



# Agri-Food & Biosciences Institute

**VETERINARY SCIENCES DIVISION**

**Chemical Surveillance Branch**

**Annual Report  
UK National Reference Laboratory  
For Marine Biotoxins**

**1<sup>st</sup> April 2019 – 31<sup>st</sup> March 2020**

**Contacts:**

**[NRL.MB@afbini.gov.uk](mailto:NRL.MB@afbini.gov.uk)**

## **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 between 1<sup>st</sup> April 2019 and 31<sup>st</sup> March 2020. 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 2019 UK-NRL work programme is provided in Appendix 2.

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 2019-2020**

The table below provides a summary of meetings attended as part of the NRL activities that took place during 2019-2020.

#### *Meetings Attended 2019-2020*

<b>Date</b>	<b>Venue</b>	<b>Subject</b>
13 <sup>th</sup> June 2019	FSS, Aberdeen	34 <sup>th</sup> UK NRL Network Meeting
24-25 <sup>th</sup> June 2019	London	UK-Harmful Algae meeting
8 <sup>th</sup> October 2019	Athlone, Ireland	Shellfish safety network meeting
5 <sup>th</sup> December 2019	FSA, London	35 <sup>th</sup> UK NRL Network Meeting

*The following meeting was not attended under FSA guidance*

24-25 <sup>th</sup> October 2019	Baiona, Spain	EURL-MB/NRL Annual workshop
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## **Collaboration with the EURL-MB**

In 2019-2020, AFBI as UK-NRL continued to participate in the EURL-MB co-ordinated phytoplankton working group. This working group was established with the aim of producing a harmonised best practice guide to monitoring of toxin-producing phytoplankton in production areas for live bivalve molluscs. Activities are conducted mainly electronically (e-WG) with the EURL-MB co-ordinating separate meetings to agree final draft documents submitted through NRLs. The UK-NRL co-ordinated all UK input/responses received from official control laboratory experts (AFBI, Cefas, SAMS) and submitted these to Dr. Pablo Serrat, chair of the Working Group.

AFBI confirmed to the EURL its intention to participate, in 2019-20, in any ongoing co-ordinated activities to assess application of LC-MS/MS methods for the detection of Tetrodotoxin (TTX), and build capability in the NRL lab network for TTX testing. An EFSA opinion recommending a limit of 44ug/kg TTX equivalents in shellfish meat was published in March 2017 which recognised that LC-MS/MS methods were most suitable for identification and quantification of TTX and its analogues.

In 2018-19, AFBI as UK-NRL participated in the EURL co-ordinated inter-laboratory exercise for TTX methodology comparison using LC-MS/MS (open to NRL laboratories only). The UKNRL returned 4/4 acceptable results for recovery uncorrected analyses in that study. A separate TTX PT exercise was not co-ordinated in 2019-20 by the EURL, however AFBI did participate in a Quasimeme TTX method assessment exercise for this toxin, as indicated in the 2019/20 work programme. This assisted in evaluation of the performance of an in-house method in 2019-20. Results are given in the PT summary section of this report (for information purposes only as this was a method assessment exercise).

### **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 (AFBI, Cefas, SAMS). The 34<sup>th</sup> meeting of the UKNRL-Network group was hosted by FSS in Aberdeen on the 13<sup>th</sup> June 2019, with the 35<sup>th</sup> meeting hosted by FSA in London on the 5<sup>th</sup> Dec 2019.

In 2019, 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 2019, with a request for results to be submitted after the closure of the EURL test submission deadline (end of June 2019), and prior to 31<sup>st</sup> July 2019. A reporting sheet was provided by the UK-NRL for reporting of test results.

The UK-NRL has collated a summary of the yearly performance for all PT exercises undertaken by the UK official control laboratories (EURL, Quasimeme, IPI), and these were circulated and discussed at UK network meetings in 2019.

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 has assisted 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 2019 a total of 20 laboratories participated, 18 of which were NRLs, and 2 OC laboratories from third countries.
- PT exercises for PSP have been organised since 2004. The exercise covered HPLC-FLD methods only (biological methods are no longer permitted for official control). The number of participants in 2019 was 18, of which 17 were NRLs, and 1 official control (OC) laboratory from a third country also submitting results. Seventeen sets of results were submitted using the pre-column method AOAC.OMA 2005.06, with 1 using the post-column method, AOAC.OMA.2011.02. The PT scheme consisted of samples derived from naturally contaminated mussels or clam, covering both the *Alexandrium* Spp. profile or the more complex *Gymnodinium catenatum* profile.

