekema *et al*, 2008
	▪ Willamette River and lower Santiam River	▪ Northern pikeminnow (*Ptychocheilus oregonensis* ▪ Smallmouth bass (*Micropterus dolomieui*) ▪ Largescale sucker (*Catastomus macrocheilus*)	Henny *et al*, 2008
	▪ The Environment of the Southern Moravia Region, Czech Republic	▪ *Barbus barbus*	Lana *et al*, 2008

(TBT) - Organochlorine pesticides (OCPs) - Pentachlorobenzene (QCB) - Octachlorostyren (OCS) - Hexachlorobenzene (HCB) - Hexachlorocyclohexane isomers (HCHs) - Chlordane compounds (CHLs, including transchlordane, cis-chlordane, trans-nonachlor and cis-nonachlor)	▪ PCB isomers and congeners (ng/g wet wt.) in fish and shellfish from Dalian, Tianjin and Shanghai in China	▪ Flathead flounder ▪ Halfbreak fish ▪ Small yellow croaker ▪ White sardine ▪ Escallop ▪ Tilefish ▪ Chubmackerel ▪ Bastard halibut ▪ Rock bream ▪ Razor clam ▪ Yellow porgy ▪ Perch ▪ Egg of Perch Pacific cutlassfish ▪ Crucian carp ▪ Octopus ▪ Squid	Yang *et al*, 2006
	▪ Organochlorine pesticides (ng/g wet wt.) in fish and shellfish from Dalian, Tianjin and Shanghai in China	▪ Dotted gizzard shad ▪ Eel ▪ Gray mullet ▪ Chub-mackerel ▪ Red tongue sole ▪ Hen clam ▪ Squid ▪ Octopus ▪ Codfish ▪ Jack knife clam ▪ Black Chinese roach	

		▪ Gurnard ▪ Small yellow croaker ▪ Pacific cut-lassfish ▪ Dotted gizzard shad	
	▪ Commercial food amended with canola oil solutions containing PCB-126 or TBT at 0, 1, or 100 ppb	▪ Channel catfish (*Ictalurus punctatus*)	Burton *et al*, 2002
	▪ Organochlorines (polychlorinated biphenyls, RDDTs, hexachlorobenzene, hexachlorocyclo hexanes, chlordanes, mirex and dieldrin) from the Shing Mun Riverin Hong Kong	▪ Tilapia (*Tilapia mossambica*) ▪ Big head (Aristichthys nobilis) ▪ Grass carp (Ctenopharyngodon idellus) ▪ Mud carp (Cirrhunus molitorella) ▪ Napolean wrasse (Cheilinus undulates) ▪ Grouper (Epinephelus akaara) ▪ Grouper (Epinephelus coioides) ▪ Gold line sea bream (Rhabdosargus sarba) ▪ Ochiai Macau sole (Heteromycteris matsubarai) ▪ Freshwater grouper (Micropterus spp) ▪ Golden thread (Nemipterus virgatus) ▪ Flat head (Platycephalus indicus) ▪ Big eye (Priacanthus tayenus) ▪ White pomfret (Stromateoides argenteus) ▪ Hair tail (Trichiurus haumela) ▪ Rabbit fish (Siganus canaliculatus)	Chan *et al*, 1999
Heavy metals (As, Cd, Cu, Hg, Pb), total mercury (THg) and persistent	▪ Willamette River and lower Santiam River	▪ *Salmo salar, Penaeus monodon, Perna viridis, Pectinidae sp., Selaroides leptolepis*	Bayen *et al*, 2005

organic pollutants (POPs) such as organochlorine pesticides, polybrominated diphenyl ethers (PBDEs) and dioxins and furans		▪ Whitefish (Prosopium williamsoni)	Henny *et al*, 2008
Polybrominated diphenyl ethers (PBDEs)	▪ Electronic waste (e-waste) recycling site in South China.	▪ Chinese mysterysnail (*Cipangopaludina chinensis*) ▪ Prawn (*Macrobrachium nipponense*) ▪ Mud carp (*Cirrhinus molitorella*) ▪ Crucian carp (*Carassius auratus*), ▪ Northern snakehead (*Ophicephalus argus*) ▪ Water snake (*Enhydris chinensis*)	Wu *et al*, 2008
Methylmercury (MeHg) - inorganic mercury, ethylmercury (EtHg) and phenylmercury (PhHg)	▪ Seafood samples from Yantai port, China	▪ Dogfish (*Squalus acanthias*) ▪ *Ruditapes philippinarum* ▪ *Sinonovacula constricta* ▪ *Neverita didyma* ▪ *Saxidomus purpuratus* ▪ *Scapharca subcrenata*	Yin *et al*, 2008
	▪ Japan	▪ Cod fish ▪ Cod muscle powder	Inagaki *et al*, 2008
	▪ Aquatic environment	▪ Tuna fish	Reyes *et al*, 2008
	▪ DA	▪ Tilapia ▪ Sword fish ▪ Mackerel ▪ Halibut ▪ Hake	Kuballa *et al*, 2008

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		▪ Bass ▪ Common mussel ▪ Shrimp ▪ Coalfish ▪ Redfish	
	▪ In the dock of Sesimbra in the Portuguese coast	▪ Swordfish (*Aphanopus carbo*) ▪ Dogfish *Tintureira* (*Prionace glauca*) ▪ Dogfish *Anequim* (*Isurus oxyrhinchus*) ▪ Ray (*Raja oxyrhinchus*) ▪ Dogfish *Carocho* (*Centroscymnus coelolepsis*) ▪ Dogfish *Lixa* (*Centrophorus squamosus*) ▪ Sapata (*Deania calceus*) ▪ Dogfish *Cação* (*Gauleorhinus galeus*) ▪ Sardine (*Sardina pilchardus*) ▪ Mackerel shad (*Trachurus trachurus*) ▪ Octopus (*Octopus vulgaris*)	Carvalho *et al*, 2008
Chlordane and DDT organochlorine Pesticides (dichlorodiphenyltrichloroethane (DDT) and its metabolites (DDTs, including o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT and p,p'-DDT)	▪ In the Hong Kong waters, in local waters from inland rivers to estuarine regions and in the South China Sea	▪ Gold line sea bream (Rhabdosargus sarba) ▪ Freshwater grouper (Micropterus spp) ▪ Grouper (Epinephelus akaara)	Chan *et al*, 1999
Halogenated aromatic hydrocarbons (HAHs) such as chlorinated dioxins, dibenzofurans and biphenyls	▪ Willamette River and lower Santiam River	▪ Northern pikeminnow (*Ptychocheilus oregonensis* ▪ Smallmouth bass (*Micropterus dolomieui*) ▪ Largescale sucker (*Catastomus macrocheilus*)	Henny *et al*, 2008

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comprise a major group of marine contaminants. Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychalogenated diphenyl ethers (PCDEs and PBDEs), and several other classes of compounds (isomers and congeners)	▪ On the Coosa River at Childersburg, the Alabama River at Eureka Landing, the Tombigbee River at Lavaca, and the Mobile River at Bucks	▪ McIntosh bass ▪ Largemouth bass (*Micropterus salmoides*)	Hinck *et al*, 2008
	▪ DDTs, including o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT and p,p'-DDT in fish and shellfish collected from Dalian, Tianjin and Shanghai in China	▪ Dotted gizzard shad ▪ Eel ▪ Gray mullet ▪ Chub-mackerel ▪ Red tongue sole ▪ Hen clam ▪ Squid ▪ Octopus ▪ Codfish ▪ Jack knife clam ▪ Black Chinese roach ▪ Gurnard ▪ Small yellow croaker ▪ Pacific cut-lassfish ▪ Dotted gizzard shad	Yang *et al*, 2006
	▪ In Hong Kong market	▪ Tilapia (*Tilapia mossambica*) ▪ *Rhabdosargus sarba* ▪ *Epinephelus akaara* ▪ *Micropterus spp* ▪ *Platycephalus indicus* ▪ *Siganus canaliculatus*	Chan *et al*, 1999

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*No information available

Table 2.2. Chemical toxins of microorganisms

Toxins of microorganisms	Microorganism	Fish/shellfish	Environmental source	Reference
Cholera (cfx) toxin and zonula occludens (zot) toxin	*Vibrio cholerae* 01, 0139 and non-01 strains	Clam, Shrimp and Salmon	Autochthonous bacterial species in aquatic environments	Ahn & Durst, 2008 Rivera *et al*, 1995
Phycotoxins	Algae, dinoflagellates, and cyanobacteria or blue-green algae	Pelagic species	Periodic blooms of algae "red tides"	Luckas, 1992
Cyanotoxins (anatoxins, microcystins, nodularins, cylindrospermopsin)	Cyanobacteria: *Microcystis sp.* (including e.g. *M. aeruginosa*, *M. flos aquae* and *M. viridis*), and *Planktothrix* (*P. agardhii* and *P. rubescens*) *Nodularia spumigena*, *Anabaena sp*., *Aphanizomenon sp*.	Mussels (*Mytilus edulis*, *Anodonta cygnea*), fish: carp (*Hypophthalmichthys molitrix*), *Tilapia rendallii*, freshwater snail (*Bellamya aeruginosa*, *Melanoides tuberculata*), crayfish (*Cherax quadricarinatus*), prawns (*Penaeus monodon*, *Palemon modestus*, *Macrobrachium nipponensis*) and red swamp crayfish (*Procamburus clarkii*), oysters (*Pinctada maxima*)	Waterblooms: Proliferations of freshwater toxin-producing cyanobacteria are simply called "cyanobacterial blooms" or "toxic algal blooms."	Ibelings & Chorus, 2007
Shiga toxin (STEC) and *E. coli* heat-stable toxin (STa), single-nucleotide polymorphism (SNP)	*Escherichia coli*	Clam and fish	Autochthonous bacterial species in marine environments	Kumar *et al*, 2004 Kalamaki *et al*, 1997
Type E toxin, Botulinum neurotoxin (BT)	*Clostridium botulinum*	Salmon		Ahn-Yoon *et al*, 2004 Wong, 1995 Wictome *et*

				al, 1999
Cytotoxin	Cyanobacteria (mainly *Cylindrospermopsis raciborskii* and *Umezakia natans*)	Mussels (*Mytilus edulis*), freshwater clams and fish such as flounder (*Platichthys flesus*) or salmon		Mankiewicz *et al*, 2003 Hitzfeld *et al*, 1999
Dermatotoxins including aplysiatoxins, debromoaplysiatoxins and lyngbyatoxins	Tropical and subtropical marine benthic cyanobacteria such as *Oscillatoria, Lyngbya* and *Schizothrix*			
Irritant toxins - Lipoppollysaccharides (LPS)	Cyanobacteria such as *Salmonella*			
Hepatotoxins	*Microcystis* (*Microcystis aeruginosa*), *Planktothrix, Anabaena, Nodularia, Nostoc* and *Umezakia*			
Diphtheria toxin (DT)	*Corynebacterium diphtheriae*	In water and maybe in shellfish and fish	NA*	Kalamaki *et al*, 1997
Tetanus toxin (TT)	*Clostridium tetani*			
Other Toxins	*Pfiesteria piscicida* and *P. shumwayae*	Shellfish and tropical fishes	Autochthonous bacterial species in aquatic environments	Bowers *et al*, 2000

*No information available

Table 3.1. Rapid methods (Biological, Chemical, Biochemical, Molecular and Immunochemical) for toxin detection				
	Biological methods	Methods	Fish/ Shellfish	Reference

A	In vivo bioassays: Deliberate exposure of test animals to contaminants or contaminated materials. Either in the field, e.g. caging studies, or in the laboratory.	Mouse bioassay (MBA)	Clams *Saxidomas giganteus*	Manger *et al*, 2003
			Clams, mussels, and oysters	Pierce & Kirkratrick, 2001
			Mussel	Fremy *et al*, 1999
			Seafood	Wictome *et al*, 1999
			Mussels	Terao *et al*, 1986
			Blue mussels: *Mytilus edulis*	Bates *et al*, 1978
		Receptor Binding Assay (RBA)	Shellfish	Report of the Joint FAO/IOC/WHO, 2004
		Cytotoxicity assays: Mouse neuroblastoma cells lines rat cerebellar neurones (neuro-2a neuroblastoma cells)	Cyanobacteria *C. raciborskii* and in shellfish	Humpage *et al*, 2007
		Hemolysis assay for the detection of sodium channel (SC)	Fish: Red tilapia *Sarotherodon mossambicus*	Shimojo & Iwaoka, 2000
		Rats bioassay: primary cultures of rat hepatocytes	Shellfish: Green-shell mussels	Kerr *et al*, 1999
		Rabbit skin permeability factor (PF) assay		
		Mosquito bioassay		Usleber *et al*, 1991

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B	In vitro bioassays- *In vitro* Functional Assays	Measure receptor-binding	Shellfish: Butter clams *Saxidomas giganteus*	Manger *et al*, 2003
	Bioassays employing cultured cells or cellular extracts.	Enzyme Inhibition		
		Changes in gene expression in cultured cells		
		Sodium channels assays	Tilapia (*Sarotherodon mossambicus*)	Shimojo & Iwaoka, 2000
		Cytotoxicity assays (Cytotoxicity bioassays utilize cell culture of neuroblastoma mouse cells to provide voltage-sensitive sodium channel receptor sites for assay of sodium channel-specific phycotoxins)	Clams, mussels, and oysters	Pierce & Kirkratrick, 2001
		Voltage-sensitive sodium channels (VSSC)	Shellfish	Inoue & Hirama, 2004
			Carnivorous fish	Takai *et al*, 2003
		Neuroblastoma assays	Molluscs	Report of the Joint FAO/IOC/WHO, 2004
		A microtiter plate-based receptor binding assay	Shellfish: Common bivalve molluscs, including *Mytilus californianus*, *Mytilus edulis*, *Saxidomus nuttalli* and *Crassostrea giga*, Clams and oysters	Doucette *et al*,1997
		Solidphase radioreceptor assay		
		Mouse synaptoneurosome assay	Shellfish: Mussels and other	Nicholson *et*

		CD1 mouse injection assay	bivalve species: scallops, geoducks, butterclams, razor clams, little clams and fresh oysters	*al*, 2002
		A protein phosphatase-based assay *in vitro* phosphatases type 1 (PP1) and 2A (PP2A)	Oyster	Honkanen *et al*, 1996
			Carnivorous fish	Takai *et al*, 1995
			Bivalve molluscs	Report of the Joint FAO/IOC/WHO, 2004
C	In vivo biomarkers: biochemical, physiological, or other types of biological changes that indicate the presence or effects of xenobiotic compounds.	Commonly used biomarkers of exposure and effect.	Fish and shellfish	Hahn, 2002
D	Cell culture bioassays	The YI adrenal cell assay	Shellfish: Clam *Ruditapes philippinarum*	Okumura *et al*, 2005
	Cells in culture are referred to variously as in vitro, in vivo, or ex vivo, depending on the perspective and bias of the investigator. Application of cell culture bioassays.	Buffalo green monkey (BGM) kidney cell cultures	*Mytilus galloprovincialis*	Croci *et al*, 1997
Chemical methods and Biochemical techniques				

