



# Agri-Food & Biosciences Institute

## VETERINARY SCIENCES DIVISION

### Chemical Surveillance Branch

## Annual Report UK National Reference Laboratory For Marine Biotoxins

**1<sup>st</sup> April 2011 – 31<sup>st</sup> March 2012**

### **Contacts:**

#### **Cowan Higgins**

Chemical Surveillance Branch, VSD

Tel 02890 525785

Email [cowan.higgins@afbini.gov.uk](mailto:cowan.higgins@afbini.gov.uk)

#### **Glenn Kennedy**

Chemical Surveillance Branch, VSD

Tel 02890 525651

Email [glenn.kennedy@afbini.gov.uk](mailto:glenn.kennedy@afbini.gov.uk)

## **Glossary**

**AFBI:** Agri-Food and Biosciences Institute

**ASP:** Amnesic Shellfish Poison (Domoic Acid)

**Cefas:** Centre for Environment, Fisheries and Aquaculture Science

**DSP:** Diarrhetic Shellfish Poison (Lipophilic Toxin group)

**EURL-MB:** European Reference Laboratory for Marine Biotoxins

**FSA:** Food Standards Agency

**HPLC-FLD:** High Performance Liquid Chromatography with fluorescence detection

**LC-MS/MS:** Liquid Chromatography coupled with tandem Mass Spectrometry

**UK-NRL:** United Kingdom National Reference Laboratory

**OCL:** Official Control Laboratory

**PSP:** Paralytic Shellfish Poison (Saxitoxin group)

**UK-NRL:** United Kingdom National Reference Laboratory

## **Introduction**

The following report provides an outline of the work of the UK-NRL over the past year. It does not seek to be a comprehensive review but aims to highlight some of the areas to which it has contributed throughout the year. The UK-NRL acknowledges the support of the FSA and the help of AFBI and Cefas in fulfilling its duties.

The FSA is designated as a Competent Authority for the purposes of Regulation (EC) 882/2004 regarding Official Feed and Food Controls. As the Competent Authority, the FSA is responsible for establishing the location and boundaries of classified production and relaying areas for live bivalve molluscs. It has responsibility for the organisation of official controls including the organisation of statutory monitoring for the presence of marine biotoxins in shellfish and toxin-producing phytoplankton in the classified production and relaying areas. The FSA is responsible for the appointment of the UK-NRL for marine biotoxins whose role is to carry out the following requirements and duties, as set out in Article 33 of Regulation (EC) 882/2004:

1. Collaborate with the European EURL-MB in their area of competence.
2. Co-ordinate, for their area of competence, the activities of official laboratories responsible for the analysis of samples.
3. Where appropriate, organise comparative tests between the official national laboratories and ensure an appropriate follow-up of such comparative testing.

4. Ensure the dissemination to the competent authority and official national laboratories of information that the EURL-MB supplies.
5. Provide scientific and technical assistance to the competent authority for the implementation of co-ordinated control plans adopted in accordance with Article 33.
6. Be responsible for carrying out other specific duties provided for in accordance with the procedure referred to in Article 33 without prejudice to existing additional national duties.

### **Co-ordination of the Activities of the Monitoring Laboratories**

As part of its on-going review activities, the UK-NRL issued a standard operating procedure for the quantitative determination of Domoic Acid (ASP) by HPLC-UV. A revised version of the standard operating procedure for the calibration of the mouse bioassay for PSP was issued and the document relating to shellfish sample and test portions was updated following the implementation of LC-MS/MS for the detection of lipophilic toxins.

The UK-NRL network group met in July 2011, at which the UK-NRL agreed to raise the issues of the use of sentinel species, sampling protocols for marine biotoxin testing and sampling procedures for phytoplankton with the European Reference Laboratory network group. It was envisaged that information from other European laboratories could help to establish best practice for sampling and the use of sentinel species. Audit performance following UKAS inspections at AFBI and Cefas were reviewed and the record of performance by both laboratories in external proficiency tests was updated and circulated.