- 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 2019, there were 21 participants, of which 19 were NRLs, and 2 OC laboratories from third countries. The PT scheme consisted of samples derived from naturally contaminated scallops.

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 the final agreed annual report will be made available on the UK-NRL website (full PT reports generated by the EURL are confidential). Results obtained by the UK-NRL were circulated to the UK NRL Network and discussed at the Network meeting held in December 2019.

Both UK OCLs participated in the Quasimeme 2019 Proficiency test programmes for ASP/PSP/LTs and in the International Phytoplankton Inter-comparison (IPI) taxonomic quiz. The UK-NRL also requests additional sample materials for the EURL-MB PT scheme tests to allow assessment of other UK OCL lab performance. These additional samples were sent to Cefas and results of the analyses submitted to the UK-NRL for assessment of performance (z-scores retrospectively calculated).

In addition to participating in PT exercises for the regulated marine biotoxins listed in Commission Regulation 853/2004, in 2019-20 the NRL also participated in a Quasimeme developmental exercise for the emerging toxin TTX. This permitted assessment of the performance of an in house method for this toxin, for which there are currently no specific regulatory limits.

A summary of all PT results is collated by the NRL, circulated to the NRL network, and performance discussed at the Network Meetings. A full summary of PT results obtained in 2019 by the UK-NRL are summarised in Appendix 1 (TTX developmental exercise results for information only).

A performance '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:

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

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 2019 for lipophilic toxins, the National Reference Laboratory reported results for individual toxins with 99% of Z scores less than 2 (satisfactory), 1% of the Z scores less than 3 (questionable) and 0% of the Z scores as  $>3$ . Performance in the EURL PT and both Quasimeme rounds was excellent.

For PSP, 83.8% of individual toxin Z scores were less than 2, 13.5% between 2 to 3, and 2.7% above 3 (1 result out of 37). Individual toxin results  $>3$  were investigated and reviewed as part of the laboratories internal quality procedures. Overall, results for Quasimeme across both PT rounds were acceptable. Questionable or the single unsatisfactory indicative z-score are associated with the EURL PT, where performance scores have been calculated as Z' scores (higher than predicted RSDs across overall NRL toxin datasets received). Overall, results for the UK-NRL in the EURL PT are deemed satisfactory by the EURL with no performance follow up required, and no impact on reported results. It is noted that the application of recovery correction to participant results by the EURL (based on laboratories original validation data - due to lack of reference material for routine IQC spiking) is not in keeping with the normal reporting of results by laboratories.

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

For TTX, satisfactory z-scores were returned in tissue samples or extracts received (Mussel or Oyster).

Performance in the International Phytoplankton Inter-comparison (Bequalm) taxonomic quiz was acceptable. All analysts passed with Analyst 1 achieving a proficient classification (100% overall score), analyst 2 a proficient score (94%), and analyst 3 a good score classification (89%).

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

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

Draft versions of the EURL harmonised best practice guide to monitoring of toxin-producing phytoplankton were circulated to the CA and OCLs. These were discussed with FSANI in May 2019, and at the UK-NRL network meeting in June 2019. The final version of these documents was requested in January 2020 from the EURL. These were circulated to the CA when received in May 2020.

The EURL has requested input from member states to assist with the drafting of a 'Guideline document for harmonisation of biotoxin monitoring'. The UKNRL has forwarded the nominated contact details from the competent authority to the EURL (in addition to the NRL) for the proposed expert working group. This e-WG is anticipated to commence in 2020-21.

The UK-NRL did not attend the annual EURL/NRL workshop in 2019 as per UK government restrictions and under FSA advice. The performance of the UK-NRL in the 2019 EURL PT schemes was discussed at the UK-NRL network meeting in December 2019.

The UK-NRL provided scientific and technical support to the competent authority and OCLs throughout the year on request. This included providing comment on proposed updates to EU legislation, completing returns to assist the CA with EU exit issues/ Brexit contingency plans, updating NRL SOPs, advising/reviewing scientific publications or validation reports.

The NRL assessed the inclusion of Pinnatoxin-G (PnTX-G) within the current LT official method used for official control. The UK-NRL informed OCLs in 2019-20 that this toxin could be included within the current LT method, and this would assist in gathering data on UK Pinnatoxin occurrence, using PnTX-G as a marker, should the CA request this. OCLs were asked to contact



the CA for approval, prior to commencing monitoring. There is a lack of EU and indeed UK occurrence data for this emerging toxin class.

