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

ASP: Amnesic Shellfish Poison (Domoic Acid)

Cefas: Centre for Environment, Fisheries and Aquaculture Science

eWG: Electronic working group

EURL-MB: European Reference Laboratory for Marine Biotoxins

EFSA: European Food Safety Authority

FSA: Food Standards Agency

HPLC-FLD: High Performance Liquid Chromatography with fluorescence detection

IPI: International Phytoplankton Inter-comparison exercise

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

LTs: Lipophilic toxins (including Diarrhetic Shellfish Poison (DSP) group)

OCL: Official Control Laboratory

PSP: Paralytic Shellfish Poison (Saxitoxin group)

PTs: Proficiency Tests

SAMS: The Scottish Association for Marine Sciences

SOP: Standard Operating Procedure

TTX: Tetrodotoxin

UK-NRL: United Kingdom National Reference Laboratory

Introduction

This report provides an outline of the work of the UK-NRL over the financial year 2017-2018. It is not a comprehensive review but highlights 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. A summary of the proposed 2017 UK-NRL work programme is provided in Appendix 2. The additional work activities listed for 2017 will be carried forward into 2018 as a result of delays in the commencement of inter-lab collaborative work on TTX or PSP methods which are beyond the NRLs control.

For the purposes of Regulation (EC) 2017/625 regarding Official Feed and Food Controls, the FSA is designated as the Competent Authority and as such 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 appointment of the UK-NRL for marine biotoxins is also the responsibility of the FSA. The role of the UK-NRL for marine biotoxins is to carry out the requirements and duties set out in Article 101 of Regulation (EC) 2017/625, namely:

1. Collaborate with the European EURL 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 supplies;
5. Provide scientific and technical assistance to the competent authority for the implementation of co-ordinated control plans adopted in accordance with Articles 109 and 112.
6. Where relevant, validate the reagents and lots of reagents, establish and maintain up-to-date lists of available reference substances and reagents and of manufacturers and suppliers of such substances and reagents;
7. Where necessary, conduct training courses for the staff of official laboratories designated under Article 37(1).

Summary of Meetings attended 2017-2018

The table below provides a summary of meetings attended as part of the NRL activities that took place during 2017-2018.

Meetings Attended 2017-2018

Date	**Venue**	**Subject**
14-18th May 2017	Conference	ICMSS Galway, Ireland
8th June 2017	Brussels	EURL-NRL Working group (TTX + EFSA opinion)
21st June 2017	London	30th UK NRL Network Meeting
16th November 2017	Belfast	31st UK NRL Network Meeting
26-27th October 2017	Baiona	EURL-MB/NRL Annual workshop

Collaboration with the EURL-MB

In 2017-2018, AFBI as UK-NRL continued to participate in the following EURL-MB co-ordinated working groups: (1) LC-MS/MS working group (2) Toxic phytoplankton working group.

The EURL convened a Working Group (WG) meeting in June 2017 to discuss the EFSA opinion on TTX, and to consider agreeing a consensus among NRLs regarding an approach that the NRL network could take to advance capability within the NRL lab network for TTX testing. The EFSA opinion (65 pages, issued 15th March 2017) recommends a limit of 44ug/kg TTX equivalents in shellfish meat and recognises that LC-MS/MS methods are the most suitable for identification and quantification of TTX and its analogues.

The EURL described their work on a single laboratory validation study by LC-MS/MS. Discussions focused on the methodology used at the EURL which was based on Turner et al. 2015 and have been shared within the EURL-NRL network. AFBI as UK-NRL agreed to participate in future collaborative inter-laboratory exercises that the EURL would co-ordinate for TTX methodology comparison.

The phytoplankton working group was established with the aim of producing 'a best practice guide to monitoring of toxin-producing phytoplankton in production areas for live bivalve molluscs'. Much of the work of this WG is conducted electronically (eWG) with meetings as required to agree final draft documents. The UK-NRL co-ordinated all UK input/responses received from official control laboratory experts and submitted these to Dr. Pablo Serrat, chair of the Working Group. The EU-RL has highlighted the necessity of the experts participating in the WG for Phytoplankton to communicate and report any input to the relevant NRL, as this activity remains under NRLs co-ordination.

Co-ordination of the Activities of the Monitoring Laboratories

The NRL organised two UK Network meetings, comprising representation from the FSA, FSS, FSANI and monitoring laboratories (SAMS, Cefas, AFBI). The 30th meeting of the UKNRL-Network group was hosted by FSA in London on the 21st June 2017, with the 31st meeting hosted by AFBI in Belfast on the 16th Nov 2017.

