Agri-Food & Biosciences Institute

VETERINARY SCIENCES DIVISION

Chemical Surveillance Branch

Annual Report
UK National Reference Laboratory
For Marine Biotoxins

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Glossary

AFBI: Agri-Food and Biosciences Institute

AESAN: Agencia Espanola de Segurida Alimentaria y Nutricion

ANSES: Agency for Food, Environmental and Occupational Health and Safety, France

ASP: Amnesic Shellfish Poison (Domoic Acid)

CEFAS: Centre for Environment, Fisheries and Aquaculture Science

DSP: Diarrhetic Shellfish Poison (Lipophilic Toxin group)

EU-NRLMB: 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

NRL: National Reference Laboratory

OCL: Official Control Laboratory

PSP: Paralytic Shellfish Poison (Saxitoxin group)

RIKILT: Institute of Food Safety, Wageningen, Netherlands.

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 EU-RLMB 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 EU-RLMB 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 an on-going review of the UK-NRL Standard Operating Procedures (SOPs), the revision of the SOPs for the Transport and Storage of shellfish for biotoxin monitoring, the screening of PSP by HPLC, the analysis of DSP by the biological method and the analysis of shellfish for the presence of ASP, were undertaken. The UK response to the redraft of the EU-RLMB SOP for the detection of lipophilic toxins by LC-MS/MS was also co-ordinated. This work was undertaken prior to the validation study co-ordinated by the EU-RL.
Two meetings of the UK-NRL network were held at FSA premises, London on 13th May and 22nd November 2010. Representatives of the monitoring laboratories undertaking biotoxin and phytoplankton analyses attended, together with FSA representatives from England, Scotland, Wales and Northern Ireland.

During 2010, two interlaboratory validation studies were undertaken on the detection of lipophilic toxins by LC-MS/MS, one study coordinated by the Institute of Food Safety (RIKILT), Netherlands and one coordinated by the EU-RLMB. The UK monitoring laboratories

participated in both studies and the results were presented by CEFAS and AFBI at the November 2010 meeting of the UK-NRL network.

Dissemination of Information from the EU-RLMB

The UK-NRL has continued to disseminate information received from the EU-RL and has responded to requests for information on test protocols used within the UK. Permission was granted by the Spanish Competent Authority (AESAN) to publish documents from the EU-RLMB on the UK-NRL website and these have been continually updated as new documents are received.

The XIIIth meeting of the EU-NRLs was hosted by RIKILT, Wageningen, the Netherlands on 22nd - 23rd October 2010. The main items on the agenda were the preliminary results from two LC-MS/MS interlaboratory validation studies, one coordinated by RIKILT and a parallel study coordinated by the EU-RLMB. The minutes of the meeting were circulated to the UK-NRL Network.

EU-RLMB Proficiency Tests

The EU-RLMB 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 2010 was 31 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 2010, there were 32 participants.

For lipophilic toxins, the EU-RLMB has organised proficiency exercises since 2000 but priority in 2010 was given to the validation of an LC-MS/MS method for lipophilic toxins, through a collaborative study. However, to assist the NRLs with their accreditation and quality systems, the EURL-MB organised a proficiency exercise for the determination of lipophilic toxins by biological methods. A total of 21 laboratories participated in the 2010 study.

Reports on the EU-RLMB proficiency tests and validation studies have been circulated throughout the year and the UK-NRL results are detailed below. The full reports are available for download from either the EU-RLMB or the UK-NRL website.

Table 1: UK results in EU-RLMB Proficiency Tests 2010

	Analyte	Sample Number	Result	Assigned	Z-Score	Method
AFBI	Lipophilic Toxins	EURLMB/10/01	Positive	Positive	N/A	MBA
AFBI	Lipophilic Toxins	EURLMB/10/02	Negative	Negative	N/A	MBA
AFBI	PSP (µg/kg)	EURLMB/10/P/01	754	1557	-1.719	MBA
AFBI	PSP(µg/kg)	EURLMB/10/P/02	451	670	-1.088	MBA
AFBI	PSP(µg/kg)	EURLMB/10/P/01	804	1557	-1.612	HPLC
AFBI	PSP(µg/kg)	EURLMB/10/P/02	503	670	-0.829	HPLC
CEFAS	PSP(µg/kg)	EURLMB/10/P/01	1139	1557	N/A	HPLC
CEFAS	PSP (µg/kg)	EURLMB/10/P/02	905	670	N/A	HPLC
AFBI	ASP (µg/g)	EURLMB/10/A/01	43.0	46.6	-0.776	HPLC
AFBI	ASP (µg/g)	EURLMB/10/A/02	3.6	3.8	-0.372	HPLC

The EU-RLMB was unable to extend the proficiency tests to include CEFAS in 2010 however, did agree to supply any surplus material to CEFAS for information and their own testing purposes. The results from CEFAS are included in Table 1 above. Table 2 below details the results obtained by CEFAS and the UK-NRL for the toxins identified in each of the test samples.