In 2019-20 the NRL participated in a Quasimeme developmental exercise for the emerging toxin TTX, to permit assessment of the performance of an in house method for this, as yet, unregulated toxin.

### **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 2019 Proficiency Test Summaries**

**AFBI EURL 2019**

Sample ID	Sample description	Assigned value	Reported value	Units	z-score
EURLMB/19/A/01	Mussel homogenate	7.3	7.2	mg/kg	-0.1
EURLMB/19/A/02	Scallop homogenate	76.5	77.9	mg/kg	0.1

**AFBI Quasimeme Round 2019.1**

Sample No	Sample ID	Sample description	Determinand	Assigned value	Reported value	Units	z-score
2019.1	QST260BT	King scallop homogenate	Total Domoic + Epi DA	27	25.98	mg/kg	-0.3
2019.1	QST261BT	Queen scallop adductor	Total Domoic + Epi DA	28.1	29.48	mg/kg	0.4
2019.1	QST262BT	Pacific oyster	Total Domoic + Epi DA	8.43	8.33	mg/kg	-0.1

**AFBI Quasimeme Round 2019.2**

Sample No	Sample ID	Sample description	Determinand	Assigned value	Reported value	Units	z-score
2019.2	QST269BT	Oyster homogenate	Total Domoic + Epi DA	17.1	18.7	mg/kg	0.7
2019.2	QST270BT	Scallop homogenate	Total Domoic + Epi DA	28.3	32.2	mg/kg	1.1
2019.2	QST271BT	Mussel homogenate	Total Domoic + Epi DA	52.1	45.9	mg/kg	-0.9

## PSP 2019 Proficiency Test Summaries

### AFBI EURL 2019

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Recovery corrected result	Units	Z'-Score
EURLMB/19/P/01	HPLC	Clam homogenate	dcGTX2&3	82.3	77.4	136	ugSTX2HCL equiv/Kg	2.50
EURLMB/19/P/01	HPLC	Clam homogenate	dcSTX	642.8	527	909	ugSTX2HCL equiv/Kg	2.20
EURLMB/19/P/01	HPLC	Clam homogenate	GTX2&3	137.4	166	252	ugSTX2HCL equiv/Kg	3.50
EURLMB/19/P/01	HPLC	Clam homogenate	GTX5 (B1)	3.43			ugSTX2HCL equiv/Kg	
EURLMB/19/P/01	HPLC	Clam homogenate	STX	24.37	14.9	17.9	ugSTX2HCL equiv/Kg	-1.1
EURLMB/19/P/01	HPLC	Clam homogenate	dcNeo	58.35			ugSTX2HCL equiv/Kg	
EURLMB/19/P/01	HPLC	Clam homogenate	GTX6 (B)	25.8	31.6	31.6	ugSTX2HCL equiv/Kg	0.9
<b>EURLMB/19/P/01</b>	<b>HPLC</b>	Clam homogenate	<b>Total STX</b>	<b>967</b>	<b>817</b>	<b>1346</b>	<b>ugSTX2HCL equiv/Kg</b>	<b>2.4</b>
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value		Units	Z-Score
EURLMB/19/P/02	HPLC	Mussel homogenate	C1&C2	150	153	232	ugSTX2HCL equiv/Kg	2.40
EURLMB/19/P/02	HPLC	Mussel homogenate	dcSTX	274	211	365	ugSTX2HCL equiv/Kg	1.60
EURLMB/19/P/02	HPLC	Mussel homogenate	GTX2&3	53.0	51.8	78.5	ugSTX2HCL equiv/Kg	1.90
EURLMB/19/P/02	HPLC	Mussel homogenate	GTX5 (B1)	194	95	233	ugSTX2HCL equiv/Kg	0.9
EURLMB/19/P/02	HPLC	Mussel homogenate	dcNeo	36.7			ugSTX2HCL equiv/Kg	
EURLMB/19/P/02	HPLC	Mussel homogenate	C3&C4	59.7	81.1	81	ugSTX2HCL equiv/Kg	1.3
EURLMB/19/P/02	HPLC	Mussel homogenate	GTX6 (B2)	148	174	174	ugSTX2HCL equiv/Kg	0.7
<b>EURLMB/19/P/02</b>	<b>HPLC</b>	<b>Mussel homogenate</b>	<b>Total STX</b>	<b>1026</b>	<b>1003</b>	<b>1312</b>	<b>ugSTX2HCL equiv/Kg</b>	<b>1.6</b>
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value		Units	Z-Score
EURLMB/19/P/03	HPLC	Shellfish	GTX2&3	358	338	512	ugSTX2HCL equiv/Kg	2.20
EURLMB/19/P/03	HPLC	Shellfish	STX	254	229	276	ugSTX2HCL equiv/Kg	0.5
<b>EURLMB/19/P/03</b>	<b>HPLC</b>	<b>Shellfish</b>	<b>Total STX</b>	<b>645</b>	<b>567</b>	<b>788</b>	<b>ugSTX2HCL equiv/Kg</b>	<b>1.2</b>

