Functional assays for marine toxins as an alternative, high-throughput-screening solution to animal tests

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Marine toxins accumulate in filter-feeding bivalves. Their presence is a risk to consumers and requires costly control systems to avoid food-safety problems. The mouse bioassay is the method currently used in most countries, but it is being challenged with regards to ethical issues and specificity.

There are two options. One uses an analytical method that identifies the analytes in the sample (e.g., liquid chromatography with mass-spectrometry detection, or fluorescence or ultraviolet detection). The drawback of this approach is that many standards are necessary, and the information does not offer any insight into the toxicology of the sample. The second option uses biological methods, which can be based on biochemicals or receptors. Biochemical methods identify only the compounds for which the binder was developed, unrelated to toxic potential, while receptor-based or functional assays recognize the potential toxicity of the toxins, hence mimicking bioassay, but they cannot identify single analytes in the sample.

The best approach is a combination of a functional assay, which provides information about sample toxicity quickly and cheaply, and a confirmatory method, which identifies the profile of toxins in the sample. This review analyzes current developments in functional assays for marine toxins.

Abbreviations: HPLC, High-performance liquid chromatography; HTS, High-throughput screening; LC-MS, Liquid chromatography mass spectrometry; SPR, surface-plasmon resonance; TEF, Toxicity equivalent factor

Keywords: Animal test; Ciguatera; Cyclic imine; Detection; Food safety; Functional assay; High-throughput screening; Marine toxin; Okadaic acid; Saxitoxin

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1. Introduction

Marine toxins comprise a large, diverse group of chemicals with many distinct structures and, in most cases, they are formed by microalgal species of dinoflagellates [1]. The complexity of their structures is notable in some cases, and many of them are ladder-like polycyclic ethers [2]. Palytoxin, the largest non-biopolymer compound in nature, or domoic acid, a glutamate analog, are examples of the chemical diversity of the marine toxins. Table 1 classifies the toxins.

Since dinoflagellates are a primary step in the food chain, they accumulate in mollusks or fish. Under certain ecological conditions of light, salinity and temperature, the dinoflagellates that produce toxins may grow in very large quantities and, for some hours, days or weeks, become the main source of food for filter-feeding mollusks. Sometimes, the existing population of dinoflagellates becomes toxic after a change in ecological conditions. As a consequence, shellfish accumulate very large amounts of toxins in their digestive tracts, so marine toxins can be a food-safety threat of great relevance, as the toxins may appear at any time of the year, and anywhere in the world. In some cases, they are extremely toxic (e.g., maitotoxin or palytoxin [3], two of the most toxic natural compounds known to date). Increases in oceanic eutrophication and commercial shipping provide ways to disperse toxic dinoflagellates, so contributing to the increased rates of intoxication worldwide [4].

The risk associated with shellfish consumption has prompted authorities

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Table 1. Marine toxins

Toxin group (common denomination)	Reference compound (TEF in Table 3)	Analogs	Biological target
Diarrheic shellfish toxins (DST or DSP))	Okadaic acid (OA)	Dinophysistoxins (DTX) 1-6	Inhibition of cytosolic phosphatases 1 and 2A [34]
Paralytic shellfish toxins (PST or PSP)	Saxitoxin (STX)	Carbamate (GTX 1-4, Neo-STX); N-sulfocarbamoyl (C 1-4, GTX 5-6); Decarbamoyl (dcGTX1-4, dcSTX, dcNeo); Benzoate (GC 1-6) [74]	Blockade of site 1 on the voltage-dependent sodium channel [75]
Azaspiracids	Azaspiracid 1 (AZ1)	Azaspiracid 1-11 [76]	Unknown target
Yessotoxin	Yessotoxin (YTX)	Adriatoxin, and several analogs of: HydroxyYTX; CarboxyYTX; HomoYTX KetoYTX; NoroxoYTX [77]	Phosphodiesterase activation [56] and possibly other targets
Palytoxin	Palytoxin	Up to 10^{21} isomers, Ostreocins, Ovatatoxins (Ovatoxins), Mascarenotoxins [78]	Blockade of Na+-K+ ATPase [78]
Domoic acid or amnesic shellfish toxin (ASP)	Domoic acid	Isodomoic acids A-H; epidomoic acid [79]	Activates kainate receptor [80]
Maitotoxin	Maitotoxin	Up to 2^{99} possible isomers [2]	Unknown target, activates calcium entry
Ciguatera	Ciguatoxin-1 (CTX1)	Caribbean ciguatoxins (C-CTX 1-2) Pacific ciguatoxins (P-CTX 1-4, three derivatives of P-CTX3 and two P-CTX4) [81] Gambiertoxins (nine analogs) Gambierol	Activates sodium channels at site 5 [81]
Brevetoxins or neurotoxin shellfish poisoning (NSP)	Brevetoxin B (PbTx2) Brevetoxin A (PbTx1)	PbTx 3-14 Brevenal [82]	Activates sodium channels at site 5 (several hundred-fold less potent than ciguatera) [83]
Cyclic imines	Gymnodimine (GYM-A) Spirolide	Gymnodimine A, B; Spirolide A-G (and desmethyl spirolides); Pinnatoxin A-D; Pteriatoxin A-C; Pinnamine; Prorocentrolide A-B; Spiro-Prorocentrimine C; Symbioimines (and probably many more imines) [84]	Reversible blockade of muscarinic/nicotinic receptor (gymnodimine and spirolides, probably same for other cyclic imines) [63,64]
Pectenotoxins	Pectenotoxin 2 (PTX2)	Pectenotoxins 1-14 Pectenotoxins seco acids	Inhibition of actin polymerization by capping the barbed end [65]

worldwide to implement monitoring systems that would prevent the appearance of these toxins in the market, hence protecting consumers. This review will describe options for methods to monitor marine toxins and developments to be expected in the future.

Monitoring marine toxins has become an important issue, from not only an economic view, but also as a quick decision tool, since the presence of a toxin bloom in controlled waters means that it is essential to close the production area, with all the necessary economic losses, demanding the shortest possible response time and the best management and decision-making processes. Although in marine toxins, progress has been slow, there has been an explosion of technical options in the past few years, which make the outlook promising in terms of future developments.

2. Bioassay

The combination of complex structures, unknown mechanisms of action, wide variations in toxin-analog structures, abundance of toxin groups and historical scarcity of standards and enough research material has made the mouse bioassay the main tool for monitoring the presence of marine toxins. Although phycotoxins were identified as a problem to consumers more than 70 years ago [5,6], progress in finding alternatives to animal tests has been minimal.

The first animal test for marine toxins was the saxitoxin bioassay [5], the only one to achieve the status of being an AOAC official method [7]. Later, in 1978, Yasumoto et al. [8] developed a bioassay for diarrheic toxins. Dependence on animal tests is a clear indication of an underdevelopment in this field. Although there has been progress in recent years, there is still no clear solution to the bioassay on the near horizon.

Basically, an animal test is an intraperitoneal injection of a shellfish extract into three mice. If two of three mice die, the test is positive, but, if only one dies, the test is negative. Depending on the solubility of the compounds, there are two types of bioassay:

- with hydrophilic solvents, for paralytic toxins (saxitoxin and analogs); and,