

End-Product Testing for Shellfish Toxins

Information for Shellfish Harvesters, Growers and Processors



Introduction

1. The Food Standards Agency administers the testing of shellfish for toxins 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 toxins, 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 sell a product.
2. This leaflet sets out to explain how the different testing methods work, their limitations, and how they should be used as part of a wider risk assessment process.

Why should I test my shellfish for biotoxins?

3. Producing safe food is the responsibility of Food Business Operators (FBOs). Shellfish growers, harvesters and processors, as food businesses, 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 toxins 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 toxins is not placed on the market.

4. As some of the case studies in **Annex 2** show, EPT can also influence the OC Monitoring Programme bringing real benefits to those businesses who proactively undertake EPT.

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

5. The law 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 businesses must identify and control the risks associated with their product.
6. 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 phytoplankton and toxin monitoring data. HACCP plans should also consider procedural CCPs (such as shucking for scallops) for biotoxin control.
7. As part of the HACCP verification process and to ensure compliance with the regulatory limits set out in **Annex 1**, EPT is considered to be an important element in the overall safety management controls for businesses marketing shellfish.
8. The recommendations and checklist contained within this leaflet will help FBOs to implement EPT for shellfish. **Annex 2** provides some practical examples as to how this can help your business.

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

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 toxins of interest?

Characteristics of the test used

Can the method detect all regulated PSP/ASP/lipophilic 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 in marketing my shellfish?

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.
- **Phytoplankton results.** Phytoplankton results from OCs carried out on behalf of the Food Standards Agency. These are available at www.food.gov.uk. Growers, harvesters and processors should use these results as an indicator as to whether increased EPT should take place. Harvesters should also consider carrying out their own phytoplankton analysis to assess trends and risks.
- **OC Monitoring Programme results from your/neighbouring sites.** An increasing trend of toxins in the area would help signal potential risk/positives.

Further information:

Food Standards Agency in Scotland – Dr Kasia Kazimierczak:

kasia.kazimierczak@foodstandards.gsi.gov.uk

Marine Scotland Science – Tel: 01224 876544

Centre for Environment, Fisheries and Aquaculture (Cefas) - Cefas Shellfish Testing:

Tel: 01305 206600. Email: CST@cefas.co.uk

Seafish - Mandy Pyke, Tel: 01964 503024. Email: m_pyke@seafish.co.uk

VeroMara – Tel: 01631 559368, email: info@veromara.com

Shetland Seafood Quality Control - Tel: 01595 772 000. E-mail: info@nafc.uhi.ac.uk

Seafood Shetland – Tel: 01595 693644. Email: sfpa@fishuk.net

The Association of Scottish Shellfish Growers – only members should contact:

Walter Speirs on: 01631 710653. Email: walter.speirs@btconnect.com

The Scallop Association - Tel: 01470 521225. Email: Scallop@btconnect.com

Shellfish Toxin Testing in Detail

Annex 1

This should be read in conjunction with the Quick Reference Table enclosed

What are the regulatory limits for biotoxins?

9. EU Regulation 853/2004 lays down the specific hygiene rules for food of animal origin; Chapter V of this regulation outlines the health standards for live bivalve molluscs. FBOs placing live bivalve molluscs on the market must ensure their product meets those standards. The limits for marine biotoxins (measured in the whole body or any part edible separately) must not exceed:
- Paralytic shellfish poisoning (PSP) toxins, 800 micrograms saxitoxin equivalents/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?

10. EU Regulation 2074/2005 (as amended) lays down following analytical testing methods for detection of marine biotoxins: high performance liquid chromatography (HPLC) for ASP and 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 and functional assays, can be used for testing of shellfish flesh for presence of marine biotoxins, provided that they are not less effective than biological assays (MBA).
11. The HPLC and LC-MS/MS methods are not suitable for implementation in shellfish premises due to the complexity of the test systems, but are available in specialist labs. Rapid antibody based assays and functional assays are available, which may be suitable for implementation by FBOs, either in-house or in specialist labs. 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 have implemented the use of in-house immunoassay testing for shellfish biotoxins.

PSP toxins

12. 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.

13. 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 procedures, they can be accepted by the FSA as indicators of due diligence and results may be used to support a case for earlier OC retesting of closed harvesting areas or a challenge to the RMP² sample.

14. The only alternative test for PSP toxins is one of the HPLC methods, one of which (2005.06 HPLC) has been approved for use as an OC method for the quantification of PSP toxins in mussels, razors, hard clams and cockles in the UK. 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 a specialised laboratory. 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

- i. 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 lethal, 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.
- ii. Currently, the best measure of total toxicity of a sample, comparable to the regulatory limits, is obtained by HPLC (preferably by the official HPLC methodology or a validated and accredited method). This method can be used for OC samples.