In 2017, the UK-NRL successfully lobbied for the inclusion of Cefas in EURL-MB proficiency testing (PT) exercises. The EURL offered official participation to NRLs and provision of additional test materials (on request) to permit NRLs to fulfil their obligations under Regulation (EC) 2017/625. The additional samples obtained from the EURL PT scheme were shipped by the UKNRL to Cefas in May 2017, with a request for results to be submitted after the closure of the EURL test submission deadline (end of June 2017), and prior to 31st July 2017. A reporting sheet was provided by the UK-NRL for reporting of test results. The UK-NRL collated a summary of results for PT exercises undertaken by the UK official control laboratories and these were circulated and discussed at UK network meetings in 2017.

The UK-NRL co-ordinated responses received from official control laboratory experts in the UK to documents circulated through the EURL phytoplankton WG. This WG is assisting the EURL in harmonising phytoplankton monitoring activities across member states through the preparation of a guideline document on toxic phytoplankton monitoring.

Proficiency Tests (PTs)

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 LTs, PSP and ASP.

- For Lipophilic Toxins (LTs), the EURL-MB has organised PTs since 2000. In 2017 a total of 23 laboratories participated, 21 of which were NRLs (18 EU-NRLs), and 2 OC laboratories from third countries.

- PT exercises for PSP have been organised since 2004. The exercise covered both biological methods and HPLC-FLD. The number of participants in 2017 was 24, of which 21 were NRLs (18 EU-NRLs), with 3 official control (OC) laboratories from third countries also submitting results.

- 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 2017, there were 23 participants, of which 20 were NRLs (18 EU-NRLs), and 3 OC laboratories from third countries.

Reports on the EURL-MB proficiency tests are circulated in October and discussed at the annual EURL-NRL workshop. The results obtained by the UK-NRL are summarised in Appendix 1 and are available on the UK-NRL website (full reports generated by the EURL are confidential). Results obtained by the UK-NRL were circulated to the UK NRL Network and discussed in full at the Network meeting held in November 2017.

Both UK laboratories participated in the Quasimeme 2017 Proficiency test programmes for ASP/PSP/LTs and in the International Phytoplankton Inter-comparison (IPI) taxonomic quiz. The UK-NRL also requested additional sample materials for the EURL-MB 2017 PT scheme tests and forwarded them to Cefas. Results of the analyses of the EURL samples were submitted to the UK-NRL for assessment of performance (z-scores retrospectively calculated). A summary report of all PT results is circulated to the NRL network prior to each Network Meeting. The results obtained by the UK-NRL are summarised in Appendix 1.

A z-score is calculated for each participant's data for each matrix / determinand combination which is given an assigned value. The z-score is calculated as follows:

$$z\text{ - score} = \frac{\text{Mean from Laboratory - Assigned Value}}{\text{Total Error}}$$

$|Z| < 2$ Satisfactory performance

$2 < |Z| < 3$ Questionable performance

$|Z| > 3$ Unsatisfactory performance

Proficiency test summary

For the EURL and Quasimeme proficiency tests in 2017 for lipophilic toxins, the National Reference Laboratory reported results for individual toxins with 87% of Z scores less than 2 (satisfactory), 9% of the Z scores less than 3 (questionable) and 4% of the Z scores as >3. Performance in the EURL PT and round 1 Quasimeme test results was acceptable overall with 95% of the Z scores being acceptable, and 5% questionable. For Quasimeme round 2, results with z-scores >2 or 3 were traced to an issue with a DTX2 toxin analytical standard which resulted in some outliers. All questionable or unsatisfactory results were investigated and reviewed as part of the laboratories internal quality procedures, with no impact on reported results.

For PSP, 94% of individual toxin Z scores were less than 2, 3% between 2 to 3, and 3% above 3 (1 result out of 32). Individual toxin results >2 were investigated and reviewed as part of the laboratories internal quality procedures. Overall, all total toxicity results were within acceptable ranges, with no impact on reported results.

For ASP (Domoic Acid) all results returned satisfactory z-scores.

Performance in the International Phytoplankton Inter-comparison (Bequalm) taxonomic quiz was acceptable. Analyst 1 achieved a proficient classification with a 100% overall performance rating, with analyst 2 achieving a pass (87.4%).

Dissemination of Information from the EURL-MB & Provision of Scientific and Technical Assistance to the Competent Authority

Minutes and reports from EURL Working Groups and workshops attended were discussed at the UK network meetings and further information circulated on request.

The EURL convened a Working Group (WG) meeting on the 8th June 2017 to discuss the EFSA opinion on TTX recommending a limit of 44ug/kg TTX equivalents in shellfish meat, and agree a consensus among NRLs regarding an approach that the NRL network could take to advance capability within the NRL lab network for TTX testing. AFBI as UK-NRL agreed to participate in the future collaborative inter-laboratory exercise that the EURL would co-ordinate for TTX methodology comparison. A summary of the discussions was presented at the UK-NRL network meeting on the 21st June 2017 and is included within the minutes of that meeting.

The UK-NRL attended the ICMSS conference held in Galway, Ireland from the 14-18th May 2017. A summary was presented at the UK-NRL network meeting on the 8h June 2017 and is included within the minutes of that meeting.