A	Numerous chemical methods such as SEM are used for the detection of toxins.	Gas chromatography (GC)		Bordajandi *et al*, 2006
		Gas chromatography (GC) with detection by electron capture or mass spectrometry (MS) –or capillary electrophoresis (ECD)	Fish: *Barbus barbus*	Lana *et al*, 2008
			Alaska butter clam *Saxidomus giganteus*	Luckas, 1992
			Dinoflagellates: *P. concavtun* and *P. lima*	Dickey *et al*, 1990
		SSID–GC–ICPMS	Cod fish	Inagaki *et al*, 2008
		Ion-spray/ electrospray (ESI)	Shellfish	Garthwaite, 2000
		Scanning electron microscopy (SEM)		
		Light microscopy (LM)		
		High-performance liquid chromatography (HPLC) with fluorimetric detection (FOD)	*Ruditapes Mphilippinarum*	Okumura *et al*, 2005
			Clams, mussels and oysters	Pierce & Kirkratrick, 2001
			Algae *Tetraselmis suesica*-Chlorophyceae and *Skeletonema costatum*	Garthwaite, 2000
			Scallops and mussels	Fremy *et al*, 1999
			Molluscan	Powell & Doucette, 1999

			Saxidomus giganteus, Molluscs (*Myrilus edulis*), shellfish, bivalves, mussels clams (*Protothaca stuminea*)	Luckas, 1992
			Blue mussel (*Mytilus galloprovincialis*)	Anderson *et al*, 1989
			Blue mussels (*Mytilus edulis*), oysters (*Crassostrea gigas*), scallops (*Pecten maximus*) and clams (*Ruditapes philippinarum*)	Lassus et *al*, 1989
			Shellfish	Cembella *et al*, 1987
			Rockfish, Salmon, Lobster, and Shrimp	Wills *et al*, 1987
			Mussels (*Mytilus edulis*) Butter clams (*Saxidomus giganteus*), Littleneck Clams littleneck clams (*Protothaca staminea*)	Sullivan *et al*, 1985
			Puffer fish: *Takifugu poecilonotus*, *T. vermicularis* and *T.radiates*	Nakamura *et al*, 1984
			Butter clams, Littleneck clams, Oysters, Mussels	Sullivan *et al*, 1983
		HPLC-ICP-MS	Tuna fish	Reyes *et al*,

				2008
		HPLC-UV and LC-MS	Shellfish: Scallops	Hess *et al*, 2005
		Gel-filtration chromatography	Shellfish	Garthwaite, 2000
		Ion-exchange chromatography	Shellfish: Mussels and phytoplankton *Dinophysis* spp., *Prorocentrum* spp., *Protoceratium Reticulatum, Lingulodinium polyedrum*	Draisci *et al*, 1999
		Thin-layer chromatography (TLC)	Shellfish: Blue mussels	Quilliam *et al*, 1998
		Ion spray liquid chromatography-mass spectrometry (LC ISP-MS)	Shellfish: mussels (*Mytilus galloprovincialis*) *and* algae such as *Dinophysis fortii, D. sacculus, D. tripos,* and another species similar to D.*acuminata*	Draisci *et al*, 1995 Garthwaite, 2000
		Alkaline oxidation method (the alkaline peroxidation technique)	Shellfish	Garthwaite, 2000
		Liquid chromatography (LC)	Phytoplankton: *Dinophysis acuta*	James *et al*, 1997
			Oysters (*Crassostrea*	Dickey *et al*,

			virginica) and phytoplankton	1992
		LC–MS/MS	Mussels and Dinoflagellate *Protoperidinium crassipes*	Krock *et al*, 2008
			Danish surf clams (*Spisola sp*.) and blue mussels (*Mytilus edulis*)	Jorgensen *et al*, 2005
				Puente *et al*, 2004
			Clams, mussels, and oysters	Pierce & Kirkratrick, 2001
				Garthwaite, 2000
		LC-MS	Mussels (*Mytilus edulis*) cockles (*Cerastoderma edule*) oysters (*Crassostrea gigas*) king scallop (*Pecten maximus*)	Stobo *et al*, 2005
			Scallops and mussels	Fremy *et al*, 1999
		LC–UV	Shellfish: mussel *Mytilus edulis*, clams *Siliqua patula*, and phytoplankton *Pseudonitzchia australis*	James *et al*, 2000
		LC-ESI-MS	*Mytilus chilensis*, *Venus antiqua*, *Pecten jacobaeus*	López-Rivera *et al*,

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			and *Pecten maximus*	2005
				Quilliam, 1995
		Micellar electrokinetic capillary chromatography (MEKC) in conjunction with laser-induced fluorescence (LIF) detection (MEKC-LIF)	Clams, mussels, and oysters	Pierce & Kirkratrick, 2001
		Micellar electrokinetic chromatography (MEKC) with ultraviolet (UV) detection	Shellfish: Mussels and dinoflagellate *Prorocentrum lima*	Bouaïcha *et al*, 1997
		Microplate fluorescence method (active phosphodiesterases)	Shellfish: Scallop *Patinopecten yessoensis* and mussels *Mytilus galloprovincialis*	Alfonso *et al*, 2004
		FI-CV-AFS	*Prionace Mglauca*, *Isurus oxyrhinchu*, *Xiphias gladius*	Carvalho *et al*, 2008
		Fluorimetric assays	Shellfish: Shell clams (*Mya arenaria*), blue mussels (*Mytilus edulis*), and scallop viscera (*Placopecten magellanicus*)	Indrasena & Gill, 1998 Louzao *et al*, 2001
		Fluorometric Detection Periodic acid, *t*-butyl hydroperoxide, and hydrogen peroxide Biosensors		
		Capillary electrophoresis (CE) combined with UV	Mussels, clams and anchovies	Zhao *et al*, 1997
	Atomic spectroscopy	Fast atom bombardment mass spectrometry (FAB-MS)	Blue mussels *(Mytilus edulis* L.)	Quilliam *et al*, 1989
		Atomic absorption spectrometry (AAS)	Dogfish and muscle	Yin *et al*, 2008
		Inductively coupled plasma mass spectrometry		

		(ICP-MS)		
		Atomic fluorescence spectrometry (AFS)		
		Atomic emission spectrometry (AES)		
Immunological assays/ immunochemical methods				
A	Immunoassays using chemical-specific antibodies prepared in sheeps and rabbits, following immunization with conjugates of the polyether toxins - Gold Labeled Immunosorbent Assay test	Enzyme-linked immunosorbent assay (ELISA)	Clam and fish	Kumar *et al*, 2004
			Shellfish: Oysters dinoflagellate *Karenia brevis* and Mammalian Body Fluid	Naar *et al*, 2002
			Clams, mussels, and oysters	Pierce & Kirkratrick, 2001
			Scallops and mussels	Fremy *et al*, 1999
			Seafood	Wictome *et al*, 1999
		Competitive direct enzyme-linked immunosorbent assay (cdELISA) and a competitive indirect ELISA (ciELISA)	*Mytilus edulis*, *Meretrix lusoria*, *Crassostrea gigas*	Yu *et al*, 2004
		The Immunoblot ELISA	Shellfish and fish	Kalamaki *et al*, 1997
		Indirect enzyme immunoassay (EIA)- IDR Histamine EIA Test	Reef fish	
		Enzyme immunoassay (EIA) in microtitration plate (ELISA) and a test strip EIA	Mussel (*Mytilus edulis*)	Kawatsu & Hamano, 2000
			Mussel and clam	Usleber *et*

				al, 1991
		Competitive enzyme-linked immunosorbent assay (cELISA)	Shellfish: Mussel	Hesp *et al*, 2005
		Enzyme activity-based assays (Development of a protein phosphatase-based assay (PP2A)	Shellfish: Oyster, marine dinoflagellates, commonly belonging to the genus *Dinophysis*	Honkanen *et al*, 1996
		Immunoaffinity chromatography (IAC)	Shellfish: Clams, mussels, and oysters	Usleber *et al*, 2001 Fremy *et al*, 1999
		Enzyme-linked immunofiltration assays		Kalamaki *et al*, 1997
		Microtiter plate enzyme immunoassay (EIA)		
		Dipstick and enzyme-linked immunofiltration assay (ELIFA) formats		
		LFIC Lateral Flow Immuno-Chromatography	Molluscs	Report of the Joint FAO/IOC/WHO, 2004
		Immunomagnetic Separation (IMS) technique	Shellfish	Kalamaki *et al*, 1997

B	The stick test immunoassay (monoclonal antibodies in the stick enzyme immunoassay) - filter paper from air or water samples	MISTAlert™ (Commercial Qualitative Assay)	Shellfish: Bivalve Molluscs (*Mytilus edulis*), scallop (*Pecten maximus*), queen scallops (*Aquapecten opercularis)* razor fish (*Ensis arcuata*) native oysters (*Ostrea edulis*), and pacific oysters (*Crassostrea gigas*)	Jellett *et al*, 2002 Mackintosh *et al*, 2002 Pierce & Kirkratrick, 2001
		Immunodiffusion/Motility Enrichment Test	Shellfish	Kalamaki *et al*, 1997
		Monoclonal antibody enzyme immunoassay (Mab)	Seafood	Wong, 1996
		Enzyme Immunoassay Stick test (Biosense®, DSP Check® test, Rougier Bio-Tech®)	Fish: *Ctenochaetus strigosus and Thunnus thynnus* Dinoflagellate: *Gambierdiscus toxicus*	Hokama *et al*, 1987
		Latex agglutination assay	Clams, mussels, and oysters	Kalamaki *et al*, 1997
C	Radioimmuno-assays (RIA) and enzyme-linked immunosorbent assays (ELISA)	The radioimmunoassay procedure mixes a known amount of radio-labeled toxin with purified antibody receptor, followed by the addition of the unknown toxin sample	Shellfish: Clams, mussels, and oysters	Pierce & Kirkratrick, 2001
			Mussels, clams, anchovies and crab	Lawrence *et al*, 1994
D	Immuno-liposome assay	A ganglioside-liposome immunoassay Gangliosides, sialic acid containing glycosphingolipids	Clam, Shrimp and Salmon	Ahn & Durst, 2008
			Salmon	Ahn-Yoon *et al*, 2004

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				Micheli *et al*, 2004 Kreuzer *et al*, 2002
	Biochemistry and molecular techniques (DNA-based methods)			
A	Polymerase chain reaction (PCR)-based assays	DNA-binding (gel shift) Native responses in cell culture (CYP1A)	Shellfish	Rivera *et al*, 1995
B	Molecular techniques based on the detection of a part of the microorganism's genome such as verocytotoxin genes *VT1* and *VT2*, *the Lamb* gene, the Sth toxin gene, the *uidA* gene, the glycine decarboxylase gone, the listeriolysin O *hly* gene, 16S and 23S rRNA	Real-Time PCR Assays	Seawater, shellfish ant tropical fishes	Bowers *et al*, 2000
		DNA-extraction method	In modified-atmosphere-packaged fish (jack mackerel)	Kimura *et al*, 2001
		DNA-DNA hybridization technique, ERIC (enterobacterial repetitive intergenic consensus sequences (ERICs)-PC	Clams, mussels, oysters and fish	Kumar *et al*, 2001 Kalamaki *et al*, 1997
		Multiplex PCR plus hybridization PCR DNA probe		
		RT-PCR		
		cc (cell culture)- RT-PCR RT-seminested-PCR	Shellfish: Autochthonous bacterial species in aquatic environments	Rivera *et al*, 1995
		RT-PCR nested-PCR plus hybridization RFLP (RV)-RNA		
		Multiplex PCR		
		AFLP-PCR (amplified fragment length polymorphism)		
		Multiplex Real-Time PCR Method	Seafood	Jinneman *et al*, 2003
		TaqMan PCR assay	Shellfish: Raw Oysters and	Lyon, 2001

		Rep-PCR	Synthetic Seawater	
		cRT-PCR		
		RADP-PCR		
		Ac-PCR		
		Minor-groove binder (MGB)	Mussels (*Mytilus edulis* and *Mytilus galloprovincialis*)	Croci *et al*, 1997
		The real-time PCR MGB		
		PCR- fluorescence resonance energy transfer (FRET)		
		Real-time PCR STEC method		

Table 3.2. Rapid methods detections of seafood toxins

Rapid detection of seafood toxins (Methods)	Toxins	Fish or shellfish	Experimental/ Procedure	Reference
Cell bioassay: antagonism cell bioassay the mouse	Saxitoxin (STX) or brevetoxin (PbTx) or ciguatoxin (CTX)	Butter clams *Saxidomas giganteus*	• Cell suspensions were inserted into microtiter 96-well culture plates using 200 µL per well of either Neuro-2a cells at $5x10^5$ cells/ml or SK-N-SH cells at ca $2x10^5$ cells/ml. • Incubation (37^o C for 6–8 h), processing with MTT and detection on a multi well scanning spectrophotometer	Manger *et al*, 2003

bioassay (MBA)			followed	
Mouse brain synaptone uro-somes (*in vitro* assay)	STX	Shellfish: Mussels and other bivalve species: scallops, geoducks, butterclams, razor clams, little clams and fresh oysters	• The brain was chopped into pieces with a razor blade and the tissue parts were homogenized in 2.5 ml saline using a Teflon homogenizer. • Resuspended material centrifugation (1000g for 15 min) and final synaptoneurosomal pellet followed • Processing at 4^{o}C	Nichols on *et al*, 2002
A microtiter plate-based receptor binding assay	STX	Shellfish: Common bivalve molluscs, including *Mytilus californianus /edulis*, *Saxidomus nuttalli* and *Crassostrea giga*. Clams and oysters. Dinoflagellate species of the genus *Alexandrium*	• Tissue homogenate centrifugation at 54,000 g for 15 min at 4°C and the supernatant discarded. • Working stock solution containing 5.0 nM [^{3}H] STX made up in 75 mM HEPES/140 mM NaCI buffer, pH 7.5. • Preparation of unlabeled STX solutions for development of a standard curve (use of a STX dihydrochloride standard). • Incubation for 1 hr at 4^{o}C and filtering (MultiScreen ^{TM} vacuum manifold)	Doucett e *et al*, 1997
Bioassay cell-based Neuro-2A neuroblas toma toxicity assay	STXs, GTX1 to GTX6 and decarbamoyl (dc)-GTX1 to dcGTX4, C toxins (C1 to C4)	Shellfish: Using the cyanobacteria, *C. raciborskii*	• Use of pure toxins the neuroblastoma assay: STX, neoSTX, dcSTX, an epimeric mix of GTX2/3, an epimeric mix of GTX1/4, and GTX5. • Neuro-2A cell–based bioassay: Addition of 25,000 cells to each well of a 96-well microtiter plate and incubation for 24 h. • Addition of veratridine (0.05 mM) and ouabain (0.5	Humpa ge *et al*, 2007

			mM), along with the sample (20 µl in a 200µl total volume), to the culture medium (RPMI-1640 with 300 mg/L of L-glutamine and without sodium bicarbonate but with 10% newborn calf serum, 110 mg/L of sodium pyruvate, 2 g/L of sodium bicarbonate, 62.5 mg/L of penicillin G, and 100 mg/L of streptomycin sulfate, adjusted to pH 7.4±0.05)	
Bioassay: mouse neuroblastoma tissue culture assay for sodium channel using red blood cells (RBCs). Hemolysis assay for the detection of sodium channel	STX, Tetrodotoxin (TTX) and (CTX-a)	Fish: Red tilapia (*Sarotherodon mossambicus*). Dinoflagellate (*Prorocentrum mexicanum*) and fish (*A. triostegus*, *M. cephalus*, and *K. sandsicensis*)	• A sodium channel activator resulted in enhanced sodium permeability and ouabain, an inhibitor of Na^+/K^+ ATPase in combination samples potentially contaminated with biotoxin. • Toxins were placed in sterilized 0.1-M acetic acid, respectively, at 0°C, and opened as required. • Veratridine, 1-mM in acidic (pH 2) distilled water, at 4°C. • Dillutions of extracts in 20% MeOH (200 µg/ml). • RBCs within 4-6 h in all the test wells	Shimojo & Iwaoka, 2000

