End-Product Testing for Shellfish Biotoxins

Information for Shellfish Growers, Harvesters and Processors

Food Standards Agency
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Introduction

The Food Standards Agency (FSA) administers the testing of shellfish for marine biotoxins in the UK as part of the Official Control (OC)[1] Biotoxin Monitoring Programme. This leaflet tells you how best to implement End-Product Testing (EPT) for shellfish biotoxins, in order to help fulfil some of your due diligence requirements to protect consumers. EPT can also add value to your product, and may give you more control over when you harvest and place the product on the market.

This leaflet sets out to explain what EPT is currently available, how the different testing methods work, their limitations and availability, and how they should be used as part of a wider risk assessment process.

Why should I test my shellfish for biotoxins?

Producing safe food is the responsibility of Food Business Operators (FBOs) under current EU Food Hygiene legislation. Shellfish growers, harvesters and processors, as FBOs, are placing food on the market and must therefore satisfy the legal requirement that all reasonable measures have been taken to ensure that it is safe.

Unlike many of the other hazards associated with food production, biotoxins are largely heat stable which means that even if the food is cooked the biotoxins will not be broken down or removed. The only way to minimise the risk of your customers becoming ill through consumption of contaminated shellfish is to ensure that a product likely to contain unsafe levels of biotoxins is not placed on the market.

How can I manage shellfish biotoxin risk in a food business?

EU Food Hygiene legislation applies in different ways to different types of food businesses. For example, whilst primary producers (such as shellfish growers and harvesters) are not subject to the legal Hazard Analysis and Critical Control Points (HACCP) requirements, which other food businesses are subject to, all FBOs must identify and control the risks associated with their product.

Biotoxins can be effectively controlled through implementation of a HACCP system. HACCP plans must be specific to different shellfish species, although many 'Critical Control Points' (CCPs) may be shared. HACCP plans should take full advantage of all available biotoxin data, including current and historic OC phytoplankton and biotoxin monitoring data. HACCP plans should also consider procedural CCPs (such as shucking for scallops) for biotoxin control.

As part of the HACCP verification process and to ensure compliance with the regulatory limits set out in the **Annexe**, EPT is considered to be an important element in the overall safety management controls for FBOs placing shellfish on the market.

[1] 'Official Controls' are those measures and/or testing programs undertaken by or on behalf of the Food Standards Agency in accordance with EU law.

The recommendations and checklist contained within this leaflet will help FBOs to implement EPT for shellfish biotoxins.

Checklist

FBOs are advised to seek further technical guidance from their service providers and/or the manufacturer of field kits if they are unsure of the characteristics of a particular test.

It is important for FBOs to determine:

Accreditation and/or quality assurance of laboratory offering the service

Does the laboratory have ISO17025 accreditation for the method applied to the shellfish species? Can the laboratory demonstrate it can detect relevant amounts of the biotoxins of interest?

Characteristics of the test used

Can the method detect all regulated Paralytic Shellfish Poison (PSP)/Amnesic Shellfish Poison (ASP)/Lipophilic toxins, including Diarrhetic Shellfish Poison (DSP) toxins?

Comparing EPT results to regulatory limit

Does the test measure total toxicity? If so, how?

Interpretation of the result

What do the results mean? Am I confident in interpreting the result and how this links to the decisions I should make when placing shellfish on the market?

What else should I consider?

EPT is one of the main ways you can demonstrate compliance with your legal obligations, but there are other factors you should consider in conjunction with your EPT, to help fully determine any potential risk to human health from your product. Other factors to bear in mind are:

- **Time of year.** In the summer months (April – October) toxicity levels are likely to be higher. Increased EPT should take place in the summer months or whenever there are indications that toxicity is likely to be high (e.g. elevated levels of phytoplankton).
- **OC Phytoplankton Monitoring Programme results.** Phytoplankton results from FSA's OC Phytoplankton Monitoring Programme are available at **www.food.gov.uk/foodindustry/farmingfood/shellfish.** Shellfish growers, harvesters and processors should use these results as an indicator as to whether increased shellfish EPT should take place.

- **OC Biotoxin Monitoring Programme results.** Biotoxin results from FSA's OC Biotoxin Monitoring Programme are available at **www.food.gov.uk/foodindustry/farmingfood/shellfish.** An increasing trend of toxic episodes within the production area would help signal potential risk.

