



## Work Package 5: Phycotoxins

### Introduction

Shellfish toxins or phycotoxins are potent natural contaminants produced by certain species of marine algae including dinoflagellates and diatoms. In recent years toxic algal blooms have increased in frequency, intensity and geographical distribution potentially due to climate change and increased shipping routes. The associated toxins accumulate within bivalve molluscs such as mussels, clams, cockles, scallops and oysters upon filter feeding.



The consumption of toxin contaminated shellfish may result in illness and even death in birds, mammals and humans. Therefore, sporadic toxic episodes are of major economic concern to both public health officials and the global aquaculture industry. Shellfish toxins are classified into five major groups. Within BioCop, WP5 is dedicated to one of the most potent groups, the paralytic shellfish poisoning (PSP) toxins.

### Paralytic Shellfish Poisoning Toxins

PSP toxins are potent neurotoxins consisting of greater than 20 structural analogues of the parent compound known as Saxitoxin.

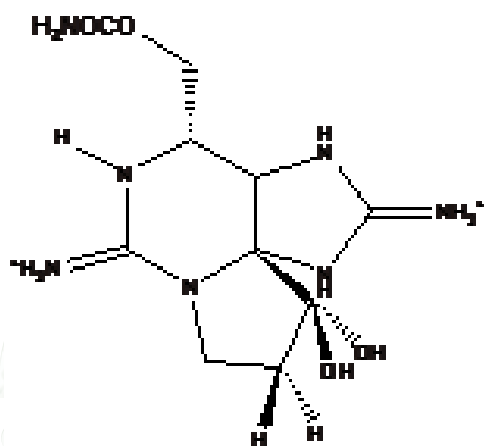


Figure 1.

These toxins affect the nervous system of mammals by blocking voltage-gated sodium channels. Symptoms will include headaches, dizziness, nausea, numbness around lips, muscular paralysis and even death by cardio respiratory failure. Hence, the monitoring and control of PSP toxins presence in shellfish is essential.

According to the regulation EC No 853/2004, the total PSP content in shellfish fit for human consumption must not exceed 800 µg per Kg of mollusc flesh. The Mouse Bioassay (MBA) is the internationally accepted method for detecting PSP toxins in shellfish at this level. However, this method is prone to interferences and ethical considerations are of increasing concern due to the sacrifice of large numbers of animals.

In 2006, the Lawrence HPLC method (AOAC Official Method 2005.06) was accepted as an alternative (EC No 1664/2006) to the mouse assay but with set limitations.

The aim of this work package was to address the lack of in-vitro tests suitable for PSP detection by developing an optical biosensor assay intended for routine use for rapid PSP determination in shellfish.

### Work Package Progress and Results

An optical biosensor assay for the detection of PSP toxins in shellfish has been developed based on the phenomenon of Surface Plasmon Resonance (SPR). SPR biosensors are capable of detecting molecular interactions between antibodies and antigens in real time without labelling either component.

This PSP assay was designed as an inhibition assay with a PSP toxin analogue immobilised onto a chip surface. When the antibody and sample are mixed and passed over this chip surface, if no toxin is present in the sample the antibody will bind to the surface.





## New Technologies to Screen Multiple Chemical Contaminants in Foods

However, when a toxin is present the antibody will bind to the toxin in solution and be inhibited from binding to the surface.

Assay development required:

- the purification of PSP toxins to be used as standards and antigens and
- the production of antibodies.

The antibodies raised against marine toxins are also useful tools for researchers in trying to trace the mechanism of action of the toxins, identify the original source of the toxin or isolate the toxins from shellfish.

The purification and determination of individual PSP toxins was performed. Several sensor chip surfaces and antibodies were produced during the project and tested for performance using the inhibition assay format. After the evaluation of a number of models a prototype kit for the SPR PSP assay was assembled with the key components of a saxitoxin sensor chip surface and a polyclonal anti-saxitoxin antibody.



**Figure 2.**

Simple rapid sample preparation protocols were optimized to minimize matrix interference and maximize toxin recovery. This assay can be used for the detection of saxitoxin and analogues in all bivalve molluscs: mussels, clams, cockles, oysters and scallops.

A single laboratory pre-validation process to ensure the assay is fit for purpose has been successfully completed. In addition seven international laboratories are currently

participating in an inter-laboratory trial of the assay to ensure that the technology can be transferred effectively between laboratories.

### Benefits of the BioCop Project

#### Consumer

Rapid and reliable assays for the detection of shellfish toxins produced within the project are necessary to ensure shellfish is fit for consumption in order to protect human health.

#### Food Industry

The shellfish industry constitutes a major resource and contributes significant revenue to the European economy. The marketability of shellfish in Europe is based on a perception of quality and a superior product image. Consequently, the quality control and assurance aspect of production has become a main focus for the industry. The analysis of bivalves for the presence of biotoxins is one of the most important tests required. Marine biotoxins have become a serious threat to the shellfish industry and may impede any further growth and development unless controlled.

The detection of marine toxins with reliable assays, which could be implemented at hazard analysis and critical control points (HACCP) in the shellfish production process, will protect the shellfish aquaculture industry from economic losses. Economic losses arise from disposing of contaminated shellfish, prolonged closure of culture areas or toxic incidents following consumption which reduce consumer trust.

#### Scientist

Routine detection of marine toxins requires the sacrifice of large numbers of laboratory animals, which in the European Union and many other countries is a conflict with ethical regulations. This SPR PSP assay offers to scientists a fast, simple and reliable method that can be used for the screening of shellfish samples without animal sacrifice, thereby reducing the number of deaths for this purpose. This effectively could reduce costs associated with animal housing and maintenance.





## Training/Workshops

North American and European workshops were held for the technology transfer of the developed SPR PSP assay for varied stakeholder audiences:

- Vancouver, Canada – all stakeholders
- Limmassol, Cyprus - European regulatory audience
- Vigo, Spain – Shellfish Industry
- York, UK – Consumer Forum

Each workshop provided an opportunity to:

- a. Disseminate the expertise within the BioCop project
- b. Demonstrate the practical application of the new SPR PSP assay to all delegates
- c. Provide a forum for extended discussion on this technology as a screening tool and its application to shellfish monitoring procedures within the EU and Canada in order to replace the MBA
- d. Learn directly from delegates how applicable this technology could be in terms of their own experiences

## Future Activity

Substantial interest and discussion about the new assay was generated at each workshop.

Future activities will include the inter-laboratory validation trials and further dissemination activities until the end of the project.

Further details are available at [www.biocop.org](http://www.biocop.org)

## Frequently Asked Questions

### Question:

- Ⓚ How do SPR biosensors work?

### Answer:

- Ⓐ The surface plasmon resonance (SPR) occurs as a thin conducting film at an interface between the media of different refractive indexes. In the Biacore systems, the media of these refractive indexes is referred to as the glass of the sensor chip and the sample solution, and the conducting film is the gold layer on the sensor chip surface. Under the conditions of a total internal reflection, the light leaks an electric field intensity, known as an evanescent wave, across the interface into the medium of lower refractive index, without actually losing net energy. At a certain combination of angle of incidence and energy, the incident light excites plasmons (electron charge density waves) in the gold film. A characteristic absorption of energy via the evanescent wave then occurs and the SPR is seen as a drop in the intensity of the reflected light. As the evanescent wave penetrates the solution, conditions for this resonance effect are very sensitive to the refractive index of the solution. Changes in solute concentration at the surface of the sensor chip can cause changes in the refractive index of solution that can be measured as changes in the SPR condition.