**z' scores: Recovery corrected evaluations are available only. (Statutory monitoring samples are not reported as recovery corrected).**

**AFBI Quasimeme Round 2019.1**

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST266BT	HPLC	Mussel	GTX-2,3	3.21	3.04	µmol/kg	-0.4
QST266BT	HPLC	Mussel	STX	1.74	1.568	µmol/kg	-0.6
<b>QST266BT</b>	<b>HPLC</b>	<b>Mussel</b>	<b>Total toxicity</b>	<b>1334</b>	<b>1263</b>	<b>µgSTXdiHCleq./kg</b>	<b>-0.4</b>
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST267BT	HPLC	Mussel	GTX-2,3	2.94	2.768	µmol/kg	-0.4
QST267BT	HPLC	Mussel	STX	1.41	1.296	µmol/kg	-0.5
<b>QST267BT</b>	<b>HPLC</b>	<b>Mussel</b>	<b>Total toxicity</b>	<b>1156</b>	<b>1101</b>	<b>µgSTXdiHCleq./kg</b>	<b>-0.4</b>
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST268BT	HPLC	Mussel	dc-Neo	0.215	0.256	µmol/kg	0.5
QST268BT	HPLC	Mussel	dc-STX	1.87	1.384	µmol/kg	-1.5
QST268BT	HPLC	Mussel	GTX-2,3	0.072	0.072	µmol/kg	0
QST268BT	HPLC	Mussel	STX	0.0841	0.064	µmol/kg	-0.3
<b>QST268BT</b>	<b>HPLC</b>	<b>Mussel</b>	<b>Total toxicity</b>	<b>762</b>	<b>592</b>	<b>µgSTXdiHCleq./kg</b>	<b>-1.5</b>

**AFBI Quasimeme Round 2019.2**

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST275BT	HPLC	Mussel	GTX-2,3	5.02	4.56	µmol/kg	-0.5
QST275BT	HPLC	Mussel	STX	3.4	3.24	µmol/kg	-0.3
<b>QST275BT</b>	<b>HPLC</b>	<b>Mussel</b>	<b>Total toxicity</b>	<b>2327</b>	<b>2224</b>	<b>µgSTXdiHCleq./kg</b>	<b>-0.3</b>
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST276BT	HPLC	Mussel	dcSTX	0.506	0.44	µmol/kg	-0.6
QST276BT	HPLC	Mussel	GTX-2,3	5.35	4.98	µmol/kg	-0.4
QST276BT	HPLC	Mussel	STX	1.27	1.11	µmol/kg	-0.7
<b>QST276BT</b>	<b>HPLC</b>	<b>Mussel</b>	<b>Total toxicity</b>	<b>1768</b>	<b>1689</b>	<b>µgSTXdiHCleq./kg</b>	<b>-0.3</b>
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST277BT	HPLC	Mussel	GTX-2,3	6.85	6.26	µmol/kg	-0.5
QST277BT	HPLC	Mussel	STX	1.88	1.66	µmol/kg	-0.7
<b>QST277BT</b>	<b>HPLC</b>	<b>Mussel</b>	<b>Total toxicity</b>	<b>2163</b>	<b>2012</b>	<b>µgSTXdiHCleq./kg</b>	<b>-0.5</b>