Following the implementation of LC-MS/MS for the detection of lipophilic toxins a review meeting was held in November 2011. Representatives of the monitoring laboratories, the Competent Authority and the NRL met by video conference to discuss a range of technical issues. The group agreed that the NRL would seek clarification from the EURL-MB Working Group on LCMS/MS, on a number of points relating to the EURL SOP and the application of quality control parameters in routine analyses.

A further network meeting was held in March 2012 at which the UK-NRL presented an update on the EURL sampling survey. The survey had been coordinated by the UK-NRL who had developed the questionnaires with assistance from the EURL-MB. A further update on

proficiency test performance was presented and the group informed that AFBI and Cefas would participate in the EURL-MB proficiency tests for 2012.

### **EU-RLMB Proficiency Tests**

The EURL-MB evaluates the performance of the EU NRLs and checks the equivalency of the methods used by the laboratories for the official control of marine biotoxins in bivalve molluscs through annual proficiency exercises for PSP, DSP and ASP. Proficiency exercises for PSP have been organised since 2004. The number of participants in 2011 was 33 and the exercise covered both biological methods and HPLC-FLD. For ASP, proficiency exercises have been organised since 2007 to evaluate method and laboratory performance, with participants requested to use the method usually employed for official control. In 2011, there were 28 participants. For lipophilic toxins, the EURL-MB has organised proficiency exercises since 2000. A total of 28 laboratories participated in the 2011 study.

Reports on the EURL-MB proficiency tests and have been circulated throughout the year and the UK results are detailed below. The full reports are available for download from either the EURL-MB or the UK-NRL website. The EURL-MB was able to extend the proficiency tests to include both the UK-NRL and Cefas laboratories in 2011 and the results are included in Table 1. Tables 2 and 3 detail the results obtained by Cefas and the UK-NRL for PSP and lipophilic toxins identified in the proficiency test samples.

**Table 1: UK results in EU-RLMB Proficiency Tests 2011**

	<b>Analyte</b>	<b>Sample Number</b>	<b>Result</b>	<b>Assigned</b>	<b>Z-Score</b>	<b>Method</b>
AFBI	Lipophilic Toxins	EURL-MB/11/L/01	Positive	Negative	N/A	MBA
AFBI	Lipophilic Toxins	EURL-MB/11/L/03	Positive	Positive	N/A	MBA
AFBI	LT-YTX (µg YTX equiv/kg)	EURL-MB/11/L/02	0.895	0.654	1.22	LC-MS/MS
AFBI	LT-OA (µg OA equiv/kg)	EURL-MB/11/L/03	749.8	747.9	0.01	LC-MS/MS
AFBI	LT-AZA (µg AZA equiv/kg)	EURL-MB/11/L/03	374.9	340.4	0.41	LC-MS/MS

	Analyte	Sample Number	Result	Assigned	Z-Score	Method
CEFAS	LT-YTX (µg YTX equiv/kg)	EURL-MB/11/L/02	0.725	0.654	0.36	LC-MS/MS
CEFAS	LT-OA (µg OA equiv/kg)	EURL-MB/11/L/03	769.4	747.9	0.1	LC-MS/MS
CEFAS	LT-AZA (µg AZA equiv/kg)	EURL-MB/11/L/03	316.4	340.4	-0.28	LC-MS/MS
AFBI	PSP (µg STX equiv/kg)	EURL-MB/11/P/01	1039	1514	-1.26	MBA
AFBI	PSP (µg STX equiv/kg)	EURL-MB/11/P/02	1329	1460	-0.36	MBA
AFBI	PSP (µg STX equiv/kg)	EURL-MB/11/P/01	1327	1514	-0.49	HPLC
AFBI	PSP (µg STX equiv/kg)	EURL-MB/11/P/02	1210	1460	-0.68	HPLC
CEFAS	PSP (µg STX equiv/kg)	EURL-MB/11/P/01	1336	1514	-0.47	HPLC
CEFAS	PSP (µg STX equiv/kg)	EURL-MB/11/P/02	1208	1460	-0.69	HPLC
AFBI	ASP (µg/g)	EURL-MB/11/A/01	15.7	16.2	-0.33	HPLC
AFBI	ASP (µg/g)	EURL-MB/11/A/02	4.7	4.8	-0.1	HPLC
CEFAS	ASP (µg/g)	EURL-MB/11/A/01	15.9	16.2	-0.21	HPLC
CEFAS	ASP (µg/g)	EURL-MB/11/A/02	4.9	4.8	0.18	HPLC