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Cell bioassay: further modifications of the cell-bioassay, MBA and HPLC	Maitotoxin (MTX)	Shellfish: Clam (*Ruditapes philippinarum*)	• Cultured MTX, a marine toxin of ciguatera fish poisoning, which limits incubation period to 6 h when applied to the microplate 15 min prior to the end of the incubation -antagonism WST-8, a dehydrogenase for the dectection of water-soluble tetrazolium salt.	Okumura *et al*, 2005
Development of a protein phosphatase-based assay (PP2A)	OA, DTX-1 (35 methylokadaic acid) and DSP toxins	Shellfish: Oyster, marine dinoflagellates, commonly belonging to the genus *Dinophysis*	• Purification of he catalytic subunit of PP2A to apparent homogeneity using G-75 in the place of Ultrogel-AcA44. • Conduction of assays at 30°C for 10 min. • Termination after addition of 100 µ1 of 1 NH_2SO_3 containing 1 mM K_2HPO_4.	Honkanen *et al*, 1996
Tissue culture assay (TC-CC) (direct microscopic observation of toxin-induced	OA	Mussels: *Mytilus edulis* and *M. galloprovincialis*	• Preparation of standard stock solution containing 20 pg OA/ml by dissolving OA standard in methanol. • Suspention of the residue in triple-distilled water containing 0.8% NaCl (w/v). 0.1 % glucose (w/v), 2% foetal bovine serum (v/v), 0.5% Tween 60 (v/v), pH 7.4. • Clarification of the suspension was clarified by addition of carbon black and, after 5 min of centrifugation at 1200rpm collection of the supernatant. • Cells were checked for morphological changes each hour for the first 7 hr of incubation, and at 24 hr.	Croci *et al*, 1997

morphological changes in (BGM) cell cultures).				
In vitro rat hippocampal slice preparation	Combination of toxins: STX, PbTx and DA	Shellfish: Green-shell mussels	• Field EPSP and fiber spike analysis, extracellular field potentials were recorded from the pyramidal cell layer and dendritic field of hippocampal region CA1. • Toxins were washed in for 30-45 min, and washed out (45-90 min).	Kerr *et al*, 1999
Histopathological studies: Sequrntial ultrastructural changes in mouse digestive organs after injections of dinophysi	DTX_1 and PTX_1	Shellfish: Mussels	• Induction of ultrastructural changes in the small intestine and liver of suckling mice by dinophysiatoxin-1 and pectrnotoxin-1. • Autopsy of animals and organs. • For electron microscopy pieces of the duodenum, the liver and kidney were put in a cold 2% paraformaldehyde- glutaraldehyde solution for 12 hr and then postfixed in 1% OsO_4, for 2 hr. • After 15 min the duodenum and upper portion of the small intestine became distended and contained mucoid, but not bloody, fluid.	Terao *et al*, 1986

stoxin-1 and pectrnotoxin-1				
Fluorescence detection (FLD)	STX: neosaxitoxin (NEO), gonyautoxin 1 and 4 (GTX 1/4), gonyautoxin 2 and 3, C toxins, and B toxins	Shell clams (*Mya arenaria*), blue mussels (*Mytilus edulis*), and scallop viscera (*Placopecten magellanicus*) Dinoflagellate, *Gonyaulax catenella*	• Testing of periodic acid, *t*-butyl hydroperoxide, and hydrogen peroxide. • Hydrogen peroxide was the most convenient and efficient oxidant (the fluorescence could be detected after incubation at 100°C for 3-5min). • Fractionation of freeze-dried powder on a Bio-Gel P2 column (2.5 3 72 cm).	Indrasena & Gill, 1998
High-performance liquid chromatography (HPLC) and MBA	STX, NEO, GTX1/4, GTX2/3, Okadaic acid (OA) and dinophysistoxin-1 (DTX1)	Shellfish: Gastropod (*Neverita didyma*) and scallop (*Argopecten irradians*) clams (*Ruditapes philipinensis*, *Cyclina sinensis*, *Pinna pectinata*), and mussels (*Perna viridis*)	• Labeling of samples and duly delivered to the laboratory for analysis, or stored at -20^{o}C. • MBA: addition of 0.1M HCl (pH: 3). • The mixture was boiled for 5min • Cntrifugation for 15min at 3000 rpm. • Ingection into three female KM strain mice. • HPLC analysis: Addition of 500 ml 0.03M periodic acid, 0.3M Na_2HPO_4 and 0.3 M ammonium formate mixture (1:1:1, v/v/v) to 100 ml shellfish, RP-18 column (4.6x150mm)	Wu *et al*, 2005
Comparison of HPLC and the standard	Neurotoxins (GTX II, GTX III, STX) N-l hydroxy toxins (NEO, GTX I-IV) sulfocarbamoyl toxins	Shellfish (Butter clams, Littleneck clams, Oysters, Mussels): The neurotoxins are produced by *Gonyaulax sp*.	• Extraction of samples • Samples assayed by the mouse bioassay. • HPLC analyses on both amino and cyano columns using methanol and ammonium phosphate buffer mobile phases.	Sullivan *et al*, 1983

AOAC - MBA	(Cl, C2, Bl)		• Preparation of toxin standards (0-10 ppm) in 0.03 M acetic acid for all of the toxins.	
HPLC and MBA	All the PSP toxins (B1, B2, C1, C2, GTX I, GTX II, GTX III, GTX IV, NEO, STX)	Shellfish: Mussels (*Mytilus edulis*) Butter clams (*Saxidomus giganteus*), Littleneck Clams littleneck clams (*Protothaca staminea*). Dinoflagellate (*Gonyaulux sp*.) blooms	• An aliquot of the supernate was removed and frozed (-20^{o}C). • The extracts were thawed and 40 µl TCA solution (40% trichloroacetic acid (PH 4.5). • Separation of the toxins on a PRP-1 column by ion interaction chromatography, using .a mobile phase containing hexane and heptanes sulfonic acids as ion-pair reagents, ammonium as the cocation and methanol as organic modifier.	Sullivan *et al*, 1985
FLD	Neurotoxins: GTX, C toxins, STX, and related compounds: NeoSTX and dcSTX	Shellfish: Mussel	• Suspension of 0.5 x 10^6 cells placed in stirred quartz microcuvette in the thermostated cell holder in a RF-500 spectro- fluorometer. • Growth of cells in 25 cm^2 tissue culture flasks at 37°C in 5% CO_2. • Subculture by transferring cells released by the application of 0.1% trypsin.	Louzao *et al*, 2001
HPLC with microplate scintillation technology and	Validation of 11-[3H] tetrodotoxin as an alternative radioligand to the [3H]-saxitoxin	Molluscan shellfish: with algal extracts dinoflagellates: *Alexandrium tamarense* and *A. lusitanicum*	• Validation of 11-[3H]-tetrodotoxin as an alternative radioligand to the [3H]-saxitoxin. • Incubation for 1 h at 4°C, filtered and washed with 200 ml ice-cold buffer. • Cetrifugation of samples for HPLC analysis (12000 rev min^{-1}, 1 min, 4°C). • Cleaned using a C-18 sep-pak light solid phase extraction cartridge.	Powell & Doucette, 1999

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Site-specific binding characteristics (Assay Characterization)			• Filtration (0.2 mm filter).	
HPLC	STX and TTX	Puffer fish: *Takifugu poecilonotus*, *T. vermicularis* and *T.radiates*. Toxic dinoflagellate *Protogonyaulax tamarensis*	• The amounts of pooled livers, ovaries and digestive tracts obtained from each species for initial extraction. • Visualization of toxins under u.v. light (365 nm) after spraying with 1% hydrogen peroxide and heating the cellulose strips at 130°C for 5 min. • Elution of Bio-Rex 70 column (1x100 cm) and the column with 500 ml of 0.04 N acetic acid and 200 ml of 1.5 N acetic acid solutions. • Electrophoresis on cellulose strips in 0.083 NTris - HCI buffer (pH 8.7) at 0.6 mA/cm for 10 min	Nakamura *et al*, 1984
Chemical assay and MBA	STX	*Mytilus edulis* (blue mussels) dinofiagellate (*Gonyaulax catenella*)	• Sonication of lyophilized dinofiagellate (*Gonyaulax catenella*) cells for 10 min. • One mouse unit of STX is the unit lethal to a 20 g mouse in 15 min.	Bates *et al*, 1978
HPLC	STX, carbamate toxins (GTX I, GTX II, GTX IV, dc-GTX II, dc-GTX-III) and Cl, C2, Bl (PSP)	Shellfish: Blue mussel (*Mytilus galloprovincialis*) and dinoflagellate *Gymnodinitun Catenatum*	• Growth of cultures in 2 liter flasks with 1 liter of seawater-based at 20°C under a 14:10h light: dark cycle, Sulfocarbamoyl toxins (ca. 90-95 mole %), 35°C Using fluorescence detector: Excitation-340 nm (l5 nm slit) and	Anderson *et al*, 1989

			Emission-400 nm (20 nm slit). • 400 microliters of the supernatant was filtered through 0.45 μm LC3S filters into a teflon capped, borosilicate auto-analyzer vial. • Column: Hamilton Co., PRP-1, 15 cm x 4.1 mm, 10 μm packing. Column: Flow 1.3 ml/ min. • Fluorescence detector Excitation-340 nm (l5 nm slit). Emission-400 nm (20 nm slit)	
HPLC	GTX_1 GTX_2, GTX_3 and GTX_4	Shellfish: Alga *Protogonyaulax* (*Gonyaulax*) *Tamarensisl catenella*, *Pmtogonyaulax catenella* species	• Cells for toxin detection were harvested. • The HPLC was recalibrated after every five samples by chromatographing a standard mixture of toxins diluted in 0.03 N HOAc	Cembel la *et al*, 1987
HPLC	GTX3 and GTXa/ epiGTXs GTX2	Shellfish: Blue mussels (*Mytilus edulis*), oysters (*Crassostrea gigas*), scallops (*Pecten maximus*) and clams (*Ruditapes philippinarum*), Algae *Tetraselmis suesica*-Chlorophyceae and *Skeletonema costatum*. Dinoflagellate *Protogonyaulax tamarensis*	• Culture in 10 liter glass bottles filled with sea-water, (30%) filtered on 0.22 um membrane. • 10 to l5 shellfish were fed with a daily rate of 0.5 liter of culture medium. • HPLC column remained at 35°C. • Post-column reaction was performed with periodic acid in a 1 ml reaction coil maintained at 90°C	Lassus *et al*, 1989
Liquid chromato graphy (LC) and HPLC	Okadaic acid (OA) and domoic acid (DA)	Shellfish: Oysters (*Crassostrea virginica*) and phytoplankton	• Establishment of viable cultures from water (29.5°C). • Sonication of suspensions for 10 min. • Filtration: 0.45 and 0.2 μm filter. • Drying vacuum at 40°C. • The preparations were redissolved in 10.0 ml of aqueous 10% acetonitrile.	Dickey *et al*, 1992

			• Storage at -60°C. • Application of HPLC.	
Liquid chromatography–multiple tandem mass spectrometry (LC-MS/MS)	OA, dinophysistoxins (DTX); pectenotoxin-2 (PTX2) and pectenotoxin-2 seco acids	Shellfish: mussels (*Mytilus edulis*) marine phytoplankton, *Dinophysis acuta*	• Separation of five DSP toxins on aC18 column using an acetonitrile-water gradient with ammonium acetate as an eluent modifier. • ESI in negative mode, was applied for the formation of the molecule in relation to ion, $[M-H]^-$, for each toxin.	Puente *et al*, 2004
Liquid chromatography with mass spectrometry (LC/MS)	OA, DTX-1, DTX-2, Yessotoxin (YTX), homoYTX, 45-hydroxy-YTX, 45 hydroxyhomo-YTX, PTX-1, PTX-2, Azaspiracid (AZA-1), AZA-2, and AZA-3	Mussels (*Mytilus edulis*), cockles (*Cerastoderma edule*), oysters (*Crassostrea gigas*), king scallop (*Pecten maximus*)	• Extraction of toxins from shellfish using Methanol-water (80% v/v). • Analyzation with a C8 reversed-phase column with a 5 mM ammonium acetate–acetonitrile mobile phase under gradient conditions (pH 6.8). • Termination of reaction with HCl (18.5 μL, 2.5M). • Filtration of extract (0.2 μm cellulose acetate filter). • The flow rates, injection volumes, and column temperatures were 0.25 ml/min, 5 μL, and 25^{o}C for the multiple toxins.	Stobo *et al*, 2005
Mass spectrometry (MS) and Biologica	Desulfoyessotoxins 1-Desulfocarboxyhomo YTX and 4-Desulfocarboxyhomo YTX	Adriatic mussels	• Measurement of NMR spectra on a Varian Unity Inova700 spectrometer. • CD3OD was applied as an internal standard. • Negative ESI spectra were achieved on a API-2000 triple quadrupole MS equipped with a turbo ion spray source.	Ciminiello *et al*, 2007

l material hampers spectroscopic analysis (NMR) Spectral Measurements			• HPLC separations were performed on a Varian apparatus. Waters 490 MS UV detector (230 nm) C18column	
LC–MS with ionspray ionization	Diarrhoeic toxins: YTX, OA and four dinophysistoxins (i.e. DTX-1, DTX-2, DTX-2B, DTX-2C), and PTXs involving PTX-2, two PTX-2 secoacids (PTX-2SAs), PTX-2SA, 7-*epi*-PTX-2SA, and AC1	Shellfish: Mussels and phytoplankton *Dinophysis sp*., *Prorocentrum sp*., *Protoceratium reticulatum, Lingulodinium polyedrum*	• Analysis on 20 CULC pump liquid chromatographies run with a total run time of 20 min. • The mobile phase was acetonitrile-water (0.1% TFA), for positive ion mode experiments and acetonitrile–water (2 mM ammonium acetate). • Separations on a microcolumn packed with Supelcosil LC18-DB, under isocratic conditions	Draisci *et al*, 1999
Normal and reversed-phase column chromatography	OA	Shellfish: Dinoflagellate, *Prorocentrum concavum* and species *P. lima*	• Samples were chromatographed on silica gel using chloroform-methanol gradients. • Reversed phase C-I8 HPLC of toxic fractions obtained from silica gel separations were achieved on a RP18 column eluted with 10% water in methanol at a flow rate of 4ml/min. • Recordence of NMR spectra on an instrument consisting of an Oxford 360 MHz superconducting magnet, a	Dickey *et al*, 1990