## Lipophilic 2019 Proficiency Test Summaries

### AFBI EURL 2019

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
EURLMB/19/L/01	LC-MS/MS	Mussel (homogenate)	Free OA	201	150.0	µg/kg	-1.2
EURLMB/19/L/01	LC-MS/MS	Mussel	Free DTX-2	165	123.0	µg/kg	-1.1
EURLMB/19/L/01	LC-MS/MS	Mussel	Total OA	1419	1266.0	µg/kg	-0.7
EURLMB/19/L/01	LC-MS/MS	Mussel	Total DTX-2	330	283.0	µg/kg	-0.8
<b>EURLMB/19/L/01</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>OA group (total)</b>	<b>1660</b>	<b>1435.8</b>	<b>µg OA eq/kg</b>	<b>-0.8</b>
EURLMB/19/L/01	LC-MS/MS	Mussel	YTX	0.20	0.20	mg/kg	0.0
EURLMB/19/L/01	LC-MS/MS	Mussel	Homo-YTX	2.10	1.90	mg/kg	-0.5
EURLMB/19/L/01	LC-MS/MS	Mussel	45-OH-YTX	0.30	0.20	mg/kg	-0.8
EURLMB/19/L/01	LC-MS/MS	Mussel	45-OH-Homo-YTX	1.20	1.50	mg/kg	1.4
<b>EURLMB/19/L/01</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>YTX group (total)</b>	<b>3.00</b>	<b>3.00</b>	<b>mg YTX eq/kg</b>	<b>0.1</b>
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	mg YTX eq/kg	Z-Score
EURLMB/19/L/02	LC-MS/MS	Mussel (homogenate)	Free OA µg/kg	44	27.8	Free OA	-1.6
EURLMB/19/L/02	LC-MS/MS	Mussel	Total OA µg/kg	81	47.2	Total OA	-1.8
<b>EURLMB/19/L/02</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>OA group (total)</b>	<b>82.1</b>	<b>47.2</b>	<b>µg OA eq/kg</b>	<b>-1.9</b>
EURLMB/19/L/02	LC-MS/MS	Mussel	AZA1 µg/kg	262	253.0	µg/kg	-0.2
EURLMB/19/L/02	LC-MS/MS	Mussel	AZA2 µg/kg	74	74.1	µg/kg	0
EURLMB/19/L/02	LC-MS/MS	Mussel	AZA3 µg/kg	55.20	46.4	µg/kg	-0.7
<b>EURLMB/19/L/02</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>AZA group (total)</b>	<b>477</b>	<b>451.3</b>	<b>µg AZA eq./kg</b>	<b>-0.3</b>
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
EURLMB/19/L/03	LC-MS/MS	Mussel (homogenate)	Free OA µg/kg	21	14.8	µg/kg	-1.2

EURLMB/19/L/03	LC-MS/MS	Mussel	Free DTX-1 µg/kg	225	229.0	µg/kg	0.1
EURLMB/19/L/03	LC-MS/MS	Mussel	Total OA µg/kg	49	23.4	µg/kg	-2.1
EURLMB/19/L/03	LC-MS/MS	Mussel	Total DTX-1 µg/kg	788	854.0	µg/kg	0.4
<b>EURLMB/19/L/03</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>µg Total OA eq/kg</b>	<b>905</b>	<b>877.4</b>	<b>µg OA eq/kg</b>	<b>-0.2</b>

### AFBI Quasimeme Round 2019.1

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST263BT	LC-MS/MS	Mussel (homogenate)	AZA-1	738	639	µg/kg	-1.1
QST263BT	LC-MS/MS	Mussel	AZA-2	210	217	µg/kg	0.3
QST263BT	LC-MS/MS	Mussel	AZA-3	125	110	µg/kg	-0.9
<b>QST263BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total AZA group</b>	<b>1304</b>	<b>1183</b>	<b>µg AZA eq./kg</b>	<b>-0.7</b>
QST263BT	LC-MS/MS	Mussel	Free DTX1	85.8	71.2	µg/kg	-1.3
QST263BT	LC-MS/MS	Mussel	Free DTX2	567	520	µg/kg	-0.6
QST263BT	LC-MS/MS	Mussel	Free OA	149	134	µg/kg	-0.8
<b>QST263BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Free OA group</b>	<b>563</b>	<b>517</b>	<b>µg OA eq./kg</b>	<b>-0.6</b>
QST263BT	LC-MS/MS	Mussel	Total DTX1	113	101	µg/kg	-0.8
QST263BT	LC-MS/MS	Mussel	Total DTX2	787	690	µg/kg	-1
QST263BT	LC-MS/MS	Mussel	Total OA	339	289	µg/kg	-1.1
<b>QST263BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA group</b>	<b>924</b>	<b>804</b>	<b>µg/kg</b>	<b>-1</b>
QST263BT	LC-MS/MS	Mussel	PTX-2	3.62	<LOQ	µg/kg	N/A
<b>QST263BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA+PTX group</b>	<b>904</b>	<b>804</b>	<b>µg OA eq./kg</b>	<b>-0.9</b>
QST263BT	LC-MS/MS	Mussel	YTX	0.227	0.2584	µg/kg	0.8
QST263BT	LC-MS/MS	Mussel	homo-YTX	1.1	1.0385	µg/kg	-0.4
QST263BT	LC-MS/MS	Mussel	45-OH-YTX	0.118	0.1524	µg/kg	1.2
QST263BT	LC-MS/MS	Mussel	45-OH-homo-YTX	0.513	0.6006	µg/kg	1.1
<b>QST263BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total-YTX group</b>	<b>1.76</b>	<b>1.7496</b>	<b>µg OA eq./kg</b>	<b>0</b>