The EURL requested input from member states to assist with the drafting of a 'Guideline document for marine biotoxins'. Nominated contacts from the competent authority were provided (in addition to the NRL) for the proposed expert working group. The UKNRL submitted FSAs

response to this query along with nominated contacts from FSA and FSS. A summary was presented at the network meeting on the 8h June 2017.

The UK-NRL attended the annual EURL/NRL workshop on 26-27th October 2017 in Baiona. The performance of NRLs in the 2017 EURL PT schemes, a review of the 2017 work programme of the EURL and activities for 2018 were presented by the EURL. The UK-NRL gave a presentation on its NRL activities for 2017 and undertook responsibility for producing minutes of that meeting. A summary was presented at the UK-NRL network meeting on the 16th November 2017.

The UK-NRL provided scientific and technical support to the competent authority and OCLs throughout the year on request. This included reviewing documentation on sub-sampling procedures by laboratories, and assessing requests for changes to laboratory standard operating procedures (SOPs).

Links

UK-NRL Web page:

The NRL website and associated links can be accessed through the following link:

https://www.afbini.gov.uk/articles/united-kingdom-national-reference-laboratory-marine-biotoxins

Updated link to EURL website:
http://www.aecosan.msssi.gob.es/en/CRLMB/web/home.html

Appendix 1

Domoic Acid 2017 Proficiency Test Summaries

AFBI EURL 2017

Sample ID	Sample description	Assigned value	Reported value	Units	z-score
EURLMB/17/A/01	Mussel homogenate	15.7	17.5	mg/Kg	0.53
EURLMB/17/A/02	Scallop homogenate	29.5	34.1	mg/Kg	0.71

AFBI Quasimeme Round 2017.1

Sample No	Sample ID	Sample description	Determinand	Assigned value	Reported value	Units	z-score
Sample 1	QST222SS	Standard solution	Total Domoic + Epi DA	0.47	0.46	mg/Kg	-0.07
Sample 2	QST223BT	Manilla clam homogenate	Total Domoic + Epi DA	22.46	22.5	mg/Kg	0.02
Sample 3	QST224BT	Q. Scallop (adductor) homogenate	Total Domoic + Epi DA	32.02	39.5	mg/Kg	1.82

AFBI Quasimeme Round 2017.2

Sample No	Sample ID	Sample description	Determinand	Assigned value	Reported value	Units	z-score
Sample 1	QST234SS	Standard solution	Total Domoic + Epi DA	2.05	2.06	mg/Kg	0.04
Sample 2	QST235BT	Oyster homogenate	Total Domoic + Epi DA	17.12	18.13	mg/Kg	0.45
Sample 3	QST236BT	Mussel homogenate	Total Domoic + Epi DA	39.42	42.99	mg/Kg	0.71

PSP 2017 Proficiency Test Summaries

AFBINI EURL 2017

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
EURLMB/17/P/01	MBA	Mussel homogenate	Total STX	1190	845	**µgSTX2HCL equiv/Kg**	**-1.49**
EURLMB/17/P/01	HPLC	Mussel homogenate	dcGTX2,3	109.2			
EURLMB/17/P/01	HPLC	Mussel homogenate	dcNeo	73.76			
EURLMB/17/P/01	HPLC	Mussel homogenate	C3C4	101.09			
EURLMB/17/P/01	HPLC	Mussel homogenate	C1C2	243.05	295.500	µgSTX2HCL equiv/Kg	0.97
EURLMB/17/P/01	HPLC	Mussel homogenate	dcSTX	154.82	137.300	µgSTX2HCL equiv/Kg	-0.48
EURLMB/17/P/01	HPLC	Mussel homogenate	GTX5 (B1)	177.02	194.250	µgSTX2HCL equiv/Kg	0.47
EURLMB/17/P/01	HPLC	Mussel homogenate	GTX6 (B2)	263.03	438.900	µmol/kg	2.51
EURLMB/17/P/01	HPLC	Mussel homogenate	**Total toxicity**	1188.58	1066	**ugSTX2HCL equiv/Kg**	**-0.54**
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
EURLMB/17/P/02	MBA	Mussel homogenate	Total STX	1714	927	µgSTX2HCL equiv/Kg	-2.58
EURLMB/17/P/02	HPLC	Mussel homogenate	GTX2&3	475.24	511.61	µgSTX2HCL equiv/Kg	0.38
EURLMB/17/P/02	HPLC	Mussel homogenate	STX	1471	1456.51	µgSTX2HCL equiv/Kg	-0.06
EURLMB/17/P/02	HPLC	Mussel homogenate	**Total toxicity**	1989.8	1968	**ugSTX2HCL equiv/Kg**	**-0.06**
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
EURLMB/17/P/03	HPLC	Cockle homogenate	GTX2&3	280.47	263.59	µgSTX2HCL equiv/Kg	-0.28
EURLMB/17/P/03	HPLC	Cockle homogenate	STX	65.34	51.57	µgSTX2HCL equiv/Kg	-0.84
EURLMB/17/P/03	HPLC	Cockle homogenate	GTX1,4	459.87	770.05	µgSTX2HCL equiv/Kg	3.24
EURLMB/17/P/03	HPLC	Cockle homogenate	**Total toxicity**	825.19	1085	**ugSTX2HCL equiv/Kg**	**1.76**

