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Shellfish Poisoning and Toxins

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ABSTRACT

Shellfish toxins are the most dangerous marine biotoxins, and produced by free living micro-algae. Shellfish toxins are concentrated in flesh, and poisoning occurs when these contaminated shellfish is consumed. They can cause paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP), amnesic shellfish poisoning (ASP) and azaspiracid shellfish poisoning (AZP), responsible for a variety of gastrointestinal and neurological symptoms in consumers. This article reviews the main types of marine toxins associated with shellfish poisoning and their toxic effects, the limitations of current regulations on marine biotoxins and the techniques used for their detection to ensuring the safety of shellfish for human consumption.

Key Words: Seafood, shellfish poisoning, microalgae, marine toxins

INTRODUCTION

Although viral and bacterial infections resulting from shellfish ingestion are more common, toxin-mediated shellfish poisoning can cause severe and life-threatening neurological effects (Isbister and Kiernan 2005). Shellfish is regarded as a 'super food', owing to their high nutritive value including high levels of poly-unsaturated fatty acids (PUFAs), especially omega-3 fatty acids. Shellfish feed on marine microalgae that contains high levels of PUFAs (Furey et al. 2010). Bivalve molluscs feed by filtering suspended particles, including phytoplankton, from the water (Turrell and Stobo 2007). Marine microalgae, especially phycotoxin producers such as several species of dinoflagellates and diatoms, are one of the main problems in the exploitation of marine resources around the world. Phycotoxins are toxic compounds that enter into the food chain as components of the phytoplankton (Campas et al. 2007). Toxic shellfish poisonings occur when algal toxins are transferred through the food chain to higher trophic levels, including humans (Takahashi et al. 2007).

Marine biotoxins including lipophilic toxins such as okadaic acid (OA), dinophysistoxins (DTXs) and associated esters, yessotoxins (YTXs), pectenotoxins (PTXs) and azaspiracids (AZAs) and hydrophilic toxins such as domoic acid (DA) and saxitoxins (STXs), may vary structures, toxicology and modes of action (Parades et al. 2011, Turrell and Stobo 2007).

Mass proliferations of phytoplankton are known as algal blooms, and blooms of toxic algae are also known as harmful algal blooms, or HABs (Camacho et al. 2007). One of the determinants of harmful algal bloom is HAB toxins produced by some algae, which accumulate in shellfish and subsequently result in poisoning of humans that consume shellfish (Yang et al. 2009). This is a major concern for public health authorities as well as for the shellfish industry, since the closure of harvesting sites due to these toxic episodes has also an important economic impact (Regueiro et al. 2011).

THE MAIN TYPES OF TOXIC SHELLFISH POISONING

Human diseases associated with exposure to harmful algal bloom toxins include paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP), amnesic shellfish poisoning (ASP) and azaspiracid shellfish poisoning (AZP), *etc*. (Yang et al. 2009).

Characteristics associated with the main types of shellfish poisoning are represented in Table 1. Additionally, Table 2 summarizes the data of reported human intoxications due to algal toxins in shellfish.

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Table 1. The main marine toxins, their sources and effects (Camacho et al. 2007, Oliveira et al. 2011)

Effects	Toxin origin	Main toxins	Food source
Paralytic shellfish poisoning (PSP)	*Alexandrium catenella, A. cohorticula, A. fundyense, A. fraterculus, A. leei, A. minutum, A. tamarense, A. andersonii, A. ostenfeldii, A. tamiyavanichii, Gymnodinium catenatum, Pyrodinium bahamense* var. *compressum*	Saxitoxins (STXs)	Clams, mussels, oysters, cockles, gastropods, scallops, whelks, lobsters, copepods, crabs, fish
Diarrhetic shellfish poisoning (DSP)	*Dinophysis acuta, D. caudate, D. fortii, D. norvegica, D. mitra, D. rotundata, D. sacculus, D. fortii, D. miles,D. norvegica, tripos, Prorocentrum lima, P. arenarium, P.belizeanum, P. cassubicum, P. concavum, P. faustiae, P. hoffmannianum, P. maculosum,Protoceratium reticulatum, Coolia* sp., *Protoperidium oceanicum, P. pellucidum, Phalacroma rotundatum*	Okadaic acid, dinophysis toxins (DTXs), yessotoxins (YTXs) and pectenotoxins (PTXs)	Mussels, scallops, clams, gastropods
Neurotoxic shellfish poisoning (NSP)	*Gymnodinium breve*	Brevetoxins (PbTxs)	Oyster, clams, mussels, cockles, whelks
Amnesic shellfish poisoning (ASP)	*Nitzschia* spp.	Domoic acid	shellfish
Azaspiracid shellfish poisoning (AZP)	*Protoperidinium crassipes*	Azaspiracids (AZAs)	Mussels, oyster