Sample EURL-MB/11/L/01 was reported positive by eleven laboratories. Preliminary analysis at the EURL-MB by LC-MS/MS (3200 Q-TRAP ABSCIEX, acidic conditions) indicated a total OA level under the LOQ. Total DTX1, total DTX2, PTX2, AZA1, AZA 2, AZA3, YTX, homo-YTX, 45-OH-YTX and 45-OH-homo-YTX were under the LOD. Analysis by MBA (two aliquots tested) showed a negative result. This situation was similar to that of 2010 for sample EURL-MB/10/L/02 where several MBA false positive results were found. Sample EURL-MB/11/L/01 was submitted to the Cawthron Institute (New Zealand) to be analysed for pinnatoxins. None were detected and investigations by the EURL-MB continue.

**Table 2: UK Results for PSP toxins by HPLC-FLD ( $\mu\text{mol/kg}$ ) identified in EURL-MB Proficiency Test 2011**

	EURLMB/11/P/01				EURLMB/11/P/02		
Toxin	Assigned	CEFAS	AFBI	Toxin	Assigned	CEFAS	AFBI
<b>GTX2,3</b>	2.01	1.88	1.68	<b>GTX2,3</b>	2.52	2.44	2.19
<b>STX</b>	3.24	2.46	2.56	<b>STX</b>	2.46	1.78	1.94
<b>Total</b> ( $\mu\text{g STX equiv / kg}$ )	1514	1336	1327	<b>Total</b>	1460	1208	1210

**Table 3: UK Results for LT toxins by LCMS/MS identified in EURL-MB Proficiency Test 2011**

	EURL-MB/11/L/01			EURL-MB/11/L/02			EURL-MB/11/L/03		
	Assigned	Cefas	AFBI	Assigned	Cefas	AFBI	Assigned	Cefas	AFBI
All toxins considered	Neg	<LOQ							
mg YTX equivalents/kg				0.65	0.73	0.90			
YTX mg/kg				0.15	0.17	0.13			
homo-YTX mg/kg				0.38	0.47	0.65			
45OH-YTX mg/kg				0.05	ND	0.01			
45OH-homo-YTX mg/kg				0.15	0.17	0.22			
mg YTX equivalents/kg				0.65	0.73	0.90			
$\mu\text{g Total OA equivalents/kg}$							748	769	750
free OA $\mu\text{g/kg}$							403	588	398

	EURL-MB/11/L/01			EURL-MB/11/L/02			EURL-MB/11/L/03		
	Assigned	Cefas	AFBI	Assigned	Cefas	AFBI	Assigned	Cefas	AFBI
µg Total AZA equivalents/kg							340	316	375
AZA1 µg/kg							167	154	186
AZA2 µg/kg							47	55	52
AZA3 µg/kg							63	46	68

### **Dissemination of Information from the EURL-MB**

In preparation for the implementation of LC-MS/MS, the EURL-MB circulated a revised version of their standard operating procedure. The UK-NRL coordinated the UK response to the proposed revision and a final version was issued in 2011. The UK-NRL attended the EURL-MB Network meeting in Vienna in October 2011, presenting information on the use of sentinel species and sampling procedures for marine biotoxin and phytoplankton sampling. The EURL requested the UKNRL to take forward a survey of the EU-NRLs and agreed to assist in the production of questionnaires for circulation to the NRLs. The questionnaires were issued by the EURL and returned to the UKNRL during February 2012. The responses are currently being assessed.