AFBI Quasimeme Round 2017.1

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST230BT	HPLC	Oyster (*Crassostrea gigas*)	GTX-1,4	0.620	0.712	µmol/kg	0.55
QST230BT	HPLC	Oyster	GTX-2,3	1.599	1.240	µmol/kg	-1.34
QST230BT	HPLC	Oyster	NEO	0.179	0.168	µmol/kg	-0.15
QST230BT	HPLC	Oyster	STX	0.565	0.520	µmol/kg	-0.36
QST230BT	HPLC	Oyster	**Total toxicity**	911	799	**µgSTXdiHCleq./kg**	**-0.91**
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST231BT	HPLC	Mussel (*M. edulis*)	dc-STX	0.556	0.440	µmol/kg	-0.94
QST231BT	HPLC	Mussel	GTX-1,4	0.628	0.760	µmol/kg	0.91
QST231BT	HPLC	Mussel	GTX-2,3	0.995	0.728	µmol/kg	-1.47
QST231BT	HPLC	Mussel	NEO	0.188	0.112	µmol/kg	-0.93
QST231BT	HPLC	Mussel	STX	0.308	0.272	µmol/kg	-0.41
QST231BT	HPLC	Mussel	**Total toxicity**	781	754	**µgSTXdiHCleq./kg**	**-0.25**
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST232BT	HPLC	Mussel (*M. galloprovincialis)*	dc-STX	2.096	1.584	µmol/kg	-1.45
QST232BT	HPLC	Mussel	GTX-5	0.138	0.112	µmol/kg	-0.38
QST232BT	HPLC	Mussel	STX	0.108	0.060	µmol/kg	-0.70
QST232BT	HPLC	Mussel	**Total toxicity**	798	611	**µgSTXdiHCleq./kg**	-1.54
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST233BT	HPLC	Mussel (*M. edulis*)	GTX-2,3	4.112	3.216	µmol/kg	-1.39
QST233BT	HPLC	Mussel	STX	3.077	3.128	µmol/kg	0.11
QST233BT	HPLC	Mussel	**Total toxicity**	2047	1882	**µgSTXdiHCleq./kg**	**-0.59**

Lipophilic 2017 Proficiency Test Summaries

AFBINI EURL 2017

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
EURLMB/17/L/01	LC-MS/MS	Mussel (homogenate)	Free OA group	41.6	30.5	OA equivalents µg/kg	-1.2
EURLMB/17/L/01	LC-MS/MS	Mussel	Total OA group	191	170.5	OA equivalents µg/kg	-0.5
EURLMB/17/L/01	LC-MS/MS	Mussel	YTX	0.58	0.60	YTX mg/kg	0.1
EURLMB/17/L/01	LC-MS/MS	Mussel	Homo-YTX	5.18	5.10	Homo-YTX mg/kg	-0.1
EURLMB/17/L/01	LC-MS/MS	Mussel	45-OH-YTX	0.53	0.60	45-OH-YTX mg/kg	0.7
EURLMB/17/L/01	LC-MS/MS	Mussel	45-OH-Homo-YTX	3.13	4.00	45-OH-Homo-YTX mg/kg	1.4
EURLMB/17/L/01	LC-MS/MS	Mussel	Total YTX group	7.54	8.2	YTX equivalents mg/kg	0.6
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
EURLMB/17/L/02	LC-MS/MS	Mussel (homogenate)	Free OA	259	207	Free OA µg/kg	-1.0
EURLMB/17/L/02	LC-MS/MS	Mussel	Free DTX-2	211	171	Free DTX2 µg/kg	-0.9
EURLMB/17/L/02	LC-MS/MS	Mussel	Free OA group	378.45	310.1	OA equivalents µg/kg	-1.0
EURLMB/17/L/02	LC-MS/MS	Mussel	Total OA	1713	1708	Total OA µg/kg	0.0
EURLMB/17/L/02	LC-MS/MS	Mussel	Total DTX-2	417	388	Total DTX2 µg/kg	-0.4
EURLMB/17/L/02	LC-MS/MS	Mussel	Total OA group	1958	1941	OA equivalents µg/kg	-0.1
EURLMB/17/L/02	LC-MS/MS	Mussel	AZA1	542	493	AZA1 µg/kg	-0.5
EURLMB/17/L/02	LC-MS/MS	Mussel	AZA2	135	141	AZA2 µg/kg	0.2
EURLMB/17/L/02	LC-MS/MS	Mussel	AZA3	36.30	33.40	AZA3 µg/kg	-0.3
EURLMB/17/L/02	LC-MS/MS	Mussel	Total AZA group	833	794	AZA equivalents µg/kg	-0.3
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
EURLMB/17/L/03	LC-MS/MS	Mussel (homogenate)	Free OA	80.0	54.5	Free OA µg/kg	-1.4
EURLMB/17/L/03	LC-MS/MS	Mussel	Free OA group	86.0	54.5	OA equivalents µg/kg	-1.6
EURLMB/17/L/03	LC-MS/MS	Mussel	Total OA	267.3	245.0	Total OA µg/kg	-0.4
EURLMB/17/L/03	LC-MS/MS	Mussel	Total DTX2	26.0	10.9	Total DTX2 µg/kg	-2.4
EURLMB/17/L/03	LC-MS/MS	Mussel	Total OA group	276.2	251.9	OA equivalents µg/kg	-0.4