Table 2: UK Results for PSP toxins identified in EU-RLMB Proficiency Test 2010

EURLMB/10/P/01					EURLMB/10/P/02				
Toxin	**Assigned**	**CEFAS**	**AFBI**	**Z-score**	**Toxin**	**Assigned**	**CEFAS**	**AFBI**	**Z-Score**
dcGTX2,3	0.44	0.60	0.29	-1.064	**GTX2,3**	0.85	1.18	0.68	-0.544
dcSTX	1.16	1.17	0.71	-1.220	**STX**	1.43	1.47	0.94	-1.079
C1,2	3.29	3.70	2.25	-0.794	**NEO****				
GTX5	5.28	6.29	5.18	-0.063	**dcSTX***		0.08		
GTX6	6.02	4.82	4.07	-0.988	**GTX1,4***		0.12		
dcNEO	1.62	0.36	nd		**GTX5**		0.07		

* EU-RLMB indicated trace levels detected.

** EU-RL indicated only 4 labs out of 31 participants detected NEO therefore this toxin was not considered for evaluation.

Interlaboratory Validation Exercises

The European Network of NRLs Working Group on the determination of marine biotoxins by liquid chromatography coupled to tandem mass spectrometry has been working since 2005 on the development and validation of a method for lipophilic toxins. The group is co-ordinated by the EU-RLMB and comprises members from the NRLs of Belgium, France, Germany, Ireland, Italy, Sweden, The Netherlands, and the United Kingdom (UK-NRL and CEFAS). Prevalidation studies conducted in 2008 and 2009 lead to revision of the standard operating procedure and the subsequent interlaboratory exercise undertaken in 2010. The aim of the validation study was to determine accuracy, repeatability and between laboratory reproducibility of the method detailed in the EU-harmonised Standard Operating Procedure for the determination of lipophilic marine biotoxins by liquid chromatography coupled to tandem mass spectrometry. The methodology allowed participants to use either, the acidic liquid chromatography conditions originally proposed by the Working Group or basic liquid chromatography conditions developed by RIKILT. The UK-NRL and CEFAS employed the basic liquid chromatography protocol. There were 12 participants from 10 European countries

and the results from the study have shown acceptable precision, interlaboratory reproducibility and recovery and the Working Group concluded that the method was reliable for the determination of lipophilic toxins in bivalve molluscs but recognised the need to extend the validation to cover the Yessotoxin-group.

As part of the EU 6th framework project 'BIOTOX", a LC-MS/MS method for the determination of lipophilic marine biotoxins was developed and in-house validated at the RIKILT Institute. This was followed up in 2010 by an interlaboratory validation study to determine accuracy, repeatability between-laboratory reproducibility of the method. The study involved the quantitative determination of the lipophilic toxin group and 12 European laboratories took part (including the UK-NRL and CEFAS). The coordinators considered the study to have shown the suitability of the method for the determination of lipophilic toxins. The full report was circulated by the UK-NRL and is available for download from the UK-NRL website.

Provide Scientific and Technical Assistance to the Food Standards Agency

The UK-NRL and the FSA undertook a review of the role of the UK-NRL and in particular, how it should interact with the UK Official Control Laboratories (OCLs). It was agreed that the UK-NRL should provide OCLs with access to the SOPs for marine biotoxins and circulate an article detailing the role of the UK-NRL in providing advice on relevant analytical methods. Revisions to the relevent procedures were prepared by the UK-NRL and placed on their web page and also supplied to the Competent Authority.

Following on from the discussions on the role of the UK-NRL for Marine Biotoxins, the Competent Authority organised the first annual meeting of all appointed UK-NRLs for the other areas of Food and Feed. The meeting was held at FSA, premises, London on 4th October 2010, at which the various UK-NRLs presented an overview of their activities and respective roles.