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST246BT	LC-MS/MS	Mussel (extract)	AZA-1	97	87.3	µg/kg	-0.8
QST246BT	LC-MS/MS	Mussel	AZA-2	20.5	22.8	µg/kg	0.8
QST246BT	LC-MS/MS	Mussel	AZA-3	36.8	32.3	µg/kg	-0.9
<b>QST246BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total AZA group</b>	<b>187</b>	<b>173.6</b>	<b>µg AZA eq./kg</b>	<b>-0.5</b>
QST246BT	LC-MS/MS	Mussel	Free DTX2	3.88	3.6	µg/kg	-0.5
QST246BT	LC-MS/MS	Mussel	Free OA	38.7	37.9	µg/kg	-0.2
<b>QST246BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Free OA group</b>	<b>41.3</b>	<b>40</b>	<b>µg OA eq./kg</b>	<b>-0.2</b>
QST246BT	LC-MS/MS	Mussel	Total DTX2	4.1	3.4	µg/kg	-1.2
QST246BT	LC-MS/MS	Mussel	Total OA	46	47.7	µg/kg	0.3
<b>QST246BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA group</b>	<b>48.4</b>	<b>49.8</b>	<b>µg OA eq./kg</b>	<b>0.2</b>
<b>QST246BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA+PTX group</b>	<b>47.9</b>	<b>49.8</b>	<b>µg OA eq./kg</b>	<b>0.3</b>
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST265BT	LC-MS/MS	Mussel (homogenate)	AZA-1	422	367	µg/kg	-1
QST265BT	LC-MS/MS	Mussel	AZA-2	119	130	µg/kg	0.7
QST265BT	LC-MS/MS	Mussel	AZA-3	84.7	72.9	µg/kg	-1.1
<b>QST265BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total AZA group</b>	<b>767</b>	<b>703</b>	<b>µg AZA eq./kg</b>	<b>-0.7</b>
QST265BT	LC-MS/MS	Mussel	Free DTX2	101	82.8	µg/kg	-1.4
QST265BT	LC-MS/MS	Mussel	Free OA	45.9	35.8	µg/kg	-1.7
<b>QST265BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Free OA group</b>	<b>107</b>	<b>85.5</b>	<b>µg OA eq./kg</b>	<b>-1.5</b>
QST265BT	LC-MS/MS	Mussel	Total DTX2	146	119	µg/kg	-1.4
QST265BT	LC-MS/MS	Mussel	Total OA	99.1	83	µg/kg	-1.2
<b>QST265BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA group</b>	<b>186</b>	<b>154.4</b>	<b>µg OA eq./kg</b>	<b>-1.3</b>
<b>QST265BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA+PTX group</b>	<b>186</b>	<b>154,4</b>	<b>µg OA eq./kg</b>	<b>-1.3</b>