AFBINI Quasimeme Round 2017.1

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST225 SS	LC-MS/MS	AZA Std. Solution	AZA-1	8.04	10.5	µg/kg	2.16
QST225 SS	LC-MS/MS	AZA Std. Solution	AZA-total	7.84	10.5	µg AZA eq./kg	2.34
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST226SS	LC-MS/MS	Lipophilic Standard Solution	AZA-1	16.8	16.9	AZA1 µg/kg	0.04
QST226SS	LC-MS/MS	Lipophilic Standard Solution	AZA-2	11.9	12.2	AZA2 µg/kg	0.14
QST226SS	LC-MS/MS	Lipophilic Standard Solution	AZA-3	8.61	9.22	AZA3 µg/kg	0.52
QST226SS	LC-MS/MS	Lipophilic Standard Solution	Total AZA group	49.5	51.7	AZA equivalents µg/kg	0.34
QST226SS	LC-MS/MS	Lipophilic Standard Solution	Free DTX1	65.7	54.3	Free DTX1 µg/kg	-1.27
QST226SS	LC-MS/MS	Lipophilic Standard Solution	Free OA group	65.8	54.3	OA equivalents µg/kg	-1.24
QST226SS	LC-MS/MS	Lipophilic Standard Solution	YTX	0.03	0.02	YTX mg/kg	-0.59
QST226SS	LC-MS/MS	Lipophilic Standard Solution	Total-YTX group	0.03	0.02	YTX equivalents mg/kg	-0.55
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST227BT	LC-MS/MS	DSP/AZP Extract	Free DTX2	48.1	42.0	Free DTX2 µg/kg	-0.92
QST227BT	LC-MS/MS	DSP/AZP Extract	Free OA	13.4	12.2	Free OA µg/kg	-0.64
QST227BT	LC-MS/MS	DSP/AZP Extract	Free OA group	43.8	37.4	OA equivalents µg/kg	-1.08
QST227BT	LC-MS/MS	DSP/AZP Extract	Total DTX2	103	93	Total DTX2 µg/kg	-0.78
QST227BT	LC-MS/MS	DSP/AZP Extract	Total OA	66.2	60.2	Total OA µg/kg	-0.67
QST227BT	LC-MS/MS	DSP/AZP Extract	Total OA group	130	116	OA equivalents µg/kg	-0.82
QST227BT	LC-MS/MS	DSP/AZP Extract	Total OA/PTX group	126	116	OA equivalents µg/kg	-0.62
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST228BT	LC-MS/MS	Mussel tissue (*M. edulis*)	Free DTX2	412	353	Free DTX2 µg/kg	-1.08
QST228BT	LC-MS/MS	Mussel tissue	Free OA	154	145	Free OA µg/kg	-0.45
QST228BT	LC-MS/MS	Mussel tissue	Free OA group	416	357	OA equivalents µg/kg	-1.07
QST228BT	LC-MS/MS	Mussel tissue	Total OA	460	400	Total OA µg/kg	-0.98