			Nicolet 118.0E FT data system and 292B pulse programmer. • Concentration of cultures by filtration at 30 days during the mid-exponential phase of growth, (4°C, centrifugation: 16,270g 20 min).	
Ion-spray liquid chromatography-mass spectrometry (LC ISP-MS)	OA and DTX-1	Mussels (*Mytilus galloprovincialis*) and algae such as *Dinophysis fortii*, *D. sacculus*, *D. tripos*, and another species similar to *D. acuminata*	• Extraction of 1 g of homogenized mussel digestive glands three times with 8 ml of acetone each time for 2 min. • Polypropylene glycols (PPG) PPG 1000 and PPG 2000. • Standard stock solutions including 10 µg/ml of OA and DTX-1 acquired from pure compounds by dilution with methanol. • Separation of DSP toxins on analytical column packed with Supelcosil LC18-DB, with a mobile phase of CH_3CN-H_{20} (90:10) (0.1% TFA) and flow rate of 0.8 ml/min. • For LC ISP-MS, a 20:1 split of the column effluent was applied. • Flow rate: 40µl/min to the MS.	Draisci *et al*, 1995
Micellar electrokinetic chromatography with ultraviole	OA and DTX-2 (DSP)	Shellfish: Mussels and dinoflagellate *Prorocentrum lima*	• HP 3D-CE system was applied. • The new column was preconditioned by flushing it with several column volumes (c.10 µ1) of borate buffer (12.5 mM, pH 9.2) and running buffer. • Analyzed at 20±1°C. • Voltage: 30 kV with positive polarity. • The residue was redissolved in 50 µ1 methanol for	Bouaïcha *et al*, 1997

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t detection (MEKC) with ultraviolet (UV) detection			analysis.	
A triple-quadrupole linear ion-trap hybrid LC-MS-MS system	Phycotoxins and novel toxigenic plankton: DA, gymnodimine, spirolides, DTXs, OA, PTXs, YTXs, and AZAs	Shellfish: Mussels. Dinoflagellate *Protoperidinium crassipes*	• Harvesting of cell pellets from the plankton net tows. • Centrifugation suspended in 500µL methanol, and transferred to a FastPrep tube with 0.9 g lysing matrix D. • A N2-22 nitrogen generator transferred N_2 at 7 bar solvent reservoir, in-line degasser (G1379A), binary pump (G1311A), refrigerated autosampler (G1329A/G1330B), and temperature-controlled column oven (G1316A). • The analytical column (50x2 mm) was packed with 3-µm BDS 120 Å and remained at 20°C.	Krock *et al*, 2008
LC with fluorimetric (SRM)	OA and its isomer, DTX-2	*Dinophysis acuta*	• Extraction of unialgal samples and analysis by LC and prodigy C_{18} column (flow-rate 0.5 ml/min). • Fluorimetric detection of toxins. • Identification of DSP toxins detected using isocratic micro LC with µLC-MS-MS and µLC-MS of the BAP with an ionspray (IS) interface couple to an atmospheric pressure ionization (API), 13-15 min for OA and DTX-2 respectively	James *et al*, 1997
HPLC	PTX-2 and OA	Shellfish: Mollusks Dinoflagellate	• Acetonitrile, methanol, trifluoracetic acid (TFA),	Draisci

followed either by UV diode-array detection (LC-UV-DAD) or LC-MS and LC-MS-MS		*Dinophysis fortii*	methylene chloride, ammonium hydroxide. • PTXs detection through analysis of the crude methanolic D. *fortii* extract by LC-UV-DAD. • The residue was maintained at -30°C. • Dissolvation with 1 ml of methanol and injection of 20 µ1. • Fractionation of DSP toxin by alumina column.	*et al*, 1996
LC/MS/ MS	DSP toxins only OA and esters of OA	Shellfish: Danish surf clams (*Spisola sp*.) and blue mussels (*Mytilus edulis*)	• Cooking and freezing of samples at -18^{o}C. • Homogenization of sample (200-400 g) in a kitchen blender. • Certification of OA standard (24.1 µg/ml) in methanol. Extraction of 1g for 3 min on a shaker with 12 ml methanol. • Addition of 500 ml 2.5M NaOH to 3ml sample and heating at 45^{o}C for 1 h. • LC system: HP 1100 system.	Jorgens en *et al*, 2005
HPLC-UV and LC-MS	DA	Shellfish: Scallops	• Manipulation and analysis of standard solutions, shellfish extracts and homogenates. • LC-MS: API 150ex, Ionspray source and coupled to an Agilent 1100 series LC and autosampler and column thermostat compartment (held at 20^{o}C). • Ion-spray voltage: 5 kV, the orifice and ring voltage: 10	Hess *et al*, 2005

			and 160 V, respectively, quantification was applied using the protonated $[M + H]^+$ ion of DA at m/z 312.	
LC–FL	DA	Shellfish: Mussel (*Mytilus edulis*) tissue, clams *Siliqua patula*, and marine phytoplankton *Pseudonitzchia australis*	• 7-fluoro-4-nitro- 2,1,3-benzoxadiazole (NBD-F) and trifluoroacetic acid and formic acid were applied. • Certification of reference shellfish material (MUS-1B) (38.3±0.8 mg domoic acid /g) and calibration solution (DACS-1C). • Sample was analyzed by LC–FL. • Mixture of NBD-F in acetonitrile (1 mg/ ml, 50 ml) and 0.1 M sodium borate (50 ml) with sample or standard solution (50 ml) for 3 min with addition of 1 M HCl (50 ml) to the mixture. (LC-10AD), column oven (CTO-10A) and fluorescence detector (RF-551). • Isocratic chromatography with acetonitrile-water (40:60) - 0.1% TFA, solvent flow (1.0 ml/min) with a Luna C_{18} column and a precolumn (30x4.6 mm, 5 mm) at 35^{o}C. • Fluorimetric detection (λ_{ex}=470, 530 nm).	James *et al*, 2000
HPLC-UV-DAD	DA	Blue mussels (*Mytilus edulis* L.), algae of the family *Rhodomelaceae*	• MSL-300 NMR spectrometer, UV absorbance detection (200nm), LC-18 solid phase extraction cartridge, pH 6-**7,** dry 0.22pm filter, built-in HP1040 DAD and HP79994 data system, DR5 solvent delivery system, variable volume (1 to 25µL) injector were used. • Columns (25cm x 4.6mm I.D. or 2.1 mm I.D.) packed with 5pm 201TP were used at 40°C, column using a 20 µL injection and linear gradient elution from 5% to 25% acetonitrile over 25min at l ml/ min, 242 nm, duration:	Quillim *et al*, 1989

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			5min.	
HPLC-UV method (LC-UV and LC-ESI-MS)	DA and its isomers, isodomoic acid D and epi-domoic acid	Mussel (*Mytilus chilensis*, *Venus antique*, *Pecten jacobaeus* and *P. maximus*)	• HPLC: a solvent delivery pump (P1000), auto-injector (AS3000), and a variable wavelength detector (UV100), 1.2 ml/min with a Luna- C18(2) column at 40^{o}C, were used. • Injected volume was 50 µl and the chromatographic run time was 15.0 min. • LC-ESI-MS: using acetonitrile-water (11:89) containing 0.035% TFA (pH 2.5), solvent flow (0.2 ml/min), Luna C18(2) column (150·2.0 mm, 5 µm) at 40^{o}C, • Injected flow at 3 µl/min, capillary temperature: 195^{o}C, source voltage: 4.20 kV, source current: 100 µA.	López-Rivera *et al*, 2005
HPLC-RIA	DA	Mussels, clams, anchovies and crab	• HPLC: 20µ1 of loop and a reversed-phase C_{18} column (LC-18, 150 x 2.1 mm I.D., 5 pm) were used. • Monitoring of effluent with a diode array UV absorbent detector set to 242 nm. • Mobile phase: 0.2% (v/v) formic acid plus 12% (v/v) acetonitrile in water (pH 3.0). • Flow-rate: 0.5 ml/min. • RIA: polyclonal antibodies derived from rabbits, 1.0-8.0 ng/ ml in PBS, pH 7.0. • Mixing of tubes with a vortex mixer and incubation (4°C).	Lawrence *et al*, 1994
HPLC and BMA	DA	Blue mussels (*Mytilus edulis*)	• Hepatopancreas: Treatment of the homogenate with a mixture of chloroform and methanol (1:1, v/v). • HPLC: Packaging of glass column (75 x 3.0cm I.D.).	Nijjar *et al*, 1992

			• Spectrophotometer: 180 and 300 nm.	
LC/MS/MS	DA		• Samples of 400 g (blue mussels) were used. • Maintainance at -18^{o}C before analysis. • 0.5 ml was taken and the methanol evaporated with nitrogen. • Dissolve of the residue in 200 ml high-performance liquid chromatography mobile phase. The LC system: HP 1100 system. • The mobile phase: acetonitrile/water (85/15) with 0.1% formic acid. • Use of a Waters Spherisorb S5 ODS1, 2.1x250 mm (25^{o}C). • Flow rate: 200µl min^{-1}. • Injected volume: 25 ml. • Mass spectrometry: Use of a triple quadrupole Z-spray mass spectrometer (electrospray capillary: 3.2 kV and the cone at 15 V).	Jorgensen & Jensen, 2004
TLC method	DA		• Strong anion-exchange (SAX) SPE columns: 3 ml portions of methanol, water and methanol-water were used. • Glass TLC plates (10x20 cm with a 250 mm thick layer of silica gel 60 with F254 indicator). • Plates: in a sealed glass chamber with a butanol–acetic acid–water system (3:1:1), were allowed to dry in a fume hood for 1 h. • Fluorescence-quenched spots: a handheld short-wave	Quillia m *et al*, 1998

			UV lamp (254 nm). DA gives a distinct yellow color.	
CE - UVD	DA and isodomoic acids	Mussels, clams and anchovies	• CE buffers were purchased: *viz.*, sodium citrate (20 mM, pH 2.0), sodium tetra-borate (20 mM, pH 9.0), sodium phosphate (20 mM, pH 9.0) and sodium3 (cyclohexylamino)-l-propanesulfonate (CAPS, 20 mM, pH 11.5). • A model PT3000 Polytron homogenizer equipped with a PT-DA- 3012/2T generator. • Injections used a 50mbar push for 0.2 min and separation potentials were 25-30 kV, 0.45 pm filter into a storage bottle and seal tightly (>30 min).	Zhao *et al*, 1997
HPLC	Histamine	Puffer fish (Rockfish, Salmon, Lobster and Shrimp)	• A modified partition column (HPX-72-0 in the -OH) with a injector, Model 6000A pump, Model 730 data module and UV detectors Model 441 operating a t 214 nm and Model 480 operating at 208 nm were used for the conduction of HPLC. • The UV wavelengths were choosen by examining the UV spectrum of each compound in a spectrophotometer. Analysis: 40 min.	Wills *et al*, 1987
HPLC	Histamine	Tuna, bonito, mackerel and eel	• Addition of 2M sodium hydroxide (1 mL) and 10 PL of benzoyl chloride mixed on a vortex mixer. • Dissolution of residue in 500 JLL of methanol. • Injection of 5 μL aliquots for HPLC analysis. • L-6200 pump, a Rheodyne Model 7125 syringe loading sample injector, a Mode1 L- 4200 UV-VIS detector set at 254 nm, and a Mode1 D-2000 integrator.	Yen & Hsieh, 1991

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			• A 100 RP-18 reverse-phasec olumn using a gradient elution system with a mixture of methanol and water in 10 min.	
NMR Spectroscopy, LC-MS and HR-MS	PbTxs: metabolites *S*-desoxybrevetoxin-B2 and brevetoxin-B2	Mussels (*Perna canaliculus*)	• Preparative separations by stepwising gradient elution on 200-mg C18. • Dissolution of *S* Desoxybrevetoxin-B2: *N*-Boc-L-cysteine (10 mg) in water (2.5 ml). • Addition of 0.05 M Na_2CO_3 (1.5 ml). • Brevetoxin-B2. *S*-Desoxybrevetoxin-B2:1 mg brevetoxin-B was dissolved in methanol (0.5 ml), and 1% H_2O_2 (4 ml). • 3 μm C18(2) 50 x 2 mm column maintained at 30°C. • Connection to Micromass Quattro Ultima triple-quadrupole mass spectrometer using electrospray ionization.	Selwood *et al*, 2008
Microplate fluorescence method (active phosphodiestera-ses)	YTX	Scallop *Patinopecten yessoensis* and mussels *Mytilus galloprovincialis*	• Quantification of the fluorescence fall in a plate reader. • Phosphodiesterase, 30:50- cyclic nucleotide specific from bovine brain, and other chemicals from Sigma. • Measurement of the PDE activity in a 96-well plate with a final volume of 100l.	Alfonso *et al*, 2004
HRGC-HRMS	PBDEs	Marine turtles, dugongs and seafood	• Column (10 m, 0.18 mm ID, 0.18 lm film thickness). • Positive identification and quantification of PBDE	Hermanussen *et*

			analytes - retention time: 0.2 s of the retention time of the corresponding 13C12-labelled-surrogate standard.	*al*, 2008
GC - ECD	PCBs	Fish: *Barbus barbus*	• Gas chromatograph was develoed with two electron capture detectors (μECD, ^{63}Ni, temp. 300^{o}C) and two columns. • Use of a HT-8 fused silica capillary column (50m x 0.22 mm x 0.25 μm) in order to analyze column of different polarity (DB-17MS, 60 m x 0.25 mm x 0.25 μm). • PTV injection: 90^{o}C for 6s, increased to 350^{o}C at 750^{o}C/min, held for 5 min and then to 220^{o}C at 10^{o}C/min. • Injection: 2 μL. Carrier gas: hydrogen - linear speed 27 cm/s.	Lana *et al*, 2008
GC-MS	PBDEs and PCBs	Mysterysnail (*Cipangopaludina chinensis*) mud carp (*Cirrhinus molitorella*) crucian carp (*Carassius auratus*), northern snakehead (*Ophicephalus argus*), water snake (*Enhydris chinensis*) mud carp, (*Cirrhinus molitorella*)	• Storage of biota samples at -20 °C until analysis. • CBs 65 and 204 for PCBs; CDE 99 (2,2',4,4',5-pentachlorodiphenyl ether), 13C12-PCB 141 and 13C12-BDE 209 for PBDEs. • ADB-XLB capillary column (30 m×250 μm i.d.×0.25 μm film thickness) was applied for the determination of the tri- to hepta-BDEs. • Quantification of PCBs by a GC coupled with mass selective detector. • A DB-5 MS column. • Injector and detector temperatures: at 260 and 230 °C, respectively. • Electron energy: 70 eV (scan time of 0.8 s).	Wu *et al*, 2008