Throughout 2010, progress was made on the validation and implementation of LC-MS/MS as a replacement for the MBA for lipophilic toxins. In support of the activities of the monitoring laboratories and to provide information to the FSA, representatives from the UK-NRL and CEFAS visited the NRL-France (ANSES). Presentations were made on the UK monitoring programme and NRL France presented information on the French monitoring programme and their implementation of LC-MS/MS. A full report was prepared and submitted to the FSA. In

support of the implementation of LCMS/MS, the UK-NRL has advised on the application of measurement uncertainty and has undertaken a review of the interlaboratory validation report submitted to the FSA in 2010.

CEFAS and the NRL undertook a comparison of the data submitted by their laboratories for the interlaboratory validation studies coordinated by the RIKILT Institute and the EU-RL. The results were presented at the UK-NRL Network meeting in November 2010. Both validation exercises were deemed to be successful, with both UK laboratories performing well. Comparison of the data submitted by the UK laboratories was very encouraging and demonstrated that the UK laboratories produced comparable results.

The UK-NRL completed a study on the application of the current methods for marine biotoxins to periwinkles. The lipophilic toxin extraction protocol for the biological method on periwinkle was acceptable in that no adverse effects were produced by the extract. Further work is required to establish the extraction efficiency of the protocol when used for periwinkle tissue. In the biological method for PSP, adverse reactions were observed making the method unsuitable for use. For ASP, there was no indication of potential coeluting peaks in the samples analysed. The extraction procedure for PSP by HPLC-FLD may be a feasible method for periwinkle matrix. There is the potential for interference from matrix components in periodate oxidation. Of more concern is the apparent low recovery and before the method could be extended for use as a screen or quantitative method for periwinkles, more extensive validation work would be required. A full report of the study was submitted to Food Standards Agency and the work was presented as a poster at the 14th International Conference on Harmful Algae (HAB2010), at Hersonissos, Crete.

Other Activities

During 2010 – 2011, the UK-NRL has provided technical advice, on behalf of the FSA, to shellfish industry representative bodies such as SeaFish and assisted in the production of fact sheets on marine toxins. Enquires from a number of European shellfish producers were received and were referred accordingly to the relevent NRLs in other member states

With the support of the FSA, the UK-NRL participated in training activities organised by the EU-RL. In May 2010, it was represented at a training course on the use of AOAC 2005.06 method for the detection of PSP by HPLC-FLD and in July 2010, at a training course on the application of LC-MS/MS for the detection of lipophilic marine biotoxins.

Meetings Attended

Date	Venue	Subject
13th May 2010	FSA, London	UK-NRL Network
15th May 2010	Vigo	EU-RL Training on AOAC 2005.06 HPLC-FLD method
8th July 2010	AFBI / London	Video Conference on Role of UK-NRL
12th -14th July 2010	Vigo	EU-RL Training on LC-MS/MS
14th -16th July	Paris	UK-NRL / CEFAS fact finding visit on implementation of LC-MS/MS for lipophilic toxins
3rd August 2010	FSA, London	Release of UK-NRL Standard Operating Procedures
4th October 2010	FSA, London	1st Meeting of Feed and Food UK-NRLs
21st-22nd October 2010	Wageningen	XIII Meeting EU-RL / NRLs
1st -5th November 2010	Crete	HAB 2010
22nd November 2010	FSA, London	UK-NRL Network

Reports

The following reports may be downloaded from the UK-NRL and EU-RLMB web pages

Minutes of the XIII Meeting of EU-NRLs, Institute of Food Safety (RIKILT), Wageningen, Netherlands.

Report on the EU-RLMB 2010 Proficiency testing on Lipophilic Marine Biotoxins Determination.

Report on the EU-RLMB 2010 Proficiency testing on Saxitoxin Group (PSP) Toxins Determination.

Report on the EU-RLMB 2010 Proficiency testing on Domoic Acid (ASP Toxins) Determination.

Report on the Interlaboratory Validation Study of the "EU-Harmonised Standard Operating Procedure for the Determination of Lipophilic Marine Biotoxins in Molluscs by LC-MS/MS.

International Validation Study of the method, Quantitative Determination of Marine Lipophilic Toxins in Shellfish using LC-MS/MS, Institute of Food Safety (RIKILT), Wageningen, Netherlands.

A Preliminary Investigation by the UK-NRL into the Applicability of Current Methods of Analysis for the detection of Marine Biotoxins in the Common Periwinkle (*Littorina littorea*).

Links

UK-NRL Web page:
http://www.afbini.gov.uk/index/services/services-diagnostic-and-analytical/marine-biotoxins-nrl.htm

EU-RLMB Web page:
http://www.aesan.msps.es/en/CRLMB/web/home.shtml