AFBI Quasimeme Round 2019.2

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST272BT	LC-MS/MS	Mussel (homogenate)	Free DTX2	407	392.4	µg/kg	-0.3
QST272BT	LC-MS/MS	Mussel	Free OA	153	142.6	µg/kg	-0.5
<b>QST272BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Free OA group</b>	<b>404</b>	<b>378.1</b>	<b>µg OA eq./kg</b>	<b>-0.5</b>
QST272BT	LC-MS/MS	Mussel	Total DTX2	706	693.7	µg/kg	-0.1
QST272BT	LC-MS/MS	Mussel	Total OA	484	475.2	µg/kg	-0.1
<b>QST272BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA group</b>	<b>920</b>	<b>891.4</b>	<b>µg OA eq./kg</b>	<b>-0.2</b>
<b>QST272BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA+PTX group</b>	<b>924</b>	<b>891.4</b>	<b>µg OA eq./kg</b>	<b>-0.3</b>
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST273BT	LC-MS/MS	Mussel (extract)	AZA-1	13.6	10.7	µg/kg	-1.6
QST273BT	LC-MS/MS	Mussel	AZA-2	3.87	3.5	µg/kg	-0.7
QST273BT	LC-MS/MS	Mussel	AZA-3	3.94	3.9	µg/kg	-0.1
<b>QST273BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total AZA group</b>	<b>25.4</b>	<b>22.5</b>	<b>µg AZA eq./kg</b>	<b>-0.9</b>
QST273BT	LC-MS/MS	Mussel	Free DTX2	60.8	53.4	µg/kg	-0.9
QST273BT	LC-MS/MS	Mussel	Free OA	11	10.5	µg/kg	-0.3
<b>QST273BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Free OA group</b>	<b>49.4</b>	<b>42.5</b>	<b>µg OA eq./kg</b>	<b>-1.1</b>
QST273BT	LC-MS/MS	Mussel	Total DTX2	74.2	68.8	µg/kg	-0.5
QST273BT	LC-MS/MS	Mussel	Total OA	18.7	16.7	µg/kg	-0.8
<b>QST273BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA group</b>	<b>63</b>	<b>57.9</b>	<b>µg OA eq./kg</b>	<b>-0.6</b>
<b>QST273BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA+PTX group</b>	<b>64.4</b>	<b>57.9</b>	<b>µg OA eq./kg</b>	<b>-0.8</b>
QST273BT	LC-MS/MS	Mussel	YTX	0.0311	0.0308	mg/kg	0
QST273BT	LC-MS/MS	Mussel	homo-YTX	0.302	0.3277	mg/kg	0.5
QST273BT	LC-MS/MS	Mussel	45-OH-YTX	0.023	0.0245	mg/kg	0.1
QST273BT	LC-MS/MS	Mussel	45-OH-homo-YTX	0.174	0.1962	mg/kg	0.6
<b>QST273BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>YTX group</b>	<b>0.432</b>	<b>0.4811</b>	<b>mg YTX eq./kg</b>	<b>0.7</b>

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST274BT	LC-MS/MS	Mussel (homogenate)	AZA-1	218	180.8	µg/kg	-1.3
QST274BT	LC-MS/MS	Mussel	AZA-2	59	59.2	µg/kg	0
QST274BT	LC-MS/MS	Mussel	AZA-3	49.6	50.9	µg/kg	0.2
<b>QST274BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total AZA group</b>	<b>392</b>	<b>358.5</b>	<b>µg AZA eq./kg</b>	<b>-0.7</b>
QST274BT	LC-MS/MS	Mussel	Free DTX2	297	284.7	µg/kg	-0.3
QST274BT	LC-MS/MS	Mussel	Free OA	67.5	57.9	µg/kg	-1.1
<b>QST274BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Free OA group</b>	<b>250</b>	<b>228.7</b>	<b>µg OA eq./kg</b>	<b>-0.6</b>
QST274BT	LC-MS/MS	Mussel	Total DTX2	439	368.6	µg/kg	-1.2
QST274BT	LC-MS/MS	Mussel	Total OA	156	125.5	µg/kg	-1.5
<b>QST274BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA group</b>	<b>435</b>	<b>346.7</b>	<b>µg OA eq./kg</b>	<b>-1.6</b>
<b>QST274BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA+PTX group</b>	<b>433</b>	<b>346.7</b>	<b>µg OA eq./kg</b>	<b>-1.5</b>

### Tetrodotoxin 2019 development exercise (Quasimeme)

Sample ID	Sample description	Assigned value	Reported value	Units	z-score
QTT003BT	Negative Oyster	-	<10	µg/kg	-
QTT004BT	Mussel homogenate	39	51.2	µg/kg	1.3
QTT005BT	Oyster homogenate	121	169.2	µg/kg	1.8
QTT001SS	Solvent standard	4.65	24.67	µg/L	28.8
QTT002EX	Mussel Extract	#	3.172	µg/L	#