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GC/LRMS-ITD in the MS/MS	PCBs, PCDDs and PCDFs	*Dicologoglossa cuneata, Solea vulgaris, Diplodus sargus, Sardina pilchardus, Lophius piscatorius, Donax trunculus, Chamelea gallina, Sepia officinalis* and *Parapenaeus longirostris*	• Analyses on a GC CP-3800 formed with an ion trap detector and an 8200CX autosampler. • Injection in the splitless mode (4 µl; injection rate: 0.5 µl/s; 100^{o}C (2min), at 200^{o}C/min to 300^{o}C; splitless time, 2 min) on a BPX-5 capillary column. • Column temperature: 60^{o}C (3min), at 30^{o}C/min to 200^{o}C (3 min), at 3^{o}C/min to 230^{o}C (15 min), at 5^{o}C/min to 270^{o}C (15 min).	Bordajandi *et al*, 2006
HRGC/HRMS	DLPCBs, PCDFs, dioxin-like PCBs	*Scomber Japonicus, Clupea pallasii, Conger myriaster, Thunnus thynnus, Trichiurus lepturus, Scomberomorus niphoniu, Parasilurus asotus, Scapharca subcrenata, Todarodes pacificus, Tapes philippinarum, Scapharca subcrenata*	• Seventeen 2, 3, 7, 8-substituted PCDD/F congeners and 12 DLPCB congeners (PCB 77, 81, 123, 118, 114, 105, 126, 167, 156, 157, 169 and 189) were used. • Digestion of edible tissues (approximately 80 g) in 200 ml of 1 N ethanolic KOH solution for 2 h by mechanical shaking. • Purification of the eluted fractions on an activated alumina column with 3% methylene chloride in hexane and 50% methylene chloride in hexane. • Capillary columns: SP-2331column.	Moon & Choi, 2008
GC and GC-ECD	PCBs, OCPs, p,p'-DDE, HCB, HCHs, CHLs and DDTs	Shellfish and chub-mackerel	• 1 M KOH in ethanol for 24h. • GC column was a DB-1 capillary column (30 m ·0.25 mm) coated with dimethyl polysiloxane at 0.25 µm film thickness. • GC–ECD: 70^{o}C (held for 1 min) -180^{o}C at 20^{o}C/min, increase to 260 C at a rate of 2^{o}C/min. • Injector and detector temperatures: 250^{o}C and 300^{o}C, respectively.	Yang *et al*, 2006

			• Helium: carrier gas at a flow rate of 2 ml/min. • Nitrogen: make-up gas at a flow rate of 60 ml/min.	
ICP-MS	Heavy metals As, Cd, Cu, Hg, and Pb	Green mussel and dogfish	• Vessel containing 10 ml concentrated HNO_3. • Ramp to 115°C in 10 min, hold for 10 min. • Analysis of samples: ICP-MS.	Bayen *et al*, 2005
GC/MS	POPs (PCBs, PBDEs, and organochlorine pesticides)	Green mussel	• DB-5ms capillary column with gas flow of helium at 35 cm/min. • The GC oven program: 50°C held for 1 min, 20°C/min to 150°C held for 5 min, 3°C/min to 250°C, 10°C/min to 300°C held for 10 min. • Operation of the detector in EI mode with SIM.	
SSID–GC–ICPMS	^{202}Hg-enriched methylmercury (MeHg)	Cod fish (*Gadus sp.*)	• Both the standard and spiked solutions were maintained at -20°C. • Addition of 20 ml of 25%wt/v KOH methanol solution. • Ultrasonication of the mixture at 60°C for 2 h. • Mechanical shaking (1h). • Addition of 1 ml of methanol, 2 g of NaCl, and 1 ml of 6 M HCl. • Ultrasonication of the resulting mixture at 60 °C for 5 min. • GC column: HP 5msi (30 m× 0.25 mm i.d., 0.25 μm film thickness). • Temperature: 70 °C for the phenyl derivatization and 100 °C (1 min hold) to 300°C (30 °C min-1, hold 2 min).	Inagaki *et al*, 2008
ICP-MS and	Methylmercury (CH_3Hg^+)	Tuna fish	• C18 reversed-phase column (150 mm×4.6 mm, 2 μm). • Equilibrated of the HPLC column with at least 50 ml of	Reyes *et al*,

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HPLC-ICP-MS			mobile phase [50 mM pyridine, 0.5% (w/v) L-cysteine, 5% (v/v) methanol at pH 3] prior to the injection of mercury compounds. • The chromatographic separation obtained with a flow rate of 1 ml /min. • HPLC: inert modular system equipped with a 709 IC pump, a software interface and a 838 autosampler	2008
HPLC-AFS (ICP-MS, UV-CVG)	Inorganic mercury, MeHg, EtHg and PhHg	Dogfish and muscle	P680 HPLC Pump. • Column CLC-ODS, 15 cm length, 6.0mm I.D., 5µm, Mobile phase A: 3% (v/v) CH_3CN, 240 mol L−1 $HCOONH_4$–HCOOH (pH 2.8), 0.01% 2 mercaptoethanol; B: 30% (v/v) CH_3CN, 240 mol L^{-1} $HCOONH_4$–HCOOH (pH 2.8), 0.01% 2-mercaptoethanol; 0–11 min: Flow rate: 1.4 ml min^{-1}, Injection 100µL. Power of UV lamp Mercury lamp, 20W. PTFE tube, 0.5m length, 0.8mm I.D., 1.2mm O.D.	Yin *et al*, 2008
GC- AED	Methylmercury (Hg^{2+})	Fish species (tilapia, sword fish, mackere, Common mussel, Shrimp, Coalfish)	• Methylmercury (II) chloride, tetramethyl ammonium hydroxide solution (25% in H_2O) and methyl *tert*-butyl. A model 6890 GC with a programmable temperature vaporizer-based injector and an AED HP-G2350. • Using split less injection mode (1 µL, 1.5 min.) and helium as carrier gas, 25 °C for 0.01 min followed by an increase of 8.0 °C/s up to 260 °C, and maintained for 10 min. • Emissions: 254 nm (Hg) and 248 nm (C) (5.0 Hz).	Kuballa *et al*, 2008
FI-CV-	MeHg	*Prionace Mglauca, Isurus*	• Digestion of samples (0.2 g wet fish) with 2.5 ml of 65%	Carvalh

AFS		*oxyrhinchu, Centroscymnus coelolepsis, Centrophorus squamosus, Gauleorhinus galeus, Xiphias gladius, Aphanopus carbo, Raja oxyrhinchus, Deania calceus, Sardina pilchardus, Trachurus trachurus, Octopus vulgaris*	HNO_3 and 1 ml of 35% H_2O_2. • A flow injection system: multichannel peristaltic pump and a six-way injection valve. • Flow rate: 2.5 ml/min of 3 *M* HCl and of the reductant solution (3% $SnCl_2$ in 15% (HCl).	o *et al*, 2008
GC-ECD	DDT and ratios of o,p'-versus p,p'-isomers in total DDT	Fish: *Gambusia affinis*	• Analyses: GCs with cool on-column capillary injection systems and autosamplers. • Analytical columns: 60-m x 0.25-mm x 0.25 mm DB-5 (5% phenyl, 95% methylsilicone) and DB-17 (50% phenyl, 50% methylsilicone;). • The hydrogen carrier gas was pressure regulated at 25 psi. Temperature: initial 60°C, immediately 150°C at 15°C/min, 250°C at 1°C/min, and 320°C at 10°C/min.	Hinck *et al*, 2008
Immunoassay Methods: microtiter plate enzyme immunoassays (ELISA and IAC)	STX and NEO for Paralytic shellfish poisoning (PSP)	Shellfish: Clams, mussels, and oysters	• The use of IAC columns for sample cleanup is is very important for trace level measurement of analytes in foods and environmental samples.	Usleber *et al*, 2001
MIST	STX for PSP™	*Crassostria gigas, Protothaca*	• Extracts tissues: were preserved at 5°C and examined by	Jellett

Alert™ test kits with the regulatory AOAC® mouse bioassay		*staminea*, *Mytilus sp*., *Panopea generosa*, *Saxidomus giganteus*, *Clinocardium sp*., *Siliqua patula*, *Mytilus trossulus*, *Chlamys sp*., *Panopea generosa*, *Chlamys sp*., *Chlamys opercularis*, *Chlamys sp*., *Mactromeris polynyma*, *Ensis sp*., *Paphies subtrianguata*, *Mya arenaria*, *Panopea generosa*, *Perna canaliculus*, *Saccostrea glomerata*, *Paphies australis*, *Tresus capax*, *Macoma nasuta*, *Mytilus edulis*, *Austovenus stutchburyi*, *Pecten novaezelandiae*	applying MIST Alerte. • Tests were evaluated after 20 min. • The samples were frozen at -20°C.	*et al*, 2002
MIST Alert™	Neurotoxins (STX and derivatives) PSP™	Sellfish: Bivalve Molluscs (*Mytilus edulis*), offshore scallop (*Pecten maximus*), queen scallops (*Aquapecten opercularis)* razor fish (*Ensis arcuata*) native oysters (*Ostrea edulis*), and pacific oysters (*Crassostrea gigas*)	• MIST Alert kit: contain strips that contain antibodies and colored particles, a band with toxin analogs, and a band of antibody detection reagent. • Addition of 100 ml of each mixture to the sample pad on the strips and examination after 20 min for the presence or absence of 'T' and 'C' lines.	Mackintosh *et al*, 2002
The conjugation of STX to horseradish	STX (dc-STX, neo-STX, GTX1, GTX4, B2)	Shellfish: Mussel and clam	• A polyclonal antiserum against STX, raised in rabbits immunized with STX coupled to keyhole limpet haemocyanin by formaldehyde processing. • Preparation of activated HRP **(**4 mg).	Usleber *et al*, 1991

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peroxidase (HRP) by a novel adaptation of the periodate method in a microtitration ELISA and in a test strip EIA				
Electrochemical immunosensors, a screen-printed electrode (SPE) system	OA, PbTx, domoic acid (DA) and TTX	Seafood and mussels	• Disposable screen-printed carbon electrode with amperometric detection of paminophenol at 300 mV vs. Ag/AgCl, formed by the label, alkaline phosphatase, was applied for signal assessment. • Analysis period: 30 min. • Purchase of Sephadex PD-10 columns.	Kreuzer *et al*, 2002
c-ELISA and LC-MS	DA	Shellfish: Mussels	• Injection of Sprague-Dawley rats with saline, 0.5, 1 or 2 mg/kg DOM.	Hesp *et al*, 2005

			• LC-MS: a 50 mg C18 SPE column and the eluent. • cELISA: anti-DOM antibodies conjugated to horseradish peroxidase for 1 h in the dark, 0.3M H_2SO_4 and absorbance at 450 nm read after 5 minutes.	
idc–EIA		Blue mussels (*Mytilus edulis*)	• An anti-domoic acid monoclonal antibody DA-3**,** EIA reader.-Model 550, C18 cartridges-Containing 360 mg octadecylsilica, Hitrap affinity column-NHS-activated, 1 ml capacity were required. • LC: 200 ml sample loop, reversed-phase ODS column with cartridge guard column, variable-wavelength UV detector set to 242 nm. • idc–EIA: 0.05 ml DA solutions as standards (0, 0.1, 1, and 10 ng/ ml in PBS), goat anti-mouse IgG peroxidase conjugate, color reaction, and absorbance was measured at 450 nm	Kawatsu & Hamano, 2000
Electrochemical immunosensor coupled to differential pulse voltammetry (DPV)		Mussels	• SPEs for the immunosensor development. • Spectrophotometric ELISA applied prior to transferring the assay to the SPEs. • Analysis period: 150 min. • A model 550-Microplate Reader was applied at 405 nm, BSA-DA conjugate (2.5µg/ml, 50 µl per well) in CB, pH 9.6. • Voltam-metry: 0-600mV, scan rate: 300 mV/s, pulse amplitude: 70mV, pulse width: 50 ms	Micheli *et al*, 2004
cdELISA		Blue mussel (*Mytilus edulis*), clam	• Conjugation of DA to KLH and BSA, were tested in four	Yu *et*

and ciELISA		(*Meretrix lusoria*), oyster (*Crassostrea gigas*)	rabbits, with two rabbits for each immune-gen (2 L of BS for 72 h at 4 °C with two changes of buffer). • iELISA**:** Addition of 0.1 ml of diluted antidomoic acid antiserum. • After incubation (37 °C for 1 h)and washing with PBS-Tween, 0.1 ml of goat anti-rabbit IgG-HRP addtition of conjugate and incubation at 37°C for 45 min was performed. • cdELISA: Incubation at 4°C, application of PBS and blocking with BSA-PBS (0.17 ml per well; 0.1% BSA in 0.01 M PBS) at 37°C for 30 min. • HPLC: 25 cm x 4.0 mm, 5 µm, C18 reverse phase column conjucted with a 4 cm x 4.0 mm, 5 µm, mobile phase.	*al*, 2004
ELISA		Shellfish and diatom *Pseudonitzschia pungens*	• Use of polyclonal antibodies (pAbs). • Microwell plates remained at 4^{o}C with 5µg/ ml DAOVA dissolved in carbonate buffer (200 ml). • After incubation and washing the plates, addition of 200 ml of a goat a-rabbit IgG labeled with biotin was performed. • Buffer for electrochemical detection: 50mM Tris,100mM NaCl, 1mM $MgCl_2$, 0.1mM $ZnCl_2$ (pH 9.0).	Kania *et al*, 2003
	PbTxs	Oysters. Dinoflagellate *Karenia brevis* and Mammalian Body Fluid	• Secondary biotinylated antibodies, streptavidine-horseradish peroxidase conjugate, and chromogenic enzyme substrate, were used. • Flat-bottomed 96-well polystyrene immunoplates were	Naar *et al*, 2002

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			coated with 100 µL of PbTx-3-bovine serum albumin (BSA) conjugate or BSA alone by incubation for 1 hr at 25°C.	
Enzyme Immunoassay Stick test	Ciguatoxin (CTX) and related polyether toxins	Fish: *Ctenochaetus strigosus* and *Thunnus thynnus* Dinoflagellate*: Gambierdiscus toxicus*	• Insertion of each stick 5 times into the flesh at 1 s/insertion. • Examination of all samples with six sticks; three in the dorsal-anterior and three in the ventral-posterior portions of the fish.	Hokama, 1988
ELISA	CTX3C	Reef fish	• Use of haptenic groups (surface area > 400 Å2) for the production of mAbs. • Immunization of Balb/c mice with KLH-conjugate mixed with RIBI adjuvant. Using to capture CTX3C and mAb 3D11 as a detector. • Conjuction of mAb 3D11 (1 mg) with HRP using an EZ-Link Plus Activated Peroxidase Kit. • Coating with 50 µL of mAb 10C9 (4.3 µg/ ml) in PBS at 4°C. • The absorbance at 450 nm was calculated with a microtiter plate reader.	Oguri *et al*, 2003
Bead-ELISA, Vero cell cytotoxicity assay, PCR and colony	Shiga toxin-producing *Escherichia coli* (STEC) and *E. coli* heat-stable toxin (STa)	Seafood: Clam and fish	• The organisms remained in Luria Bertani (LB) broth in a shaker incubator at 37^{o}C. • Growth of standard E. coli strains carrying cloned stx_1 and stx_2 genes in LB-medium.	Kumar *et al*, 2004