QST228BT	LC-MS/MS	Mussel tissue	Total DTX2	678	615	Total DTX2 µg/kg	-0.72
QST228BT	LC-MS/MS	Mussel tissue	Total OA group	887	769	OA equivalents µg/kg	-1.00
QST228BT	LC-MS/MS	Mussel tissue	Total OA/PTX group	871	769	OA equivalents µg/kg	-0.88
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST229BT	LC-MS/MS	Mussel extract (*M. edulis*)	AZA-1	90.2	92.6	AZA1 µg/kg	0.21
QST229BT	LC-MS/MS	Mussel extract	AZA-2	19.4	22.1	AZA2 µg/kg	1.10
QST229BT	LC-MS/MS	Mussel extract	AZA-3	35.0	40.8	AZA3 µg/kg	1.30
QST229BT	LC-MS/MS	Mussel extract	Total AZA group	176	189	AZA equivalents µg/kg	0.61
QST229BT	LC-MS/MS	Mussel extract	Free DTX2	3.92	3.20	Free DTX2 µg/kg	-1.19
QST229BT	LC-MS/MS	Mussel extract	Free OA	39.4	37.2	Free OA µg/kg	-0.40
QST229BT	LC-MS/MS	Mussel extract	Free OA group	43.0	39.1	OA equivalents µg/kg	-0.67
QST229BT	LC-MS/MS	Mussel extract	Total OA	43.9	35.3	Total OA µg/kg	-1.36
QST229BT	LC-MS/MS	Mussel extract	Total DTX2	3.74	3.60	Total DTX2 µg/kg	-0.25
QST229BT	LC-MS/MS	Mussel extract	Total OA group	46.4	36.6	OA equivalents µg/kg	-1.51
QST229BT	LC-MS/MS	Mussel extract	Total OA/PTX group	44.2	36.6	OA equivalents µg/kg	-1.23

AFBINI Quasimeme Round 2017.2

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST237SS	LC-MS/MS	Multi-toxin standard	AZA-1	12.68	11.37	µg/kg	-0.73
QST237SS	LC-MS/MS	Multi-toxin standard	Total AZA group	12.870	11.370	AZA equivalents µg/kg	-0.81
QST237SS	LC-MS/MS	Multi-toxin standard	Free OA	82.720	68.200	µg/kg	-1.29
QST237SS	LC-MS/MS	Multi-toxin standard	Free OA group	83.65	68.20	OA equivalents µg/kg	-1.32
QST237SS	LC-MS/MS	Multi-toxin standard	PTX-2	28.44	29.43	µg/kg	-0.65
QST237SS	LC-MS/MS	Multi-toxin standard	YTX	0.05	0.04	mg/kg	-0.61
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST238BT	LC-MS/MS	DSP/AZP extract	AZA-1	39.94	30.50	µg/kg	-1.83
QST238BT	LC-MS/MS	DSP/AZP extract	AZA-2	9.16	7.60	µg/kg	-1.24
QST238BT	LC-MS/MS	DSP/AZP extract	AZA-3	13.63	12.90	µg/kg	-0.41
QST238BT	LC-MS/MS	DSP/AZP extract	Total AZA group	76.32	62.20	AZA equivalents µg/kg	-1.41
QST238BT	LC-MS/MS	DSP/AZP extract	Free DTX1	1.90	1.60	µg/kg	-0.72
QST238BT	LC-MS/MS	DSP/AZP extract	Free OA	15.26	13.50	µg/kg	-0.83
QST238BT	LC-MS/MS	DSP/AZP extract	Free OA group	17.010	15.100	OA equivalents µg/kg	-0.75
QST238BT	LC-MS/MS	DSP/AZP extract	Total DTX1	2.62	2.50	µg/kg	-0.27
QST238BT	LC-MS/MS	DSP/AZP extract	Total OA	19.590	16.800	µg/kg	-1.06
QST238BT	LC-MS/MS	DSP/AZP extract	Total OA group	22.440	19.300	OA equivalents µg/kg	-1.02
QST238BT	LC-MS/MS	DSP/AZP extract	Total OA group + PTX group	21.40	19.30	OA equivalents µg/kg	-0.70
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST239BT	LC-MS/MS	DSP/AZP Extract	45-OH-homo-YTX	0.18	0.18	mg/kg	-0.10
QST239BT	LC-MS/MS	DSP/AZP Extract	45-OH-YTX	0.02	0.03	mg/kg	0.29
QST239BT	LC-MS/MS	DSP/AZP Extract	homo-YTX	0.31	0.29	mg/kg	-0.45
QST239BT	LC-MS/MS	DSP/AZP Extract	YTX	0.03	0.03	mg/kg	-0.10
QST239BT	LC-MS/MS	DSP/AZP Extract	Total YTX group	0.43	0.43	YTX equivalents mg/kg	0.04