# not given - study organiser indicated stability issue with this extract

**AFBI - International Phytoplankton Inter-comparison (IPI) 2019**  
(Phytoplankton Proficiency Testing Scheme)

<b>IPI Taxonomy Quiz 2019</b>	<b>Analyst 1</b>	<b>Analyst 2</b>	<b>Analyst 3</b>
<b>Overall summary result &gt;</b>	100% (proficient)	93.37% (proficient)	89.06% (good)
<b>Species ID (individual z-scores &gt;)</b>	<b>Z-score</b>	<b>Z-score</b>	<b>Z-score</b>
Akashiwo sanguinea	0.7	1.1	0.7
Prorocentrum micans	0.1	0.3	0.7
Pseudo-nitzschia seriata gp	-0.2	0.0	0.4
Azadinium spinosum	0.7	1.1	0.3
Chaetoceros danicus	0.3	0.9	-0.3
Chaetoceros curvisetus	0.0	0.2	-0.7
Gonyaulax spinifera	0.1	0.5	0.4
Corethron hystris	0.1	0.3	1.9
Thalassiosira tenera	0.6	0.3	0.0
Heterosigma akashiwo	0.7	0.6	1.2
<b>The IPI quiz assesses both enumeration and individual species identification. An overall summary result is also given for each analyst as follows: test score &gt;90% is deemed proficient, &gt;70% is deemed a pass.</b>			

## Appendix 2



# Agri-Food & Biosciences Institute

## VETERINARY SCIENCES DIVISION

### Chemical Surveillance Branch

## Work Programme UK National Reference Laboratory For Marine Biotoxins

**2019-20**

### **Contacts:**

#### **Dermot Faulkner**

Chemical and Immunodiagnostic  
Sciences Branch, VSD

Tel 02890 525617

Email [dermot.faulkner@afbini.gov.uk](mailto:dermot.faulkner@afbini.gov.uk)

#### **Steven Crooks**

Head of Chemical and  
Immunodiagnostic Sciences Branch

Tel 02890 525 625

Email [steven.crooks@afbini.gov.uk](mailto:steven.crooks@afbini.gov.uk)

## **Glossary**

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

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

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

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

**FSA:** Food Standards Agency

**HILIC:** Hydrophilic Liquid Interaction Chromatography

**IPI:** International Phytoplankton Inter-comparison exercise

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

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

**NRL:** National Reference Laboratory

**OCLs:** Official Control Laboratories

**PTs:** Proficiency tests

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

**TEF:** Toxic Equivalence Factor

**TTX:** Tetrodotoxin

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

### **National Reference Laboratory Annual Report**

The annual report for 2019-2020 will be drafted and submitted to the Competent Authority for comment in May 2020.

### **NRL Standard Operating Procedures**

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

### **Proficiency tests 2019**

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.

In 2018, the UK-NRL was successful in requesting additional sample materials to help with inter-comparison at internal UK level, on the basis that this was necessary to fulfill NRL requirements and duties as set out in Article 101 of Regulation (EC) 2017/625. This allowed additional performance checking of OCLs by the UK-NRL in 2018-19, with results of these analyses being sent to the UK-NRL for assessment of OCL performance.

In April 2019, the UK-NRL requested additional materials for the EURL-MB PT scheme to help with inter-comparison at internal UK level. These were received in May 2019 and forwarded to Cefas for analysis of ASP, LTs and PSP group toxins. Results will be submitted to the UK-NRL for retrospective assessment of OCL performance, pending receipt of final EURL PT reports issued to NRLs.

## **Meetings**

*EURL – NRLs Workshop 2019:* Baiona, Spain (October 2019) by EURL.

*EURL technical working Group:* A single technical working group has been proposed to deal with analytical toxin methods or emerging issues. This may be reconvened in 2019-20, to consider new data on TEFs, implementation and application of new technology, modifications to methods, emerging toxin issues or measurement uncertainty. The NRL will continue to participate in this working group as required.

*EURL working Group on Phytoplankton:* The NRL will continue to participate and be represented at meetings of the working Group in 2019-20. 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 ‘Guideline document for marine biotoxins’:* The NRL will participate and 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 2019. The first will be in June 2019 and will be held at FSS in Aberdeen. The second will be held at the beginning of November 2019 at FSA headquarters, London.

## **Additional work activities 2019-20**

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

The UK-NRL will participate in a proficiency test assessment exercise for TTX testing by HILIC-MS/MS (Quasimeme PT 2019-20)

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 assess the potential for extending the scope of the routine Lipophilic marine toxins method (LTs) applied in both OCLs in the UK, to include Pinnatoxin screening (based on parent analogue PnTX-G).