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hybridisation for the detection of stx_1 and stx_2 genes				
A ganglioside-liposome immunoassay	Cholera toxin (CT) from *Vibrio cholerae*	Seafood samples were obtained from a local supermarket: Clam, Shrimp and Salmon	• Preparation of ganglioside-incorporated liposomes by extrusion freezing and thawing, from a mixture of DPPC, DPPG, cholesterol and GM1, in a molar ratio of 40.3:4.2:40.9:1.3. • Preparation of test strips.	Ahn & Durst, 2008
Ganglioside-liposome immunoassay	Botulinum neurotoxin (BT) produced by *Clostridium botulinum*	Salmon	• In a sandwich-based, hybrid receptor immunoassay, BT detection can be performed through the formation of a colored band on a nitrocellulose membrane strip. • The assay can be performed in 20 min. • Immobilization of anti-BT antibodies in narrow zones on plastic-backed nitrocellulose (NC) membrane sheets.	Ahn-Yoon *et al*, 2004
Mab	*Clostridium botulinum* type E toxin	Seafood	• Generation of hybridomas secreting specific Mab against the type E epitope. • Selection of 5 potent stable hybndomas, which were cloned, propagated, and preserved in liquid nitrogen.	Wong, 1996
Vitro assays: MBA,	Botulinum protein neurotoxin (types A to G)		• Dilution of culture supernatants to 1 ng ml 31 (neurotoxin) in 50 mM, 150 mM NaCl, 1 mg ml31 bovine serum albumin, pH 7.4 and preservation at -70°C.	Wictome *et al*, 1999

highly specific zinc endoproteases and ELISA			• Formation of a peptide substrate representing residues 60 to 94 of human VAMP isoform 1. • Preparation of columns wth cyanogens bromide activated Sepharose 4B. • The columns were maintained at 4^{o}C.	
ELISA	PCBs (PCB-126) and TBT	Channel catfish (*Ictalurus punctatus*)	• Determination of ethoxyresorufin *O*-deethylase activity. • 210µl assay buffer (150 ml of 2.67 mM 7-ethoxyresorufin in 50 mM Tris–1M NaCl buffer, pH 7.8). • A microplate reader and mouse monoclonal antibody C10-7 were used at 405. • Visualization of alkaline phosphatase activity by adding *p*-nitrophenol phosphate dissolved in diethanol- amine buffer (1 mg/ml, pH 9.0).	Burton *et al*, 2002
Real-Time PCR Assays and SEM	Toxins from *Pfiesteria piscicida* and *P. shumwayae*	Seawater, shellfish ant tropical fishes	• Elution of DNA with 100 ml of elution buffer and maintainance at 220°C. • The primers and probes were formed by applying the Primer Express software and an alignment of 100 dinoflagellates ribosomal DNA sequences.	Bowers *et al*, 2000
Multiplex Real-Time PCR Method	Shiga Toxin Genes *stx1* and *stx2* and STEC O157:H7/H^{-} Serotype	Seafood	• The combined stringent magnesium concentration (2 mM) and temperature (63°C for 25 s) led to false-negative result for *stx2* with strain EDL 933.	Jinnem an *et al*, 2003
PCR	Shiga-toxigenic Escherichia coli (STEC)	Fresh fish, clams and water	• Homogenization of 25 g of samples in 225 ml modified EC broth and incubation for 6 h at 37°C.	Kumar *et al*,

	stx, hlyA and rfb_{O157} genes		• Escherichia coli EDL 933 was used as positive control. PCR, primers: 5'GCG ATC CCA A 3', 5' CCG CAG CCA A 3', 5'AAC GCG CAA C 3' and 5' GTG GAT GCG A 3'. *E. coli* were grown in 3 ml Luria Bertani broth for 18 h and centrifuged at 10 000 g for 10 min. Lysate was amplified in a 50 µl reaction mixture containing 1'PCR buffer, 200 µmol l^{-1} deoxynucleotide triphosphate mix, 0·5 µmol l^{-1} of each primer and 2·5 U taq polymerase	2001
PCR	Cholera (cfx) and *zonula occludens* (zot) toxin from *Vibrio cholerae* 01, 0139 and non-01 strains	Shellfish: Autochthonous bacterial species in aquatic environments	• Thiosulphatelcitrateibile salts/sucrose (TCBS) agar was used. • Collection of cells from an 18-h 106ml LB culture and resuspention in 9.5 ml of TE buffer amended with 250 µl 10% SDS solution and 2.5 µl proteinase K. • ctx primers: CTX1 and CTX_3. • zot primers: ZOT_1 and ZOT_4.	Rivera *et al*, 1995
TaqMan PCR assay	Cholera toxin (*ctx*), zonula occludens toxin (*zot*), and accessory cholera toxin (*ace*). *Vibrio cholerae* O1, O139, Non-O1, and Non-O139	Shellfish: Raw Oysters, and Synthetic Seawater	• Duration of the procedure: 3 h. • Quantification of *Vibrio* cells. • Growth of *Vibrio* species in BHI broth supplemented with 3% (wt/vol) NaCl at 35°C, (dilution into APW, and plated onto TBSC and BHI). • The PCR mixture was maintained at 50°C for 5 min and denatured at 95°C for 10 min.	Lyon, 2001
PCR	*Clostridium botulinum* neurotoxin type E (bont/e) gene)	In modified-atmosphere-packaged fish (jack mackerel)	• Application of the hydrolysis of an internal fluoregenic probe and monitoring of the increase in the intensity of fluorescence during Pc.	Kimura *et al*, 2001

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			• Achievement of accurate and quantification of the *C. botulinum* type E toxin gene.	

Table 4.1. The limit of detection of methods (LOD)

Methods	Toxin	The limit of detection (LOD)	References
Mouse bioassay	STX, TTX and CTX	0.2 μg/ml STX equiv. /100 g	Manger *et al*, 2003
			Sullivan *et al*, 1985
	OA and its derivatives (DTX and DTXJ)	< 1 μg/g	Croci *et al*, 1997
	Botulinum toxin (BT)	1μg	Lipps, 2002
	PSP toxins	0.4 μg/g	Wright, 1995
	DA	> 50 pg/g	
	B l-2	6.1 μM	Luckas, 1992
	Cl and C2	59 μM and 3.9 μM	
	GTXI, II, III, IV	0.5 μM, 1.0 μM, 0.6 μM, 0.5 μM respectively	
	NEO and STX	0.4 μM and 0.5 μM	
Mouse or rat bioassays	AZAs	0.16 mg/kg	Report of the Joint FAO/IOC/WHO, 2004
Histopathological studies: Sequrntial ultrastructural changes were studied in mouse digestive organs	Dinophysis-toxin-1 (DTX_1) and Pectenotoxin-1 (PTX_1)	50 μg/kg	Terao *et al*, 1986
Cell-bioassay	Maitotoxin	0.07 MU equiv./ ml	Okumura *et al*, 2005
	Saxitoxin	0.2 MU equiv./ ml	
Neuroblastoma assays	PbTx-3	0.03 mg/kg	Report of the Joint FAO/IOC/WHO,

			2004
1. Bioassay: mouse neuroblastoma tissue culture assay for sodium channel using RBCs. 2.HPLC	STX, TTX, and CTX-a	1. 0.3 -50 µg/ml STX- TTX0.010 -10 µg/ml for CTX 2. 0.004 µg/kg STX, 0.010 µg/kg TTX, 0.12 µg/kg CTX	Shimojo & Iwaoka, 2000
1) Bioassay cell-based Neuro-2A neuroblastoma toxicity assay 2) LC-MS/MS	Neurotoxic STXs, GTX1 to GTX6 and decarbamoyl (dc)-GTX1 to dcGTX4, C toxins (C1 to C4)	1) 72 µg/g 2) 2.0 µg/g	Humpage *et al*, 2007
Tissue culture assay: cytotoxicity technique witch based on the direct microscopic observation of toxin-induced morphological changes in BGM cells	OA	0.5 ng /100 µl	Croci *et al*, 1997
1) Microplate scintillation technology 2) HPLC Protocol: fluorescent	TTX and STX	1) 5 ng STX equiv./ mL 2) A wavelength of 330 nm 3) 50 nM STX equiv.	Powell & Doucette, 1999

derivatives by post-column oxidation, both toxins 3) HPLC			
1) Mouse bioassay 2) HPLC	All the PSP toxins (B1, B2,C1,C2, GTX I, GTX II, GTX III, GTX IV, NEO,STX)	1) 35 µg saxitoxin (STX)/100g 2) < 10 to 56 µg STX/100g	Sullivan *et al*, 1985
Mouse bioassay and ELISA	Botulinum neurotoxin	10 pg of neurotoxin/ ml (0.5 MLD50/ ml)	Wictome *et al*, 1999
Comparison of HPLC and Mouse Bioassay	Neurotoxins (GTX II, GTX III,STX) N-l hydroxy toxins (neosaxitoxin and gonyautoxins I and IV) sulfocarbamoyl toxins (Cl, C2, Bl)	1) 20 µg per 100g meat/ GTX II, GTX III and STX (HPLC) 2) 200 µg/100g meat GTX I, GTX IV and NEO (HPLC) 3) 80 µg toxin/100g (Bioassay)	Sullivan *et al*, 1983
HPLC	Histamine	0.05- 0.04 (µg) (208-214 nm)	Wills *et al*, 1987
	GTXs	80 µg/100 g	Lassus *et al*, 1989
	STX	7.1×10^6 µg STX Equiv./ cell	Anderson *et al*, 1989
HPLC-FLD	OA	40 ng/g	Lawrence & Scott, 1993
HPLC-IMS	OA	10 ng/g for OA toxin	Pleasance *et al*, 1990
HRGC/HRMS	PCDDs /Fs	0.004 pg/g wet	Moon & Choi, 2008
	HCDD/Fs	0.01 pg/g wet	

	OCDD/F and non-ortho PCBs	0.02 pg/g wet	
	mono-ortho PCBs	0.04 pg/g wet	
HPLC	GTX_1, GTX_2, GTX_3 and GTX_4	0.17 pg STX equivalents $cell^{-1}$	Cembella *et al*, 1987
	STX	40 μg saxitoxin per 100 g	Luckas, 1992
	STX	0.014 Mμ (20-μl injection)	
	NE0	0.065 μM (20-μl injection)	
	GTX	0.006 μM (20-μl injection)	
	C l-2		
	Bl	0.040 μM (20-μl injection)	
	B2	0.150 μM (20-μl injection)	
	GTX2/3	0.2 -1.9 mg/100 g tissues	Wu *et al*, 2005
	OA	3.2 - 17.5 mg/100g tissues	
HPLC (pH 2.5)	DA and its isomers	< 25 ng/ml (0.62 μg/g)	López-Rivera *et al*, 2005
HPLC	^{202}Hg	0.46±0.02 μg/L	Reyes *et al*, 2008
	Hg^{2+} and CH_3Hg^+	0.78±0.08 μg/L	
HPLC-UV	Brevetoxins	0.2 ng/ ml	Naar *et al*, 2002
HPLC-UV or -MS	DA	0.6-2.5 μg/g	Hess *et al*, 2005
HPCE-UV	PSP toxins	200 nm	Thibault *et al*, 1991
	DA	0.3 ng	Quilliam *et al*, 1989
HPCE-RIA		0.05-0.1 μg/g	Lawrence *et al*, 1994
HPLC–AFS	Hg	0.085 μgL^{-1}	Yin *et al*, 2008

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	MeHg	0.033 μgL^{-1}	
	EtHg	0.029 μgL^{-1}	
	PhHg	0.038 μgL^{-1}	
CE with ion-spray MS	OA	16 ng	Pleasance *et al*, 1992
CE - UVD	DA	220 ng/ ml or 3pg or 150 ng/g	Zhao *et al*, 1997
LC–MS with ionspray ionization	OA, GTX, DTXs PTXs and AC1	at *m/z* 803 for toxin	Draisci *et al*, 1999
LC/MS	OA, DTXs, YTXs, PTXs, AZAs	40 µg/kg for OA, PTX-2, and AZA-1 100 µg/kg for YTX	Stobo *et al*, 2005
	DA	< 0.008 µg DA/ml (0.2 µg DA/g tissue)	López-Rivera *et al*, 2005
LC–MS/MS	OA, DTX, PTX2 and pectenotoxin-2seco acids	< 1 pg 0.002 µg/ml 0.007 µg/ml OA 0.054 µg/ml DTX2	Puente *et al*, 2004
LC and HPLC	OA and DA	0 .162 µg/g OA 2 .1 pg/cell DA	Dickey *et al*, 1992
LC with fluorimetric (SRM) and (µLC-MS-MS)	OA and its isomer, dinophysistoxin-2 (DTX-2)	0.025 ng	James *et al*, 1997
LC-UV-DAD	PTX-2 and OA	200 nm	Draisci *et al*, 1996
LC–FL	DA	1 ng/ ml	Quilliam *et al*, 1989
LC–UV	OA	0.5 µg/g tissue	James *et al*, 2000
	DA	0.02-0.03 µg/g	

LC-ISP-MS	OA, DTX-I and their isomers	0.04 μg of toxin per g	Draisci *et al*, 1995
LC-MS-MS	DA, OA, DTX 1-2, PTX-2, PTX-2 seco acid, PTX-11, YTX, AZA-1	0.3 ng/NT DA 0.085 ng/NT OA 0.15 ng/NT DTX 1-2 0.25 ng/NT PTX-2 0.07 ng/NT PTX-2 0.13 ng/NT PTX-11 0.05 ng/NT YTX 0.015 ng/NT AZA-1	Krock *et al*, 2008
	OA	12 μg kg^{-1}	Jorgensen *et al*, 2005
	OA and DTX1	5 μg kg^{-1}	Jorgensen & Jensen, 2004
GC- AED	Hg^{2+}	6.1 μg/kg	Kuballa *et al*, 2008
GC/LRMS-ITD in the MS/MS	PCBs, PCDDs and PCDFs	0.03–0.3 μg/l	Bordajandi *et al*, 2006
GC-IT-MS	All organochlorine	0.1 ng g^{-1}	Chan *et al*, 1999
GC/MS	Chlordane	0.04 ng/g ww	Bayen *et al*, 2005
	DDTs	0.04 to 0.09 ng/g ww	
	PCNB	0.04 ng/g ww	
	PCBs	0.01 to 0.2 ng/g ww	
	PBDEs	0.01 to 0.03 ng/g ww	
	Methoxychlor	0.12 ng/g ww	
GC- ECD	PCBs	1 μg/kg	Lana *et al*, 2008

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	o,p'-DDE	0.81 ng g^{-1} ww	Hinck *et al*, 2008
	o,p'-DDD	0.10 ng g^{-1} ww	
	o,p'-DDT	0.10 ng g^{-1} ww	
	p,p'-DDE	2.4 ng g^{-1} ww	
	p,p'-DDD	0.18 ng g^{-1} ww	
	p,p'-DDT	0.47 ng g^{-1} ww	
	pentachlorobenzene	0.07 ng g^{-1} ww	
	hexachlorobenzene	0.14 ng g^{-1} ww	
	total PCBs;	61 ng g^{-1} ww	
TLC-UV	DA	0.3 µg	Quilliam *et al*, 1998
HRGC-HRMS	PBDEs	0.01-3.4 ng g^{-1} lipid weight	Hermanussen *et al*, 2008
MEKC with UV	OA and dinophysistoxin-2	40 pg 190 nm. of UV	Bouaïcha *et al*, 1997
A neurophysiological method: in vitro rat hippocampal slice preparation	Saxitoxin (PbTX3) brevetoxin and DA	50 nM	Kerr *et al*, 1999
ELISA	Botulinum toxin	0.04µg	Lipps, 2002
	Brevetoxins (PbTx)	2.5 µg/100 g	Naar *et al*, 2002
	CTX3C	5 ng/ ml (5 nM)	Oguri *et al*, 2003
	DA	0.6 ng/ml	Micheli *et al*, 2004
cdELISA		0.02 ng/ ml	Yu *et al*, 2004
ciELISA		<25 ng/g	