QST239BT	LC-MS/MS	DSP/AZP Extract	AZA-1	13.94	12.90	µg/kg	-0.56
QST239BT	LC-MS/MS	DSP/AZP Extract	AZA-2	3.93	3.70	µg/kg	-0.38
QST239BT	LC-MS/MS	DSP/AZP Extract	AZA-3	4.10	3.80	µg/kg	-0.50
QST239BT	LC-MS/MS	DSP/AZP Extract	Total AZA group	26.20	24.80	AZA equivalents µg/kg	-0.38
QST239BT	LC-MS/MS	DSP/AZP Extract	Free DTX2	66.42	37.40	µg/kg	-3.33
QST239BT	LC-MS/MS	DSP/AZP Extract	Free OA	12.14	10.70	µg/kg	-0.88
QST239BT	LC-MS/MS	DSP/AZP Extract	Free OA group	54.110	33.100	OA equivalents µg/kg	-2.89
QST239BT	LC-MS/MS	DSP/AZP Extract	Total DTX2	78.80	38.40	µg/kg	-3.64
QST239BT	LC-MS/MS	DSP/AZP Extract	Total OA	18.560	16.100	µg/kg	-0.95
QST239BT	LC-MS/MS	DSP/AZP Extract	Total OA group	67.720	39.100	OA equivalents µg/kg	-3.07
QST239BT	LC-MS/MS	DSP/AZP Extract	Total OA group + PTX group	66.01	39.10	OA equivalents µg/kg	-2.96
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST240BT	LC-MS/MS	Mussel homogenate	45-OH-homo-YTX	0.55	0.57	mg/kg	0.22
QST240BT	LC-MS/MS	Mussel homogenate	45-OH-YTX	0.13	0.15	mg/kg	0.53
QST240BT	LC-MS/MS	Mussel homogenate	homo-YTX	1.03	0.96	mg/kg	-0.47
QST240BT	LC-MS/MS	Mussel homogenate	YTX	0.22	0.22	mg/kg	-0.02
QST240BT	LC-MS/MS	Mussel homogenate	Total YTX group	1.54	1.61	YTX equivalents mg/kg	0.3
QST240BT	LC-MS/MS	Mussel homogenate	AZA-1	694.31	593.00	µg/kg	-1.14
QST240BT	LC-MS/MS	Mussel homogenate	AZA-2	196.76	178.00	µg/kg	-0.73
QST240BT	LC-MS/MS	Mussel homogenate	AZA-3	117.31	111.00	µg/kg	-0.41
QST240BT	LC-MS/MS	Mussel homogenate	Total AZA group	1235.74	1069	AZA equivalents µg/kg	-1.06
QST240BT	LC-MS/MS	Mussel homogenate	Free DTX1	88.5	65	µg/kg	-1.99
QST240BT	LC-MS/MS	Mussel homogenate	Free DTX2	539.13	340	µg/kg	-2.75
QST240BT	LC-MS/MS	Mussel homogenate	Free OA	143.78	124	µg/kg	-1.05
QST240BT	LC-MS/MS	Mussel homogenate	Free OA group	578.62	393	OA equivalents µg/kg	-2.34
QST240BT	LC-MS/MS	Mussel homogenate	Total DTX1	112.03	85	µg/kg	-1.73
QST240BT	LC-MS/MS	Mussel homogenate	Total DTX2	761.04	397	µg/kg	-3.6
QST240BT	LC-MS/MS	Mussel homogenate	Total OA	312.65	257	µg/kg	-1.34
QST240BT	LC-MS/MS	Mussel homogenate	Total OA group	895.57	580	OA equivalents µg/kg	-2.66

QST240BT	LC-MS/MS	Mussel homogenate	Total OA group + PTX group	881.21	580	OA equivalents µg/kg	-2.53
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST241BT	LC-MS/MS	Mussel homogenate	Free-DTX2	431.29	253	µg/kg	-3.04
QST241BT	LC-MS/MS	Mussel homogenate	Free OA	148.56	129	µg/kg	-1.01
QST241BT	LC-MS/MS	Mussel homogenate	Free OA group	424.15	281	OA equivalents µg/kg	-2.41
QST241BT	LC-MS/MS	Mussel homogenate	Total DTX2	700.09	428	µg/kg	-3
QST241BT	LC-MS/MS	Mussel homogenate	Total OA	437.07	399	µg/kg	-0.66
QST241BT	LC-MS/MS	Mussel homogenate	Total OA group	863.08	656	OA equivalents µg/kg	-1.79

AFBINI - International Phytoplankton Inter-comparison (IPI) 2017
(Phytoplankton Proficiency Testing Scheme)

Date	Analyst Code	Phytoplankton In sample	Species ID	z-score
2017	87	Akashiwo sanguinea	correct	0
2017	87	Scrippsiella trochoidea	correct	0.24
2017	87	Tieris sinensis	correct	-0.58
2017	87	Azadinium spinosum	correct	0.06
2017	87	Chaetoceros danicus	correct	0.64
2017	87	Pseudo-nitzschia pungens	correct	0.44
2017	87	Ceratoneis closterium	correct	0.82
2017	87	Chaetoceros curvisetus	correct	0.66
2017	87	Prorocentrum mexicanum	correct	0.78
Result of International Phytoplankton Intercomparison (Bequalm) taxonomic quiz score: Analyst 87 achieved a proficient classification (100% score); test score >90% is deemed proficient.				

