



Agri-Food & Biosciences Institute

**VETERINARY SCIENCES DIVISION**

**Chemical Surveillance Branch**

Annual Report  
UK National Reference Laboratory  
For Marine Biotoxins

**1<sup>st</sup> April 2020 – 31<sup>st</sup> March 2021**

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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 between 1<sup>st</sup> April 2020 and 31<sup>st</sup> March 2021. It 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, Cefas and SAMs in fulfilling its duties. A summary of the 2020-21 UK-NRL work programme is provided in Appendix 1.

For the purposes of Retained 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 2020-2021**

The table below provides a summary of meetings attended as part of the NRL activities that took place during 2020-2021 to date.

#### ***Meetings Attended 2020-2021***

<b>Date</b>	<b>Venue</b>	<b>Subject</b>
12 <sup>th</sup> June 2020	TC	36 <sup>th</sup> UK NRL Network Meeting
18 <sup>th</sup> August 2020	TC	FSA/FSS/UKNRL meeting (UK review meeting -biotoxin guide e-WG)
18-19 <sup>th</sup> November 2020	VC	EURL-MB/NRL Annual workshop
3 <sup>rd</sup> December 2020	TC/VC	37 <sup>th</sup> UK NRL Network Meeting

**TC – Teleconference**

**VC – Videoconference**

### **Collaboration with the EURL-MB**

In 2020-2021, AFBI as UK-NRL continued to participate in the EURL-MB co-ordinated biotoxin guide e-working group. This working group was established with the aim of producing a harmonised best practice guide to monitoring of marine biotoxins in production areas for live bivalve molluscs. Work commenced in 2020, with chapter 2 of the guide (sampling plans) circulated electronically from the EURL-MB to e-WG members. The UK-NRL and CA met in August (Tele Conference) to discuss chapter 2 of the guide and the UK-NRL co-ordinated all UK input/responses, submitting these to the EURL-MB.

The UKNRL, at the request of the EURL, took part in a technical review of the EURL SOP for PSP toxin analysis detailing a semi-quantitative approach (based on AOAC.2005.06), and provided comments to the EURL.

AFBI has previously confirmed to the EURL its intention to participate in any ongoing co-ordinated activities to assess application of LC-MS/MS methods for the detection of the emerging toxin Tetrodotoxin (TTX). No proficiency test (PT) exercise for TTX was conducted in 2020-21 by the EURL, however AFBI did participate in a Quasimeme TTX PT exercise for this toxin in January 2021 (as indicated in the 2020/21 work programme). This assisted in evaluation of the performance of an in-house method in 2020-21 with performance summary results listed in Appendix 1 of this report. The UK-NRL achieved optimal performance and achieved 100% satisfactory z-scores across all three samples sent for this PT 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). All meetings took place remotely due to COVID restrictions. The 36<sup>th</sup> meeting of the UKNRL-Network group was hosted by the UK-NRLMB by TC on the 12<sup>th</sup> June 2020, with the 37<sup>th</sup> meeting hosted by TC/VC on the 3<sup>rd</sup> Dec 2020.

In 2020, the UK-NRL requested for the inclusion of Cefas in EURL-MB proficiency testing (PT) exercises, to permit the UKNRL to fulfil its obligations under Regulation (EC) 2017/625. However due to COVID restrictions and the resulting reduced laboratory capacity, the EURL was unable to provide additional test materials for laboratories outside of the designated EU-NRLs. On this occasion the UK-NRL provided shellfish extracts for analysis to Cefas, with a request for results to be submitted after the closure of the EURL test submission deadline (22<sup>nd</sup> September 2020). A reporting sheet was provided by the UK-NRL for reporting of test results.

The UK-NRL 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 2020. OCLs provided updates on UKAS audits at the network meetings. All OCLs demonstrated ongoing competence within their remits in relation to official control testing with no follow up actions required.

The UK-NRL took into consideration responses received from official control laboratory experts in the review of a number of UKNRL SOPs/documents, which were circulated within the NRL group. The UK-NRL also circulated draft e-WG documents and upcoming proposed legislative updates to the NRL group.

The UK-NRL, obtained additional positive PSP incurred shellfish materials (from Portugal-NRL) through the EU-NRL network in support of the UK OCL programme.

### **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. The UK-NRL registered for the 2020 EURL PT schemes and samples were received in July 2020 (delayed from May). UK-NRL results were reported before the 22nd September deadline.

Final reports on the EURL-MB proficiency tests were circulated in November to NRLs 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 also circulated to the UK NRL Network and discussed at the Network meeting held in December 2020. The UK-NRL had no follow up actions from the EURL & successfully demonstrated competence in all EURL PTs.

Both UK OCLs participated in the Quasimeme 2020 Proficiency test programmes for ASP/PSP/LTs. The International Phytoplankton Inter-comparison (IPI) taxonomic quiz was postponed in 2020 due to COVID and is the only available scheme available to NRLs and OCLs. The UK-NRL also requests additional sample materials for the EURL-MB PT scheme tests to allow assessment of other UK OCL lab performance. On this occasion sample extracts 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 2020-21 the NRL also participated in a Quasimeme PT 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, but where an EFSA scientific opinion has determined that a concentration below 44µg/kg was considered not to result in adverse effects in humans.

A summary of all PT results was collated by the NRL, circulated to the NRL network, and performance discussed at the Network Meetings in 2020. A full summary of PT results obtained in 2020 by the UK-NRL are summarised in Appendix 1.