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Annexe

Shellfish Biotoxin Testing in Detail

(This should be read in conjunction with the Quick Reference Guide enclosed)

What are the regulatory limits for biotoxins?

Regulation (EC) No. 853/2004 lays down the specific hygiene rules for food of animal origin; Chapter V of this regulation outlines the health standards for shellfish. FBOs placing shellfish on the market must ensure their product meets those standards. The regulatory limits for marine biotoxins (measured in the whole body or any part edible separately) must not exceed:

- Paralytic shellfish poisoning (PSP) toxins, 800 micrograms /kg;
- Amnesic shellfish poisoning (ASP) toxins, 20 milligrams domoic acid/kg;
- Diarrhetic shellfish poisoning (DSP) toxins and pectenotoxins (PTX) together, 160 micrograms of okadaic acid equivalents/kg;
- Yessotoxins, 1 milligram yessotoxin (YTX) equivalents/kg and
- Azaspiracid shellfish poisoning (AZP) or Azaspiracids, 160 micrograms azaspiracid (AZA) equivalents/kg.

What shellfish biotoxin tests are available to FBOs?

Commission Regulation (EC) No. 2074/2005 (as amended) lays down regulatory methods for the detection of marine biotoxins to be used by competent authorities for OC monitoring: high performance liquid chromatography (HPLC) for ASP, HPLC and biological testing for PSP, liquid-chromatography with tandem mass spectrometry (LC-MS/MS) for DSP/lipophilic toxins. Furthermore ASP Enzyme Linked Immunosorbent Assay (ELISA) is permitted to be used for screening purposes. Additionally, other internationally recognised methods, such as antibody based assays (immunoassays) and functional assays, can be used for testing shellfish flesh for presence of marine biotoxins, provided that they are not less effective than the regulatory biological methods (MBA).

The HPLC and LC-MS/MS methods are not suitable for implementation in shellfish establishments due to the complexity of the test systems, but are available in specialist laboratories. Rapid antibody based assays and functional assays are available, which may be suitable for implementation by FBOs, either in-house or in specialist laboratories. Always ask the manufacturer whether or not these assays can provide a practical and cost effective solution to EPT for FBOs. A small number of FBOs across the UK have implemented the use of in-house immunoassay testing for shellfish biotoxins.

PSP toxins

PSP toxins are a group of many different but related chemicals. Saxitoxin is the most potent of the suite of PSP toxins, but at least 11 other PSP toxins have been detected in UK shellfish to date. All can potentially contribute to the total toxicity of the sample. Essentially there are two types of testing methods currently suitable for EPT:

- Antibody based tests, which are subdivided into two types:
  - ELISA kits, such as R-Biopharm RIDASCREEN® Fast PSP SC and Biosense Abraxis® Saxitoxin (PSP) kits;
  - lateral flow tests (dip-stick style tests), such as Jellett® PSP Rapid Test;
- Analytical methods based on HPLC.

The antibody based tests give a quick response at relatively low cost. Assays such as the Jellett® PSP Rapid Test provide an indication of the presence/absence of PSP toxins, while ELISA kits, such as the RIDASCREEN® and Abraxis® give a semi-quantitative result. However, because antibodies are very specific, not all toxins are equally measured and there is a general lack of response to many of the PSP toxins other than saxitoxin. In other words, the quantitative results obtained from such tests do not usually represent all the PSP toxins present. Therefore, FBOs should bear in mind that the kits cannot provide a measure of total toxicity in one sample of shellfish and are not directly comparable to the regulatory limit. However, EPT trends from these semi-quantitative tests are useful as part of HACCP plans and they can be accepted by the FSA as indicators of due diligence.

The HPLC method (2005.06 HPLC) measures the concentration of individual PSP toxins and provides a total toxin content that compares well to the regulatory limit in these species. However, HPLC methods require sophisticated instrumentation, and are therefore only available at specialised laboratories. They are typically more time consuming, more expensive per sample and FBOs may be more limited in their use for routine application, in terms of accessibility of laboratories offering this service.