Date	Analyst Code	Phytoplankton In sample	Species ID	z-score
2017	1	Akashiwo sanguinea	correct	-0.08
2017	1	Scrippsiella trochoidea	correct	0.34
2017	1	Tieris sinensis	correct	4.2
2017	1	Azadinium spinosum	correct	0.11
2017	1	Chaetoceros danicus	correct	1.11
2017	1	Pseudo-nitzschia pungens	correct	1.38
2017	1	Ceratoneis closterium	correct	0.22
2017	1	Chaetoceros curvisetus	correct	0.77
2017	1	Prorocentrum mexicanum	correct	0.38
Result of International Phytoplankton Intercomparison (Bequalm) taxonomic quiz score: Analyst 1 achieved a pass classification (87.4% score); test score >70% is deemed a pass.				

Appendix 2

Agri-Food & Biosciences Institute

VETERINARY SCIENCES DIVISION

Chemical Surveillance Branch

Work Programme
UK National Reference Laboratory
For Marine Biotoxins

2017

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Glossary

AFBI: Agri-Food and Biosciences Institute

NRL: National Reference Laboratory

Cefas: Centre for Environment, Fisheries and Aquaculture Science

LTs: Lipophilic toxins (including Diarrhetic Shellfish Poison (DSP) group)

EURL-MB: European Reference Laboratory for Marine Biotoxins

FSA: Food Standards Agency

TEF: Toxic Equivalence Factor

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

HILIC: Hydrophilic Liquid Interaction Chromatography

MU: Measurement Uncertainty

PSP: Paralytic Shellfish Poison (Saxitoxin group)

UK-NRL: United Kingdom National Reference Laboratory

OCLs: Official Control Laboratories

PTs: Proficiency tests

IPI: International Phytoplankton Inter-comparison exercise

TTX: Tetrodotoxin

National Reference Laboratory Annual Report

The annual report for 2017-2018 will be drafted and submitted to the Competent Authority for comment in May 2018.

NRL Standard Operating Procedures

The NRL Standard Operating procedures will be reviewed and updated, if required.

Proficiency tests 2017

Official control testing is carried out at two laboratories (AFBI and Cefas) making UK proficiency tests / ring trials of limited value. Both UK laboratories participate in marine biotoxin proficiency schemes organised by Quasimeme and share the data with the UK-NRL and the Competent Authority (FSA). Similarly, OCLs undertaking phytoplankton analysis participate in the International Phytoplankton Inter-comparison exercise (IPI) and share the data with the NRL and the Competent Authority.

The UK-NRL has negotiated participation of Cefas in the EURL-MB proficiency tests in the past. The EURL indicated in both the 2015 and 2016 programmes that participation will be limited because of resource issues, and that the number of non-NRLs taking part will be restricted.

In April 2017, the UK-NRL again requested that both UK laboratories be included in the 2017 programme. A response from the EURL-MB was received by the UK-NRL in April 2017

confirming CEFAS' full participation in the EURL-MB PT for PSP toxins with the HILIC LC-MS/MS method. The EURL is willing to help with our activities as UK-NRL and although they cannot include Official Control Laboratories in the PTs under the EU Commission budget, they have offered additional samples to help with inter-comparison at internal UK level. These have been requested and forwarded to Cefas. This will allow additional performance checking of OCLs by the UK-NRL in 2017, with results of these analyses being sent to the UK-NRL for assessment of OCL performance.

Meetings

EURL – NRLs Workshop 2017: Baiona, Spain (October 2017) by EURL.

EURL working group on LC-MS/MS: The working group may be reconvened in 2017.

EURL working Group on PSP: The working group may be reconvened in 2017 to discuss performance in the 2017 EURL proficiency test, consider new data on TEFs for PSP analogues, and consider the implementation and application of new technology and modifications to methods.

EURL working Group on Phytoplankton: The NRL will continue to participate and be represented at meetings of the working Group in 2017-18. Most of the work is conducted electronically and the NRL will continue to co-ordinate all UK responses and input from UK experts.

EURL working Group on MU: The NRL will continue to participate in this working group as required. Work to date has been conducted electronically and the NRL will continue to co-ordinate all UK responses and input from UK experts as required.

CEN/TC275/WG 14 on Marine Biotoxins: AFBI will continue to support the Competent Authority in its standardisation activities through active participation in the BSI / CEN processes.

UK-NRL Network Group: Two meetings to be held in 2017. It is proposed that two meetings will be held in 2017. The first will be in June 2017 and will be held at FSA headquarters, Aviation House, London. This will be the first meeting after renewal of the NRL contract for 2017-2019. The second will be held at the beginning of November 2017 at AFBI-VSD, Belfast.

Additional work activities 2017

The UK-NRL is willing to participate in the Cefas inter-laboratory collaborative validation study for PSP toxins by HILIC LC-MS/MS

The UK-NRL will undertake the setup and single lab validation of a Tetrodotoxin (TTX) LC-MS/MS based screening method in house.

The UK-NRL will participate in any EURL organised inter-laboratory method assessment exercise for TTX testing by LC-MS/MS.