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ELISA or ABC system		0.6 ng/ ml (ppm) or 0.8-300 ppb DA	Kania *et al*, 2003
ELISA the test strip assay EIA	STX	3 ng/g or 7 pg/ml or 0.35 pg/assay (ELISA) and 4 ng/g or 200 pg/ml (EIA)	Usleber *et al*, 1991
Electrochemical immunosensors	OA, PbTx, DA and Ttx	2 ppb DA, 1.5 ppb OA, 0.016 ppb Ttx, ppb PbTx	Kreuzer *et al*, 2002
Electrochemical immunosensor- DPV	DA	5 ng/ml	Micheli *et al*, 2004
Enzyme Immunoassay Stick test	Ciguatoxin (CTX)	0.125 ug/kg	Hokama, 1988
Immunoassay Methods	STX, NEO, dc-STX, GTX 2/3	7 pg/ ml STX (EIAs) 200 pg/ ml NEO (EIAs) 80 ng/g STX (ELIFA) 250 ng/g dc-STX, GTX 2/3, and NEO (ELIFA)	Usleber *et al*, 2001
Ganglioside-liposome immunoassay	Cholera toxin	3×10^3 fg/ ml	Ahn & Durst, 2008
Ganglioside-liposome immunoassay and mouse bioassay	Botulinum neurotoxin (BT)	15 pg/ ml (immunoliposome assay) 10 pg/ ml (mouse bioassay)	Ahn-Yoon *et al*, 2004
PP2A	OA	20 µg OA/ 100 g (0.2 pg/g)	Honkanen *et al*, 1996
Fluorimetric assay (FLD)	Saxitoxin	1 ng saxitoxin equivalents/ml	Louzao *et al*, 2001
	STX, NEO, GTX 1/4, GTX 2 and 3, C toxins, and B toxins	1.01 ng/ml	Indrasena & Gill, 1998
FI-CV-AFS	Mercury (Hg)	0.035 mg/g	Carvalho *et al*, 2008

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Microplate fluorescence method	YTXs	>0.5 μM (fluorescence by high performance liquid chromatography 2 μM (mousse bioassay)	Alfonso *et al*, 2004
A microtiter plate-based receptor binding assay	STX	5 ng saxitoxin/ml 40μg STX equiv./100 g tissue (mouse bioassay)	Doucette *et al*, 1997
Mab	*C.botulinum* type E toxin	1 MLDs/ml (5-10 pg/ml)	Wong, 1996
MIST Alert™ and mouse bioassay	STX	>40 μg STX equiv./100 g (MIST Alert™) 80 μg STX equiv./100 g (MBA)	Mackintosh *et al*, 2002
MIST Alert™ test kits with mouse bioassay		40 μg STX equiv./100 g (MIST Alert™) 32 μg STX equiv./100 g (mouse bioassay)	Jellett *et al*, 2002
Spectrometry	PSP toxins	40-80 pg PSP per 100 g	Kania *et al*, 2003
PP2A	OA	0.02 μg/g OA	Honkanen *et al*, 1996
ICP-MS	As	0.05 μg/g ww	Bayen *et al*, 2005
	Pb	0.18 μg/g ww	
	Cd	0.11 μg/g ww	
	Cu	0.30 μg/g ww	
	Hg	0.02 μg/g ww	
HRP system	DA	0.2 ppb	Ciminiello *et al*, 2007
Real-Time PCR Assays	Toxins from *Pfiesteria piscicida*	0.6 cell	Bowers *et al*, 2000
Multiplex Real-Time PCR Method	Shiga toxin-producing *E. coli* (STEC)	6 CFU/ reaction	Jinneman *et al*, 2003
PCR assay	Clostridium (botulinum neurotoxin type E (bont/e) gene)	10^2 CFU/ml	Kimura *et al*, 2001
TaqMan PCR assay	*Vibrio cholerae* O1, O139, Non-O1, and Non-O139	6 CFU/g and 10 CFU/ ml	Lyon, 2001

Table 5.1. Outbreaks of seafood toxins, assessment of toxins in seafood and clinical symptoms of toxicity

Outbreak	Responsible toxin	Fish or shellfish	Year/ date	Region / Country	Symptoms	Deaths/ incidents	Corrective actions of incidents/Pharmacology	Reference
Ciguatera fish poisoning (CFP)	Ciguatoxins (CTXs)	Amberjack	23/7/1774	New Hebrides	Heating and reddening of the face, with headache and inflammation of the whole skin; vomiting and diarrhea followed, with pangs in the intestines; pain in the body	Patients: 6 men	Recovery in 4 days	Watters, 1995
CFP. During the British Naval invasion of Mauritius		Blackish fish (Vieille)	1801	Island of Rodriquez, Mauritius	Convulsions, potential death	150 men	-	Russell & Egen, 1991
CFP		Mackerel	1967	Queensland	While viscera such as liver, intestines and gonads	2 cases	No available preventive or remedial medical treatment	Lehane & Lewis,

								2000
		Tropical fish (barracudas, groupers, sea perch, moray eels, sharks)	1980-1990	Western Pacific, France	Symptoms of poisoning appear 2-30 h after ingestion of toxic fish. Numbness, a prickling sensation in the lips, tongue and throat, general weakness and nausea, cramps, abdominal pains, vomiting and profuse diarrhea) and neurological disorders: itching (pruritus), articular pains and asthenia, can lead to paralysis, coma and death	Several cases	Stomach pumping and early administration of apomorphine. Antispasmodics and antiemetics, and vitamins Br, Bg, B12 complex, colchicine, acetylsalicylic acid and calcium gluconate injections. Acetylcholine secretion (lidocaine and tocainide, phentolamine and calcium gluconate	Bourdy *et al*, 1992
		Barracuda	6/3/1982	Bahamas	Nausea, vomiting, and muscle weakness	Numerous crew members	Evacuation for hospitalization and treatment	Clive r, 2007
		Clam, *Ruditapes philippina-rum* and barracuda	1983	Manila, Indonesia	Consistence with ciguatera fish poisoning	30 persons, 4-65 years	Pralidoxime	Streft aris & Zenet os, 2006
CFP An Ontario		Dried barracuda brought back from Jamaica and red	1983	Canada	Neurologic symptoms	30 persons	Hospitalized without direct danger	Cana da Healt

market		snapper						h, 1997
CFP Both had eaten fish at a local restaurant		Barracuda	29/10/1985	Vermont	Consistence with ciguatera fish poisoning	2 persons, a woman 48 years and a man 30 years	Tocainide and glucocorticoids	Clive r, 2007
CFP		Serranidae fish: Acanthuridae and Siganida (*Variola louti*, *Plectropomus sp*., *Epinephelus sp*.)	1986-1994	Island of Reunion (SW Indian ocean) Mascareignes islands	Gastrointestinal symptoms: diarrhea, nausea/vomiting and abdominal pain. Neurological symptoms: paresthesia, with numbness and tingling of the extremities, and dysesthesia with inversion of sensation of cold/warm	159 Outbreaks		Quod & Turqu et, 1996
		Barracuda	1990	Coast of North Carolina	Typical symptoms	2 persons	Antiemetic	Leha ne & Lewis , 2000
		Barracuda, dolphin fish (mahimahi)		North Carolina coastal waters	Gastrointestinal and neurologic symptoms	10 persons	Treating with a syndrome resembling	Morri s *et*

		and yellow-fin tuna					CFP	*al*, 1990
		Amberjack (*Seriola* sp.)	1993	Florida	Prickling of lips, tongue and throat, numbness, headache, arthralgia, erythema		Antiemetics and antidiarrheals, phentolamine, reserpine and calcium gluconate	Clive r, 2007
		Serranidae and *Labridae*		Alijos Rocks, 300 miles off East Magdalena Bay, Southern Baja California		A fishing boat crew	-	Lech uga-Deve ze *et al*, 1995
		Shark		Madagascar	Burning perioral pain, parasthesias, ataxia, cranial nerve palsies, coma, convulsions, and respiratory distress	188 persons, Fifty patients (27%) died	-	Sobel & Paint er, 2005
CFP Montre al restaura nt		Oven-baked barracuda	1996	Quebec	Neurologic symptoms: paresthesia in the form of numbness in the hands and feet, pruritus, and sensitivity to air, epigastric burning, copious vomiting, diarrhea, abdominal pain, shivering and dyspnea	5 persons	diphenhydramine chlorhydrate (Benadryl®), loperamide (Imodium®), oxygen and intravenous medication	Cana da Healt h, 1997
CFP.		Amberjack		Hawaii	Gastrointestinal symptoms:	69-year-old	Atropine 1 mg IV,	Noon

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Consumption of amberjack					Nausea, vomiting, diarrhea, abdominal pain. Neurologic symptoms: Paresthesias hot/cold temperature sensation reversal, weakness, arthralgias, myalgias, dental pain, sensation of loose teeth, blurred vision, photophobia, neck stiffness, headache, chills, diaphoresis, pruritis of hands and feet. Cardiovascular symptoms: bradycardia, hypotension	Japanese-American woman	Promethazine (Phenergan), 10 mg IV, was given for the nausea and vomiting.	e, 1996
CFP								
		Coral trout (*Plectropomus* sp.)	1997	Australia	Gastrointestinal and neurological symptoms	Australian family	Oral treatments	Lehane & Lewis, 2000
		Barracuda in Mexico		Anti-Poison Center of Marseille, France		30 persons	250 ml of 20% mannitol	De Haro *et al*, 1997
		Barracuda and red snapper caught near the Cay Sal	21/10/1997	Texas	Gastrointestinal symptoms (nausea, vomiting, diarrhea, or abdominal cramps) and	17 people	No hospitalization, supportive measures	Smith & Hoka

		Bank of the Bahamas			neurologic symptoms (muscle pain, weakness, dizziness, numbness or itching of the mouth, hands, or feet)			ma, 1998
CFP in dinner party in Houston, Texas		Snapper and barracuda fillets both caught from an oil-rig platform off the Texas Gulf Coast	1998	Houston, Texas	Gastrointestinal symptoms, including diarrhea, abdominal pain, nausea, and vomiting, arm and leg weakness, muscle aches and stiffness, burning on urination and hot-cold temperature sensation reversal	Two cases (woman aged 50 years and a man aged 56 years)		MMWR, 2006
CFP		Reef fish, especially barracuda			Diarrhea, vomiting, and cramping, hypotension, bradycardia, and tachycardia	101 outbreaks: 374 persons, 30 of whom were hospitalized and 1 of whom died	Intravenous treatment with mannitol	Sobel & Painter, 2005
		Coral trout and reef fish	3-4/ 1999	Hong Kong	Gastrointestinal symptoms	200 people affected and 100 hospitalised	Hospital	Lehane & Lewis, 2000
		Barracuda	2000	Cuba	Weakness, pruritus, perioral and distal extremity paresthesias	Four Italians	Oral fluids, antiemetics, and antihistamines. Intravenous mannitol	Buter a *et al*, 2000

	C-CTX-1 and C-CTX-2	Dinoflag. *Gambierdiscus toxicus*: barracudas, jacks, snappers, groupers	2001	Western Atlantic and the Caribbean waters	Gastrointestinal, cardiovascular, and neurological signs and symptoms	Numerous outbreaks	Intravenous injection of D-mannitol	Pottier *et al*, 2001
CFP. In a tribe from Lifou	Cyanobacteria as *Hydrocoleum K tzing*	Giant clams (*Tridacna spp*.)	2001-2005	Loyalty Islands Province, New Caledonia	Gastrointestinal disorders, general fatigue, pain in the limbs and joints, reversal of hot and cold sensations and tingling sensation upon contact with water. Sometimes, cardiovascular symptoms like hypotension were also noted	35 people	Herbal medicine: Over 64 species of plants are used for anti-diarrheal, anti-dysenteric or antispasmodic effects, for arthralgias and myalgias, analgesia, and antiallergic effects	Laurent *et al*, 2008
CFP	CTXs	Reef fishes: *Cheilinus undulatus*, *Epinephelus coioi*des, *Plectropomus areolatus*, *Plectropomus leopardus*	2004	Hong Kong	Gastrointestinal and neurological symptoms. Hypothermia (rectal body temperature below 33^{o}C), diarrhoea, reduced locomotor activity, body weight depression at 4 days, and mouse death time within 24 h	823 outbreaks involving 3159 persons	Vasopressors, vitamins B1, B6, B12 complex, and C, calcium gluconate, colchicine, corticosteroids and aspirin	Wong *et al*, 2005
CFP. Barracuda		Barracuda	10/8/2004	South Carolina	Gastrointestinal symptoms such as nausea, vomiting, and diarrhea and neurologic	Two persons	Intravenous fluids, promethazine for nausea, gatifloxacin,	MMWR, 2006

caught approximately 60 miles southeast of Charleston, South Carolina					symptoms such as weakness, tingling, pruritus and tooth pain, heartbeat, hypotension, dizziness, severe, generalized pruritus		and low doses of dopamine	
CFP. Two fishermen captured a 26-kg amberjack		Amberjack (*Seriola Rivoliana*)	1/2004	Coast of the Canary Islands, Spain	Diarrhea, nausea/vomiting, metallic taste, heart rhythm disturbances, systemic, fatigue itching, dizziness and neurologic manifestations (myalgia, peripheral paresthesia, perioral numbness and reversal of hot and cold sensations)	5 people	Hospitalized	Pérez-Arellano *et al*, 2005
CFP		Eggs of barracuda fish	2005	Southern Taiwan	Nausea, vomiting, watery diarrhea, and myalgias about 1 hour. Numbness of the lips and extremities followed the gastrointestinal symptoms about 2 hours after ingestion.	Three members of a family	Atropine totaling 40 mg over 2 days	Hung *et al*, 2005

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					Further symptoms: hyperthermia, hypotension, bradycardia, and hyperreflexia			
		Big reef fish *Diagramma labiosum*	2006	Groote Eylandt in the East Arnhem region of the Northern Territory	Muscle and abdominal pains, nausea, vomiting, diarrhea. Numbness of: mouth, headache, sweating, difficult walking, weakness, muscle pains, chills dizziness, numbness of feet, inability to move arms/legs, temperature inversion, fever, salivation, shortness of breath, numbness of hands, burning /pain with cold water, itchy skin	14 cases (Indigenous Australians)	Royal Darwin Hospital (RDH) for observation and management. All people were treated normal saline to maintain blood pressure and cardiotropics (atropine)	Opa *et al*, 2006
Scombroid fish poisoning (SFP)	Histamine	Tuna fish	1966-1991	Switzerland	Most patients had erythema (87%), half complained of headache and one third had gastroin-tetinal symptoms.	76 incidents	-	Maire *et al*, 1992
SFP. In a school lunch program			1970-1973	United States	Patients, 86% experienced nausea, 55% diarrhea, 44% headaches and 32% rashes	40 children. In 1973, more than 200 consumers		Clive r, 2007

SFP	Histamine and saurine	Canned mackerel and smoked kahawai	1973	Wellington area	Gastrointestinal and neurological symptoms	Three incidents	Anti-histamine therapy	Foo, 1975
HFP (Histamine fish poisoning)	Histamine	Tailor (*Pomatomus saltatrix*), salmon (*Arripis truttaceus*), tuna	1973-1975	Australia	Rash, urticaria, oedema and localised inflammation), gastrointestinal (nausea, vomiting, diarrhoea), haemodynamic (hypotension)	Several incidents	Medical attention. Treatment not always necessary	Lehane & Olley, 2000
HFP		Canned skipjack tuna	1974	Solomon Islands in New Zealand	Neurological (headache, palpitations, tingling, burning, itching) bronchospasm and respiratory distress		Antihistamine treatment (diphenhydramine, chlorpheniramine, cimetidine) and induced emesis	
SFP		Tuna and mackerel	1976-1986	Britain	Gastrointestinal and neurological symptoms	250 suspected incidents	Antihistamines, hydration, antiemetics	Hwang *et al*, 1995
SFP. A Japanese restaurant	Histamine *K. pneumoniae*	Tuna sashimi	1978	San Francisco		1 outbreak	-	Taylor & Speckhard, 1983
SFP. Three restaura	Histamine	Pacific amberjack fish	31/12 1985 and 4/1/1986	Alabama and Tennessee	Diastolic hypotension, and bronchospasm	10 people	Antihistamine, hydration, antiemetics	Clive r, 2007