DSP, AZP and other lipophilic toxins

15. DSP and AZP toxins, along with other toxins, such as yessotoxins and pectenotoxins, are usually collectively referred to as lipophilic toxins and are all regulated under European regulations. 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 pectenotoxin 2;
- Yessotoxins: e.g. yessotoxin, 45 OH yessotoxin; homo yessotoxin.

16. 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.

17. The first two types of methods 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 toxins, 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.
18. It is important to note that due to the differences between each of the 4 families of lipophilic toxins, there is no single antibody or functional assay that is capable of detecting the whole regulated suite of lipophilic toxins.
19. LC-MS/MS is currently the only method that is capable of measuring DSP, AZP and other lipophilic toxins in a single analysis, and can be used for OC samples. However, different laboratories offer different methods and if samples are submitted for this analysis it is important to clarify with the laboratory which toxins can be detected and quantified. As is the situation with the HPLC for PSP, 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

- i. 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.
- ii. 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. This method can be used for OC samples.

ASP toxins

20. ASP toxins include domoic acid and related toxins.
21. 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® DSP Rapid test kit;
- Analytical methods based on HPLC.

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

23. HPLC will give accurate measure of toxicity of a sample; both HPLC and ELISA test can be used for OC samples.

Recommendation

- i. 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.
- ii. HPLC is currently the only method which will allow an accurate measurement of toxicity of a sample, comparable to the regulatory limits; it can be used for OC samples.

Case Studies

Annex 2

Case Study 1. EPT helped me harvest earlier.

An Official Control (OC) sample taken from a loch on the west coast on a Monday returns a positive result for DSP in mussels on the Wednesday. Normally, the area would remain closed to all harvesting until 2 consecutive negative OCs results are found 7 days apart. This can mean a delay of up to 2 weeks until the area reopens.

However, harvester John McDonald has noted that the phytoplankton count for Dinophysis has decreased in his area over the previous 2 weeks. He has also noted increases in other PSP and ASP plankton producing species. Taking this into consideration he has decided to submit regular samples to an accredited laboratory for a Jellet Rapid® test for PSP, a functional pp2A assay for DSP and an ELISA screening test for ASP. The harvester's sample was taken on the same day as the OC sample and the results of this test were all indicative of very low levels of toxins in the sample. On the same day that the area was closed, the harvester submitted an application to his local authority to re-open the site earlier, and the FSA received that application on the same day. A further OC sample was taken on Thursday and the result declared negative on Monday. A further OC sample was taken on Monday and the result declared negative on Wednesday. The area immediately reopened, 7 days earlier than might otherwise have been the case given the routine monitoring programme frequency.

Applications to open earlier or challenge the OC sample for additional monitoring purposes will follow only where food businesses can demonstrate that they have been proactively monitoring the levels of biotoxins or toxicity in their area prior to that area being closed.

Case Study 2. EPT helped me open my area for my own species.

Oyster grower Ben Smith operates in an area currently represented by mussels. He has noted that, whilst the area is currently open, many of the OC results have returned signs indicative of DSP and that the phytoplankton count has been edging up in the past few weeks. He began a weekly programme of EPT on representative samples of his oysters. A few weeks later the area closed due to positive testing for DSP in mussels. The harvester submitted an application to both challenge the RMP and opened earlier on the basis of his EPT results. The application was successful and the harvester was able to open his site as an RMP in his own right for oysters and 8 days earlier than he might otherwise have done.

Case Study 3. EPT ensured that product could remain on the market despite area closure.

Both John McDonald and Ben Staggs have regular EPT. Both placed their product on the market Monday and Tuesday. The OC sample was taken on Monday and closed the area on Wednesday. Had no EPT been done, both harvesters may have had to initiate an expensive product recall on everything that was harvested and placed on the market between the OC sample being collected and the result announced. On the basis that their testing indicated that their produce was likely to be within regulatory limits, all product could remain on the market.

Case Study 4. EPT provides customer reassurance in scallop processor and demonstrates HACCP compliance.

In the summer time, there is a much higher risk of ASP in both shucked and whole scallops. Approximately 80% of whole wild king scallops in the summer months will return positive ASP results through the statutory monitoring programme. “Shucking” is a skill and whilst, if well done, it will remove most of the toxicity of the product, levels of toxins can be so high, that even shucked product may be over regulatory limits.

A processor in Argyll has recognised the risks associated with ASP and has initiated a ‘positive release’ initiative whereby every batch of product is tested before it is placed on the market. This provides verification of the operators own HACCP plan and provides added reassurance to a range of caterers and restaurants across the UK.



Food
Standards
Agency

food.gov.uk

Buidheann
Inbhe-Bìdhe

The Food Standards Agency in Scotland

6th Floor
St Magnus House
25 Guild Street
Aberdeen
AB11 6NJ

tel: 01224 285100

email: scotland@foodstandards.gsi.gov.uk