Table 2. Reported cases of the different types of shellfish poisoning

Types of poisoning	Date	Implicated food	Number of illness	Location	Reference
PSP	1990	Mussels	6	Massachusetts	CDC, 1991
PSP	1991	Mussels	24	Venezuela	Barbera-Sanchez et al. 2004
NSP	1996	Shellfish	3	Florida	Poli et al. 2000
DSP	2002	Blue mussels	403	Belgium	De Schrijver et al. 2002
AZP	1998	Mussels	20-30	Ireland	James et al. 2004
AZP	2000	Mussels	12-16	UK	Twiner et al. 2008
DSP	2002	Mussels	32	Portugal	Vale et al. 2003
DSP	2009	Mussels	45	France	Hossen et al. 2011

Paralytic shellfish poisoning (PSP)

Due to their world-wide incidence, PSP toxins pose the most serious threat to public health and cause and unmeasurable economic damage (Campas et al. 2007). These toxins responsible for seafood-borne illness are the neurotoxins known collectively as the saxitoxins (STXs), also referred to as PSP toxins. Major toxin sources include certain species of microalgae, notably marine dinoflagellates of the genera *Alexandrium* (formerly *Gonyaulax*), *Gymnodinium* and *Pyrodinium* (Etheridge 2010). Subsequent ingestion of filter-feeding molluscan bivalves, accumulate toxins by ingesting toxic dinoflagellates, can result in serious illness or even death in humans (Wang et al. 2011). PSP-toxins, having a skeleton of 3,4,6-trialkyltetrahydropurine, are fast-acting neurotoxins that can inhibit transmission of nerve impulses by blocking voltage-gated sodium channels in nerves, skeletal, and cardiac muscle fibres, and can lead to death, due to respiratory paralysis (Wong et al. 2009).

Symptoms resulting from these toxins include tingling sensation of the lips, mouth and tongue, numbness of extremities, paresthesias, weakness, ataxia, floating/dissociative feeling, nausea, shortness of breath, dizziness, vomiting, headache, dysphagia and dysarthria (Etheridge 2010).

Diarrhetic shellfish poisoning (DSP)

Although not a life-threatening condition, diarrhetic shellfish poisoning is a worldwide problem for the bivalve aquaculture and fisheries industries (Svensson 2003). Okadaic acid (OA) and analogues, including the dinophysis toxins DTX-1, 2 and 3, are mainly responsible for diarrhetic shellfish poisoning (DSP) syndrome (Garthwaite 2000). The toxins are produced by dinoflagellates belonging to the genera *Dinophysis* and *Prorocentrum*, and bivalves including mussels accumulate the toxins from ingestion of the algae (Duinker 2007). DSP is caused by the ingestion of shellfish that have bioaccumulated toxin in their viscera from dinoflagellates (Novak 1998). The main symptoms caused by consumption of DSP-contaminated shellfishes include diarrhoea, vomiting, and abdominal pain. Other symptoms associated with the disease include nausea and headache (Aifeng et al. 2006). Although no serious acute toxicities of OA and DTXs were reported, the chronic effects of the toxins, such as tumor promotion resulting from the inhibition of protein phosphatase, caused much concern (Aifeng et al. 2006, Takahashi et al. 2007).

Neurotoxic shellfish poisoning (NSP)

The toxins of the neurotoxic shellfish poisoning (NSP) are one of the most characteristic and spectacular classes of compounds produced by microalgae, and known as brevetoxins (Daranas et al. 2001). These polyether toxins are produced by the dinoflagellate *Gymnodinium breve* (Garthwaite 2000). NSP is characterised by a combination of gastrointestinal effects (abdominal pain, nausea, and diarrhaea) and neurological effects (paraesthesia, “temperature reversal”, myalgia, vertigo, and ataxia) (Isbister and Kiernan 2005).

Amnesic shellfish poisoning (ASP)

The causative chemical, which causes amnesic shellfish poisoning, is domoic acid, an amino acid produced by the pinnate diatoms (*Pseudonitzschia* spp). The toxin can be bio-concentrated by filter-feeding shellfish that graze on these phytoplankton and, thus can then enter the human food chain (James et al. 2005, Jeffery et al. 2004). Amnesic or encephalopathic shellfish poisoning differs from most other neurotoxic marine poisoning because the main effect is to the central nervous system (CNS). It is a toxic encephalopathy that is characterised by severe memory loss and confusion (Isbister and Kiernan 2005). Other toxic reactions to DA in humans include abdominal cramps, vomiting and diarrhoea. It has been documented to affect, not only humans, but marine organisms such as marine mammals and sea birds (Takahashi et al. 2007).

