EuroProxima

Marine Biotoxins

Competitive ELISA kits for the quantitative detection of saxitoxin, okadaic acid and domoic acid in shellfish tissue

Marine biotoxins, a world-wide risk

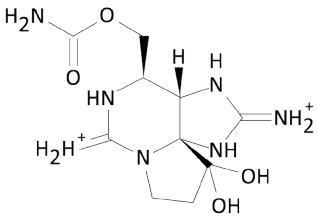
10,000 people in France were affected by diarrheic shellfish poisoning symptoms caused by domestically produced mussels [1]. In Spain a Michelin-starred chef was arrested for offering scallops retrieved from the black market; these scallops harvested high level of the toxin that cause amnesic shellfish poisoning [2]. Consumption of contaminated mussels lead to an outbreak of diarrhetic shellfish poisoning in the Zhejang province in China[3]. The California Department of Health published an official warning after local harvested mussels were found to contain high level of paralytic shellfish poisoning toxins [4]. These are just some quotes of the risks caused by marine biotoxins that occur all over the world. These risks will increase in the nearby future due to eutrophication of surface water, marine transport, aquaculture and global climate change [5]. All these issues give rise to growth of algae and therefore to the production of marine biotoxins.

Marine biotoxines, or phytotoxins, are naturally occurring compounds produced by algae and phytoplankton. Filter feeders such as clams, mussels, scallops and oysters can consume large quantities of these algae when environmental conditions result in harmful algal blooms, the so-called 'red tides'. High concentrations of toxins then accumulate in these animals causing illness amongst people who eat them. Shellfish poisoning is a very potent toxicity and extremely dangerous. For some toxins, doses at ppb level can be lethal.

Until now, five syndromes called shellfish poisoning can be distinguished, i.e. paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP) and azaspiracid shellfish poisoning (AZP). A variety of symptoms have been reported to arise from these poisonings, including stomach cramps, abdominal pain, diarrhea, headaches, memory loss, paralysis and death. PSP is associated with a wide number of derivatives of saxitoxin (STX). DSP is caused by a group of polyether toxins including okadaic acid (OA), the dinophysistoxins, pectenotoxin and yessotoxin. ASP is caused by domoic acid (DA) [6,7]. NSP and AZP are relative rare syndromes that are caused by respectively brevetoxins and azaspriracids.

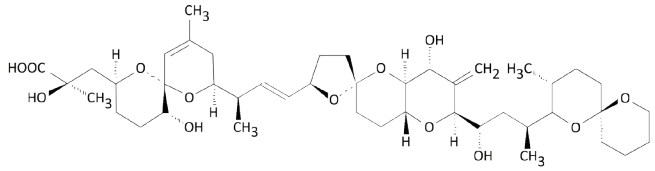
Structure, occurrence and toxic effects

Saxitoxin (STX)



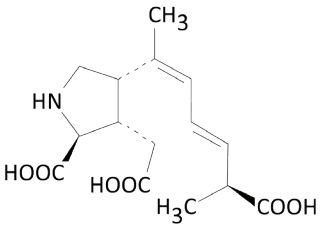
STX is one of the most potent natural toxins known. It acts as a selective sodium channel blocker and is produced by certain species of marine dinoflagellates and cyanobacteria.

Okadaic acid (OA)



OA, a polyether toxin, is a potent tumor promoter. It was named from the marine sponge Halichondria okadai, from which it was first isolated. OA and its analogues, the dinophysistoxins (DTX1, DTX2, and DTX3), together form the group of OA-toxins. These toxins can be found in various species of shellfish. Inhibition of serine/threonine phosphoprotein phosphatases is assumed to constitute the mode of action of OA-group toxins.

Domoic acid (DA)



DA is an amino acid containing the structure of glutamic acid and resembling kainic acid. It was first pinpointed as a problem in marine mammals in 1998, when many California sea lions died along the Central California coast. DA is produced by the phytoplankton Pseudo-nitzschia.

Legislation

In the European Union, Regulation (EC) no 853/2004 establishes that live bivalve mollusks should not contain marine biotoxins exceeding the following limits: 800 µg/kg for the PSP toxins (STX and analogues), 20 mg/kg DA equivalents for the ASP toxins, and 160 µg/kg OA equivalents for OA, dinophysistoxins, and pectenotoxins together [8]. Furthermore, European legislation has forbidden the use of a mouse bioassay for the detection of marine biotoxins from 31 december 2014 [9]. The ELISA is a recognized and competent substitution which complies to this regulation.

References

1. *R. Lawley (2013) Okadaic acid toxins. www.foodsafetywatch.org.*
2. *L. Abend (2008) Spanish scallop scandal. www.gourmet.com.*
3. *Chen T, Xu X, Wei J, Chen J, Miu R, et al. (2013) Food-Borne Disease Outbreak of Diarrhetic Shellfish Poisoning Due to Toxic Mussel Consumption: The First Recorded Outbreak in China. PLoS ONE 8(5): e65049. doi:10.1371/journal.pone.0065049*
4. *20130228 www.foodsafetynews.com.*
5. *James KJJ, Carey B, O'Halloran J, van Pelt FN, Skrabáková Z. Shellfish toxicity: human health implications of marine algal toxins.Epidemiol Infect. 2010 Jul;138(7):927-40.*
6. *M. Dubois, L. Demoulin, C. Charlier, G. Singh, S.B. Godefroy, K. Campbell, C.T. Elliott, and Ph. Delahaut (2010). Development of ELISAs for detecting domoic acid, okadaic acid, and saxitoxin and their applicability for the detection of marine toxins in samples collected in Belgium. Food Additives Contam. 27(6): 859-868.*
7. *K, Campbell, A-C. Huet, C. Charlier, C. Higgins, P. Delahaut, and C.T. Elliott (2009). Comparison of ELISA and SPR biosensor technology for the detection of paralytic shellfish poisoning toxins. J. Chromatogr. B 877: 4079-4089.*
8. *European Commission 2004. Commission regulation 853/2004/EC of 29 April 2004 laying down specific hygiene rules for food of animal origin. Off. J. Eur. Comm. L226: 22-82.*
9. *European Commission 2011. Commission regulation 15/2011 of 10 January 2011, amending Regulation (EC) No 2074/2005 as regards recognised testing method for detecting marine biotoxins in live bivalve molluscs.*