Recommendation

- Antibody based kits are suitable for EPT, but the results only provide an indication of the levels of PSP toxins that may be present in shellfish. Since PSP toxins are potentially fatal, samples giving a positive result with antibody based kits should generally be considered an increased risk to human health. ELISA kits will provide a semi-quantitative result which can be used as an indication of the measure of saxitoxin and is useful for monitoring the trends for PSP toxins in harvesting areas.
- Currently, the best measure of total toxicity of a sample, comparable to the regulatory limits, is obtained by HPLC (preferably by the OC HPLC method 2005.06).

DSP, AZP and other lipophilic toxins

DSP and AZP toxins, along with other toxins, such as yessotoxins and pectenotoxins, are usually collectively referred to as lipophilic toxins. Each group of the lipophilic toxins is actually a family of closely related compounds, rather than one single chemical, as indicated below:

- DSP toxins: e.g. okadaic acid (OA) and dinophysistoxins (DTX1, DTX2, DTX3);
- AZP toxins: e.g. azaspiracid 1, azaspiracid 2 and azaspiracid 3;
- Pectenotoxins: e.g. pectenotoxin 1 and pecetenotoxin 2;
- Yessotoxins: e.g. yessotoxin, 45 OH yessotoxin and homo yessotoxin.

Essentially there are three types of testing methods currently suitable for EPT:

- Antibody based tests, which are subdivided into two types:
  - ELISA kits, such as UBE Industries DSP-Check; Biosense Abraxis® Okadaic Acid (DSP) and Rougier Bio-Tech® tests;
  - lateral flow kits, such as Jellett® DSP Rapid test kit;
- Functional assay - Protein Phosphatase Inhibition Assay (PP2A), such as Zeu-Inmunotec Toxiline-DSP and Sceti K.K. DSP rapid kit;
- Analytical methods based on LC-MS/MS.

Antibody based assays and functional assays are relatively fast and cheaper than any other available method. However, different tests quantify the different DSP toxins with a different degree of efficiency and accuracy. For example, although the antibody and functional methods can detect okadaic acid and dinophysistoxins, they both tend to detect okadaic acid better than the dinophysistoxins. They also do not provide information on whether yessotoxins, pectenotoxins or AZP toxins are present. In other words, care must be taken when interpreting a negative result in an antibody based assay or a functional assay as it may not mean shellfish are free of all regulated lipophilic toxins.

It is important to note that due to the differences between each of the four families of lipophillic toxins, there is no single antibody or functional assay that is capable of detecting the whole suite of regulated lipophilic toxins.

LC-MS/MS is currently the only method that is capable of measuring DSP, AZP and other lipophilic toxins in a single analysis. However, different laboratories offer different methods and if samples are submitted for analysis it is important to clarify with the laboratory which toxins can be detected and quantified. The LC-MS/MS test requires specialist equipment and is time consuming. It will be more expensive than the other tests and you may be restricted in the laboratories that can offer such service.

Recommendation

- Antibody based kits are suitable for EPT but only as a qualitative screen (i.e. presence or absence test) to identify shellfish likely to contain DSP toxins (okadaic acid and dinophysistoxins). ELISA kits will provide a semi-quantitative result which can be used as an indication of the measure of toxicity and is useful for monitoring the trends for DSP toxins in harvesting areas.
- Currently, only LC-MS/MS is capable of measuring all regulated lipophilic toxins and this is the only method which will provide total level samples toxicity which is comparable to the regulatory limits.

ASP toxins

ASP toxins include domoic acid and related toxins.

Currently there are two types of testing methods suitable for EPT:

- Antibody based tests, which are subdivided into two types:
  - Biosense® ASP ELISA (AOAC official method 2002.06)
  - lateral flow kits, such as Jellett® ASP Rapid test kit;
- Analytical methods based on HPLC.

Assays such as the Jellett® ASP Rapid Test provide an indication of the presence/absence of ASP toxins, while Biosense® ASP ELISA will give a semi-quantitative result and can be used for screening purposes

HPLC will give accurate measure of toxicity of a sample.

Recommendation

- Antibody based kits are suitable for EPT, but the results only provide an indication of the levels of ASP toxins that may be present in shellfish. ASP ELISA test will provide a semi-quantitative result which can be used as an indication of the measure of ASP toxins and is useful for screening purposes and it can be used for OC samples.
- HPLC is currently the only method which will allow an accurate measurement of toxicity of a sample, comparable to the regulatory limits.

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