Azaspiracid poisoning (AZP)

Azaspiracid poisoning (AZP) is the most recently discovered human toxic syndrome associated with shellfish (Furey et al. 2010, Vale 2004). AZP toxins are relatively thermostable nitrogen-containing compounds, azaspiracid (AZA) (Campas et al. 2007) and *Protoperidinium crassipes* has been identified as a potential producer of them (Twiner et al. 2008). Around 30 analogues are known but not all of them are completely characterized (Parades et al. 2011), and AZA1 is the most toxic to human followed by AZA2 and AZA3 (Alvarez et al. 2010). AZA3 seems to be easily degraded compared to the others, so that might be the reason why it naturally occurs in lower concentrations than AZA1 and AZA2, which are stable until 70ºC in acidic conditions (Parades et al. 2011). Toxicology studies have demonstrated that AZA1 targets several tissues causing atrophic lamina propria and shortening and erosion of villi in the intestine, necrosis of lymphoid tissues, fatty liver and lung tumors (Vilarino et al. 2007). Similar to DSP toxins, human consumption of AZA-contaminated shellfish can result in severe acute symptoms that include nausea, vomiting, diarrhea, and stomach cramps (Kilcoynea and Fux 2010). These toxins have become widespread in multiple European countries as well as Morocco and eastern Canada (Alvarez et al. 2010, Twiner et al. 2008).

REGULATIONS AND DETECTION METHODS FOR MICROALGAL TOXINS

According to the European Union Directive 91/492/EEC, the content of PSB toxins must not exceed 80 µg/100 g of shellfish flesh in accordance with the biological testing method. Using the HPLC method, the EU permits a maximum level of 20 µg/g for the total ASP toxins content in the edible parts of mollusc (Council of the European Communities 1991). In 2002, the European Commission set a maximum level for okadaic acid (OA), dinophysistoxins (DTXs) and pectenotoxins (PTXs) present at same time in edible tissues of 160 µg of okadaic

acid equivalent/kg shellfish. The European Commission also decided to set the upper safe limit of AZP toxins in bivalve molluscs, echinoderms, tunicates and marine gastropods, to 160 µg of azaspiracid equivalents/kg (European Commission 2002).

The risk management linked to phycotoxin contamination of bivalve molluscs is based on monitoring of toxins in material intended for human consumption (Rossini 2005). Increasing and serious concerns about seafood safety and human health protection have made evident the necessity of rapid, robust, specific and sensitive analytical methods for the detection of phycotoxins (Campas et al. 2007) and several methods are currently used to assess the contamination of seafood by toxic agents.

Among the different bioassays, the in vivo mouse bioassays are the most commonly used. In spite of being laborious and time-consuming, inconsistencies, lack of specificity and questionable ethical justification of mouse bioassays, this technique is useful as preliminary toxicity screening (Rossini 2005).

Antibody-based immunoassays are potentially useful for accurate, sensitive and routine determinations of marine toxins. These assays typically include enzyme-linked immonosorbent assay (ELISA) or radioimmunoassay (RIA) (Camacho et al. 2007). Several immunosensors, including electrochemical, optical, cell and tissue biosensors, have also been used for immunodetection of marine toxins (Vilarino et al. 2010). Biosensors are characterised by the simplicity of use, even for non-skilled personnel, and the low cost. At present, biosensors should be seen as bioanalytical tools for preliminary screening the toxicity of a sample (Campas et al. 2007).

Chromatographic techniques such as high performance liquid chromatography (HPLC) have been widely developed for the detection of seafood toxins, and allowed separation, highly selective identification and sensitive quantification of the different toxins present in a sample (Campas et al. 2007). The analytical techniques including more recently liquid mass spectrometry (LC-MS) has led to rapid advances in understanding of production, accumulation, depuration and chemistry of the toxins (Garthwaite 2000, Hess 2010, Isbister and Kiernan 2005).

Apart from these, algal monitoring procedure has been successfully used in a number of countries as an early warning system and relies upon a good understanding of the toxigenic species present in the marine environment (Garthwaite 2000).

CONCLUSIONS

To ensure consumer safety, it is essential the monitoring of toxin levels in all commercially harvested shellfish using the new analytical techniques such as HPLC, LC-MS, immunoassay, cellular bioassays and molecular probes. Furthermore, an effective risk assessment programs for the identification of the environmental conditions and organisms responsible for toxin production in shellfish harvesting areas must be developed to prevent shellfish toxin contamination.

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