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nts								
SFP		Frozen mahi mahi imported from Taiwan	7 /1987	Albuquerque in New Mexico	Nausea, vomiting, diarrhea, headache, fever, flushing, and rapid pulse rate	Two people	Patients treated with Benadryl, activated charcoal, and ipecac in a hospital emergency room.	
		Puffer fish	1987	Britain	Rash, diarrhoea, flushing and headache	258 incidents. Of 240 fish samples from these incidents, 101 contained 5 mg histamine /100 g fish	Antihitamine therapy	Lehane & Olley, 2000
	Ichthyosarcotoxism	Cape yellowtail (*Seriola lalandii*)	1990	Tygerberg, South Africa	Skin rash, diarrhoea, palpitations, headache, nausea and abdominal cramps, paraesthesia, an unusual taste sensation and breathing difficulties	10 incidents, involving 22 patients		Muller *et al*, 1992
HFP	Histamine	Smoked fish	3/1990-6/1993	New Zealand	Typical symptoms: allergy	19 outbreaks	Carboxyl, imidazole, amine	Lehane & Olley, 2000
SFP.		Australian salmon, *Arripis truttaceus*	1992	Western Australian	Erythema and urticaria of the skin, facial flushing and sweating, palpitations, hot	7 patients	Parenterally administered promethazine	Smart, 1992

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					flushes of the body, headache, nausea, vomiting and dizziness.		(antihistamines) or repeated doses of promethazine	
SFP. The suspected fillets were from a seafood supplier of Pingtung Prefecture in southern Taiwan		The flesh of *Istiophorus platypterus*	7/1994	Changhua Prefecture, Western Taiwan	Rashes, urticaria, nausea, vomiting, diarrhea, flushing, tingling and itching of the skin	12 people	-	Hwang *et al*, 1995
SFP. Questionnaire interviews were given to persons		Fish of Makaira *Euthynnus*	1996	Taiwan	Flush, dizziness, blurred vision and skin rashes after eating lunch	55 people	Antihistamines	Wu *et al*, 1997

who ate lunch in the same cafeteria								
SFP		Tuna	1997	United Kingdom	Headache, flushing, rash, and diarrhoea	Two cases (45 and 31 male year old)	Antihistamines (chlorpheniramine and cimetidine)	Stell, 1997
SFP at one kindergarten		Puffer fish	25/11/1997	Taiwan	Hyperemia, particularly on the face and neck, nausea and vomiting, abdominal pain, pruritus, headache and dizziness and diarrhea	94 cases	Antihistamines	Wu & Chen, 2003
SFP		Blue fish, sardines, anchovies, amberjack, and mahi-mahi	1998-2002	United States	Tingling and burning sensations around the mouth, headache, facial flushing, palpitations, profuse sweating, truncal rash and pruritis, abdominal cramps, nausea, and diarrhea	167 outbreaks 703 persons, 38 of whom were hospitalized		Sobel & Painter, 2005
Histamine poisoning at a restaura		Yellowfin tuna (*Thunnus cdbacares*)	2001	Yokohama, Kanagawa	Palpitations, dyspnea, headache and facial flushesb without diarrhea or vomiting	Eight cases	200 mg of hydrocortisone sodium succinate	Ohnuma *et al*, 2001
					Headache, flushing, palpitations, peppery tast,	Six patients	200 mg of hydrocortisone sodium	

nt					nausea, diarrhea and erythema		succinate, Saiseikai Kanagawaken Hospital	
SFP		Canned mackerel	12/ 2001	Taipei Prefecture, northern Taiwan	Rash, urticaria, nausea, vomiting, diarrhea, flushing, and tingling and itching of the skin	3 patients		Tsai *et al*, 2005
HFP		Yellow fin tuna (*Thunnus albacares*) with wasabe and Japanese spices	2003	Australia	Abnormal Taste, paraesthesiae or numbness diarrhoea, headache, rash and hypotension	Four patients (41, 52, 59 and 27 years old)	Metoprolol 50 mg bd, aspirin 150 mg daily, trandolapril 1 mg daily and Specific treatment (a combination of H1 and H2 blockers promethazine 10-50 mg and ranitidine 150-300 mg)	Hall, 2003
					Circumoral paraesthesia and diarrhoea	Two persons	Symptoms had resolved by midnight	
SFP		Escolar fish (*Lepidocybium flavobrunneum*)	11/8/2003	California, USA	A peppery taste, numbness of the tongue, headache, flushing and sweating, dizziness, nausea, diarrhoea, and shortness of breath	42 people	H2-antagonist such as cimetidine or ranitidine, acetaminophen, diphenhydramine	Feld man *et al*, 2004
		Cold-smoked tuna	2004	Denmark	Gastrointestinal and neurological symptoms	3 cases – 11 persons		Embo rg & Dalga ard,

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								2006
		Billfish: *Makaira nigricans*	5/2004	Pingtung, southern Taiwan	Rash, nausea, diarrhea, and flushing, but all recovered within 24 h	59 people		Tsai *et al*, 2007
		Xiphias gladius	12/2004	Taichung, central Taiwan				
SFP at an Icelandic restaurant in a mixed salad		Raw tuna	2005	Iceland	Erythema over the face and neck within two hours, sweating, a feeling of intense thirst and palpitations	4 cases		Sigmundsdóttir *et al*, 2005
SFP		Tuna (*Thunnus thynnus*)	3 /2006	Chiayi Prefecture, southern Taiwan	rash, nausea, diarrhea, flushing, and tingling and itching of skin	7 cases		Chen *et al*, 2008
		Tuna, mackerel, skipjack, bonito, marlin	2007	Napoli, Italy	Slurred speech, blurred vision, loss of muscular strength, wheezing, tachypnea, marked hypotension	2 persons: 18-16 years old	Patient went to a hospital equipped with a hyperbaric chamber. Fluid therapy corrected the DO_2/VO_2 unbalance	Iannuzzi *et al*, 2007
PSP (Paralytic shellfish	STX	Shellfish	1970-1990	Canada	A tingling sensation around the lips, gums, and tongue, numbness in fingertips and toes, which progresses to the arms, legs and neck within 4-	60 persons	Continuous mechanical support of respiration is advisable in severe cases	Report of the Joint FAO/

poisoning)					6 hours			IOC/WHO, 2004
	Saxitoxin	*Mytilus edulis*	1981	Prince Edward Island, Canada	Vomiting, respiratory difficulties and paralysis, hypoxia and hypercapma	106 illnesses and 3 human deaths	Ozone treatments, mechanical ventilation, gastric decontamination	Shumway, 1990
PSP and SFP	Saxitoxin and tetradotoxin	Horseshoe crabs and mussels	1981-1987	Thailand	Gastrointestinal and neurological symptoms	8 outbreaks	Appropriate preventive	Swadiwudhipong *et al*, 1989
PSP	Saxitoxin (STX)	Clam soup	1987	Guatemala	Weak hypotensive, death, cardiovascular collapse, despite respiratory support	187 cases with 26 deaths	In severe cases respiratory paralysis is common, and death may occur if respiratory support is not provided.	Clive r, 2007
	STX	Crustaceans, gastropods and fish	1998-2002	United States	Tingling or numbness begin periorally and spread to the neck and face, headache, nausea, vomiting, and diarrhea	43 persons	No antidote exists, and supportive treatment, including artificial ventilation (when necessary), is the mainstay of therapy	Sobel & Painter, 2005
Puffer	Tetrodotoxins	Puffer fish and	1974 -1983	Pacific around	Numbness of face and	646 incidents,	Usually within 3 h.	Whitt

fish poisoning (PFP)	(TTXs)	fugu		China and Japan	extremities, floating sensation, weakness, ascending paralysis, respiratory failure, cardiovascular collapse, death	and of them 179 deaths	Artificial respiration	le & Gallacher, 2000
PFP. They consummated of frozen pufferfish imported from Taiwan		Pufferfish and mislabelled as angler fish	1977	Italy	Bright red, sometimes itchy rash on face neck and chest hot flushing and sweating, tingling of the lips, mouth and tongue, headache, burning in the mouth and peppery taste	Three deaths	No known antidote; Supportive therapy: gastric decontamination and aggressive airway management	Clive r, 2007
PFP		Pufferfish and fugu	1983 -1992	Japan	Ingling of the lips and tongue, general warmth, euphoria and exhilaration, perioral paresthesia, nausea and dizziness, respiratory failure, bradycardia and hypotension	449 cases and 49 deaths	No antidote for tetrodotoxin; treatment is aimed at limiting the absorption of tetrodotoxin by gastric lavage	Mines *et al*, 1997
		Fugu (puffer fish) brought from Japan	29/4/1996	California	Onset of symptoms began approximately 3-20 minutes after ingestion	Three cases	All three persons were transported by ambulance to a local	Clive r, 2007

							emergency department	
	Histamine	Fish	1996	Taiwan	Facial flush, dizziness, diarrhoea, headache, conjunctival hyperaemia and hypotension. Loss of vision in association with atrial tachycardia	Two outbreaks	Antihistamine therapy including return of vision, was rapid	Lehane & Olley, 2000
	TTX	Puffer fish: blowfish, toadfish, globefish, balloonfish, patkafish and fugu	2002	Khulna, Bangladesh	Perioral paraesthesia weakness of both lower limbs, paraesthesia, headache, difficulty in respiration, nausea and vomiting, blurring of vision, and vertigo	Eight families: 37 patients. Eight patients died	There is no specific treatment reffered: Medical help	Ahasan *et al*, 2004
Amnesic shellfish poisoning (ASP)	Domoic acid (DA)	Blue mussels (*Mytilus edulis*)	1987	Prince Edward Island, in eastern Canada	Gastrointestinal and neurological symptoms	156 cases: 22 individuals were hospitalized and three elderly patients eventually died	Supportive	Clive r, 2007
		Mussels		The Prince Edward Islands, Canada	Vomiting, cramps, diarrhea, agitation, seizures, coma, profuse respiratory secretions, and circulatory instability	107 persons: 19 persons were hospitalized and 4 persons died		Sobel & Painter, 2005;

								Hummert *et al*, 1997
Diarrhetic shellfish poisoning (DSP)	Okadaic acid (OA) and dinophysistoxins (DTXs)	Mussels originating from Ireland	1995	Netherlands	Acute diarrhoea, nausea, vomiting, abdominal pain, and chills	8 people	Inhibition of essential protein phosphatases. Not detected by bioassay.	Whittle & Gallacher, 2000
NSP	Brevetoxin	Barracuda, grouper, sea bass, snapper, and amberjack	1998-2002	United States	Symptoms typically resolve within 48 h after onset	2 outbreaks: persons, 1 of whom was hospitalized	Supportive treatment	Sobel & Painter, 2005
Haff disease: people ate fish	Cyanobacterial toxin	Cyanobacterial blooms *Oscillatoria spp.*	1920s-1940s	Polish and Swedish coasts	Gastrointestinal and neurological symptoms	Large numbers of human cases of illness and deaths	Antibiotics include cyclic molecules	Codd *et al*, 1999
Haff disease in a supermarket (Califor	Biotoxins	Buffalo fish (*Ictiobus cyprinellus*)	1984-1992	United States	Myalgia, Muscular stiffness, Pain to light touch, Dry mouth, Painful breathing, Chest pain, Nausea or vomiting, Numbness of thighs, Numbness of whole	Two cases (Texas 1984) four cases (Texas 1996) two cases (Los Angeles,	All patients were hospitalized, none died, and the median hospital stay was 3 days. Treatment consisted of Intravenous fluids, in	Bucholz *et al*, 2000

nia) or alive from a fish tank at a market (Missouri)					body	California, 1985) two cases (San Francisco, California, 1986)	addition to mannitol or bicarbonate	
Cyanobacte-rial	Cyanobacterial toxins	Solomon Dam *C. raciborskii*	1979	Palm Island, Queensland, Australia	Vomiting, headache, abdominal pain with tender hepatomegaly, lethargy, diarrhoea, acidosis, and injury to the liver, kidneys, lungs, adrenals and intestine	139 children and 10 adults	Intravenous therapy	Codd *et al*, 1999
Cyanobacterial bloom	Lyngbya majuscula	Surgeon fish butter fly fish damsel fish and puffer fish	1983	Lahaina, Maui, HI	Dermatitic, respiratory and eye effects	1 outbreak		Osborne *et al*, 2001
Cyanobacterial poisoning	Cyanobacterial toxins	Blooms of *Microcystis* and *Anabaena*	1993	Bahia, Brazil	Gastroenteritis outbreak	2000 cases of gastroenteritis, including 88 deaths	Copper sulphate as an algicide during water treatment	Codd *et al*, 1999
Untreated water		*Planktothrix agardhii* (*Oscillatoria*	1994	Sweden	Gastroenteritis outbreak	70 to 80 people	Water treatment	

from the eutrophic River Kavlinge an, the outlet of Lake Vombsjon		*agardhii*)						
Cyanobacterial poisoning (blue-green algae)	Cyanobacterial blooms	*Lyngbya majuscula* *Cylindrosp-ermopsis raciborskii*	1996-97 1997-98	Moreton Bay Queensland	Rashes, blisters, allergic reactions resembling hay fever, asthma, conjunctivitis, and ear and eye irritation	Hundreds of swimmers and fishermen		
Cyanobacterial poisoning	Cyanobacterial hepatotoxins	*Microcystis*, *Anabaena*, *Anabaenops Cylindrospermopsis Raphidiopsis* and *Nodularia*	2000	China and Brazil	Gastroenteritis, nausea, vomiting, fevers, flu-like symptoms, sore throat, blistered mouth, ear and eye irritation, rashes, myalgia, abdominal pains including painful hepatomegaly, pulmonary consolidation, visual disturbances, kidney damage, and liver damage	Several deaths	Improve water treatment to longer term, including inreservoir remedial action to reduce cyanobacterial growth	Codd *et al*, 2005

31% by finfish, Norwalk virus 42%, scombrotoxin 44% of outbreaks	*Vibrio vulnificus* and *V. parahaemolyticus*	Whitefish, finfish (clams, oysters, mussels), bluefish, tuna, salmon, shrimp, crab, lobster seafood chowder	1980-1994	New York	Mild gastrointestinal symptoms, numbness and tingling	3959 illnesses, 76 hospitalizations, and 4 deaths		Wallace *et al*, 1999
Kanagawa phenomenon: a typical outdoor street market	*Vibrio parahaemolyticus*	Raw oyster	8-9/ 1999	Galicia (northwest Spain)	Gastrointestinal symptoms	64 cases	Nine case patients were hospitalized. Antibiotic susceptibility	Lozano-León *et al*, 2003

Legend

Figure 1: Effects of depuration time on domoic acid contents of mussels (*Mytilus galloprovincialis*) stored at 22°C (Blanco et al., 2002) and accumulation of the same toxin in tissues of mussels (*Mytilus edulis*) exposed for 4 days to toxic *Pseudo-nitzschia multiseries* (at 12°C) (Mafra et al., 2010).