Competitive ELISA

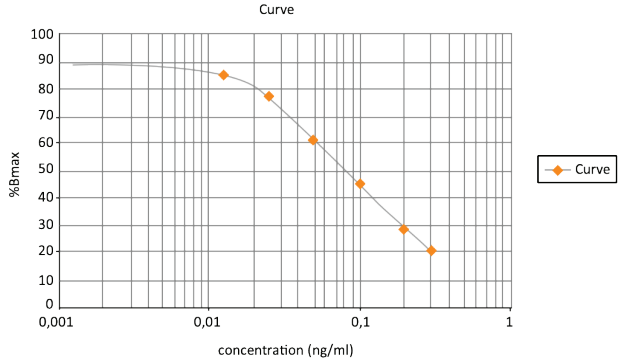
EuroProxima has developed 3 competitive ELISA's for the detection of respectively the toxins STX, DA and OA in shellfish. The ELISA's are easy to perform and fast in comparison with other techniques like HPLC. The scientific team of EuroProxima has validated a simple sample preparation for shellfish matrices. Choosing for an ELISA for marine biotoxins is responding to the expected increase of samples with a cost-effective screening method. This screening can be followed up by a phosphatase inhibition assay for among other OA toxin. This latter test is then interpreted as a risk analysis: giving information about the activity of the toxin.

Principle

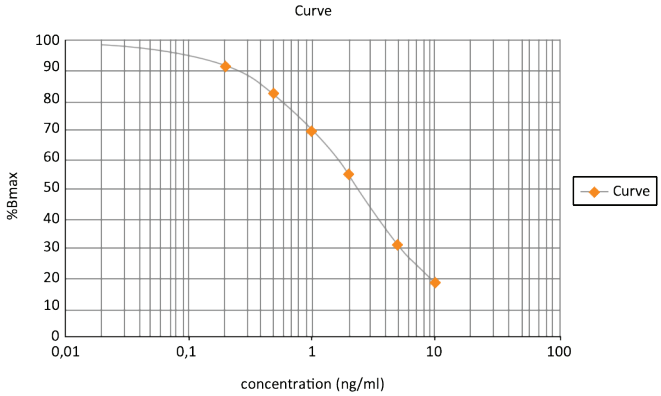
The microtiter plate consists of 12 strips, each containing 8 wells, precoated with sheep antibodies to rabbit IgG (SAR). Rabbit polyclonal antibodies to the toxin, horseradish peroxidase (HRP) labelled toxin as well as toxin standard solutions or samples are added to the precoated wells. The rabbit anti-toxin antibodies are bound by the immobilized SAR antibodies and simultaneously the toxin-HRP and toxin present in the standard or sample compete for binding to the anti-toxin antibody. After an incubation step of 30 minutes, the non-bound reagents are removed in a washing step. The amount of bound toxin-HRP is visualized by adding a substrate/chromogen solution (H_2O_2/TMB) which transforms the colourless chromogen into a coloured product. The reaction is stopped by adding sulfuric acid and the colour intensity is measured photometrically at 450 nm. The optical density is inversely proportional to the toxin concentration in the sample.

Linearity

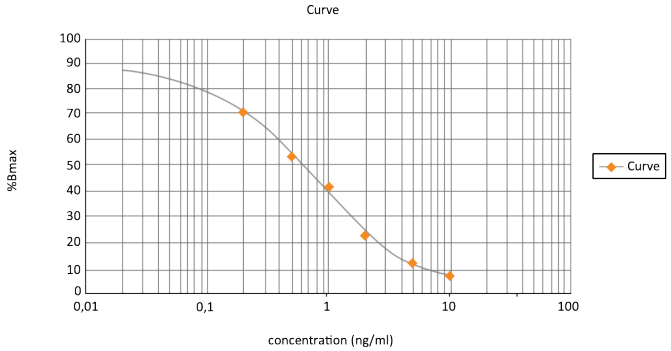
The standard curve for the different marine biotoxin ELISA's is presented. This curve shows the linearity of the assay.



STX standards: 0, 0.0125, 0.025, 0.05, 0.1, 0.2, 0.3 ng/ml



OA acid standards; 0, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 ng/ml



DA acid standards; 0, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 ng/ml

Sensitivity

The detection capability (CCβ) is defined as the smallest concentration of the analyte that can be identified in a sample with an error probability of less or equal to 5%. The CCβ for three common shellfish matrices are presented for the different marine biotoxins.

	CCβ STX	CCβ OA	CCβ DA
Mussels	4 ppb	40 ppb	60 ppb
Oysters	3 ppb	40 ppb	150 ppb
Scallops	-	-	60 ppb

Ordering information

For ordering the marine biotoxin ELISA tests, please use the following catalogue codes:

Saxitoxin ELISA, code 5191SAXI
Okadaic acid ELISA, code 5191OKA
Domoic acid ELISA, code 5191DOMO