Following discussion with the EU-NRLs, the EURL Network agreed to the replacement of the EURL Steering Committee with an Advisory Group which would be contacted by the EURL-MB on specific issues as they arose. It was agreed that the advisory group would be comprised of representatives from the NL, GER, GRE and the UK-NRLs.

The NRL represents the UK on the EURL-MB Working Group on LC-MS/MS. The group met in December 2011 to review progress on the introduction of LC-MS/MS for the detection of lipophilic toxins. The issues which the NRL were asked to raise were satisfactorily addressed and the outcome presented to the UK-NRL Network at its meeting in March 2012. The terms of reference of the Working Group have been extended to cover emerging toxins and the group will meet as part of a EURL-MB seminar on emerging toxins, to be held in May 2012.

### **Provide Scientific and Technical Assistance to the Competent Authority**

The UK-NRL and FSA attended the Resolution meeting at DIN (the German Standardisation body) in Berlin on 1<sup>st</sup> June 2011. The meeting was to discuss UK's and other WG 5 member's comments on the draft CEN document 16204 on 'Determination of Lipophilic Algal Toxins

(DSP Toxins, Yessotoxins, Azaspiracids, Pectenotoxins) in Shellfish and Shellfish products by LC-MS/MS'. This draft European Standard drawn up by the Technical Committee CEN/TC 275 has been submitted for vote as CEN Formal Vote on FprEN 16204 Foodstuffs - Determination of Lipophilic Algal Toxins (DSP Toxins, Yessotoxins, Azaspiracids, Pectenotoxins) in Shellfish and Shellfish products by LC-MS/MS.

The 31st Session (April 2011) of the Codex Committee on Fish and Fishery Products (CCFFP) agreed to establish an electronic working group (e-WG), led by Canada, open to all members and observers. The mandate of the group was to propose criteria/parameters for screening methods for biotoxins in the standard for live and raw Bivalve Molluscs, to consider whether the criteria developed for both reference and confirmatory and screening methods should reside in the Code of Practice. The Group commenced work in December 2011. The UK-NRL, together with the other UK representatives, submitted proposals to the working group in March 2012 and further submissions are in preparation.

In preparation for the forthcoming mission of UK shellfish hygiene controls, the UK-NRL has provided information to the Competent Authority as part of the initial desk study. It is anticipated that the FVO inspectors will visit the UK-NRL on the 17<sup>th</sup> April 2012.

### **Other Activities**

The UK-NRL has provided advice to the shellfish industry representative organisation on the revision of their leaflet on Paralytic Shellfish Poisoning and attended the Food Standards Agency in Scotland's stakeholders meeting, held in June 2011.

### **Meetings Attended**

<b>Date</b>	<b>Venue</b>	<b>Subject</b>
18 <sup>th</sup> May 2011	VC	CEN Standardisation of LC-MS/MS
1 <sup>st</sup> June 2011	Berlin	CEN Resolution Meeting on LC-MS/MS
13 <sup>th</sup> June 2011	Belfast	Northern Ireland Shellfish Safety Group, NRL update
30 <sup>th</sup> June 2011	VC	FSA in Scotland, Stakeholders meeting
21 <sup>st</sup> July 2011	London	UK-NRL Network Meeting, Aviation House
19 <sup>th</sup> -21 <sup>st</sup> October 2011	Vienna	EURL-MB/ NRL Network meeting
11 <sup>th</sup> November	VC	FSA/Cefas/AFBI QC parameters for LC-MS/MS
15 <sup>th</sup> December 2011	Brussels	EURL-MB WG on LC-MS/MS
30 <sup>th</sup> March 2012	London	UK-NRL Network Meeting, Aviation House



## **Links**

UK-NRL Web page:

<http://www.afbini.gov.uk/index/services/services-diagnostic-and-analytical/marine-biotoxins-nrl.htm>

EURL-MB Web page:

<http://www.aesan.msps.es/en/CRLMB/web/home.shtml>