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:

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

$|Z| < 2$  Satisfactory performance

$2 < |Z| < 3$  Questionable performance

$|Z| > 3$  Unsatisfactory performance

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

The UK-NRL attended the annual EURL/NRL workshop by video-conference in 2020 (after FSA advised this was permitted), and presented on some of the work of the UK-NRL. The UK-NRL gave an update on this meeting at the UK network meeting in December 2020 and a summary was included within the minutes of the network meeting.

A draft version of chapter 2 (sampling plans) of the EURL harmonised best practice guide to biotoxin monitoring of toxin-producing phytoplankton was circulated to e-WG members in 2020, which included the UK CA and UK-NRL. The UK-NRL and CA met in August (TC) to discuss chapter 2 of the guide and the UK-NRL co-ordinated all UK input/responses, submitting these to the EURL-MB.

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 queries, updating NRL SOPs, advising/reviewing scientific publications or reports.

In 2020-21 the NRL continued participating in a Quasimeme PT exercise for the emerging toxin TTX, to permit assessment of the performance of an in house method for this, as yet, unregulated toxin. In support of scientific development and advancement of the capability of the UK-NRL network in monitoring for emerging toxins, the UK-NRL undertook research activities and collaborations as detailed below.

## **Research activities and collaborations:**

### ***Pinnatoxins:***

The UK-NRL continued a survey of NI shellfish for Pinnatoxins in 2020 (using the marker PnTX-G) by LC-MS/MS (routine & archive samples), through extension of the scope of the official method for LTs. The Pinnatoxins are a recognised emerging toxin group (non-regulated at present). A summary of the work and findings was presented to FSA in 2020. The UKNRL recommended to the CA that both OCLs should include PnTX-G in the current LT method toxin suite for surveillance/horizon scanning of emerging toxins, even if not accredited at present. This would support additional surveillance for this toxin class across the UK, with minimum or no additional cost to the monitoring programme. Further work of the UK-NRL is ongoing to extend the UKAS scope of the LT method to include PnTX-G.



### ***Tetrodotoxin (TTX)***

The UK-NRL participated in a TTX ring trial exercise in January 2021, co-ordinated by Cefas, to support ongoing research in methods of analysis for emerging toxins using LC-MS/MS techniques.

### **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 2020 Proficiency Test Summaries

#### AFBI EURL 2020

Sample ID	Sample description	Assigned value	Reported value	Units	z-score
EURLMB/20/A/01	Scallop homogenate	9.8	10.2	mg/kg	0.2
EURLMB/20/A/02	Scallop homogenate	16.7	12.6	mg/kg	-1.12

#### AFBI Quasimeme Round 2020.1

Sample No	Sample ID	Sample description	Determinand	Assigned value	Reported value	Units	z-score
2020.1	QST278BT	King scallop homogenate	Total Domoic + Epi DA	59.00	69.77	mg/kg	1.4
2020.1	QST279BT	King scallop adductor muscle	Total Domoic + Epi DA	27.30	32.26	mg/kg	1.4
2020.1	QST280BT	Razor clam	Total Domoic + Epi DA	24.70	26.40	mg/kg	0.5

#### AFBI Quasimeme Round 2020.2

Sample No	Sample ID	Sample description	Determinand	Assigned value	Reported value	Units	z-score
2020.2	QST287BT	Mussel homogenate	Total Domoic + Epi DA	42.70	48.86	mg/kg	1.1
2020.2	QST288BT	King scallop adductor muscle	Total Domoic + Epi DA	17.50	24.66	mg/kg	3.2
2020.2	QST289BT	Cockle homogenate	Total Domoic + Epi DA	17.90	21.06	mg/kg	1.4

## PSP 2020 Proficiency Test Summaries

### AFBI EURL 2020

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Recovery corrected result	Units	Z'-Score
EURLMB/20/P/01	HPLC	Mussel homogenate	GTX2&3	526	448	678	ugSTX2HCL equiv/Kg	1.48
EURLMB/20/P/01	HPLC	Mussel homogenate	STX	1085	850	1025	ugSTX2HCL equiv/Kg	-0.35
<b>EURLMB/20/P/01</b>	<b>HPLC</b>	<b>Mussel homogenate</b>	<b>Total STX</b>	<b>1659</b>	<b>1704</b>	<b>1704</b>	<b>ugSTX2HCL equiv/Kg</b>	<b>0.18</b>
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value		Units	Z-Score
EURLMB/20/P/02	HPLC	Mussel homogenate	dcGTX2&3	64.79	0	0	ugSTX2HCL equiv/Kg	
EURLMB/20/P/02	HPLC	Mussel homogenate	C1&C2	252	248	375	ugSTX2HCL equiv/Kg	2.03
EURLMB/20/P/02	HPLC	Mussel homogenate	dcSTX	145.0	96.8	167.0	ugSTX2HCL equiv/Kg	0.65
EURLMB/20/P/02	HPLC	Mussel homogenate	GTX5 (B1)	203	169	201	ugSTX2HCL equiv/Kg	-0.06
EURLMB/20/P/02	HPLC	Mussel homogenate	dcNeo	63.19	32.75	33	ugSTX2HCL equiv/Kg	-1.46
EURLMB/20/P/02	HPLC	Mussel homogenate	C3&C4	72.5	55.12	55	ugSTX2HCL equiv/Kg	-0.94
EURLMB/20/P/02	HPLC	Mussel homogenate	GTX6 (B2)	314	494	494	ugSTX2HCL equiv/Kg	2.43
<b>EURLMB/20/P/02</b>	<b>HPLC</b>	<b>Mussel homogenate</b>	<b>Total STX</b>	<b>1132</b>	<b>1325</b>	<b>1325</b>	<b>ugSTX2HCL equiv/Kg</b>	<b>1.02</b>

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

**AFBI Quasimeme Round 2020.1**

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST284BT	HPLC	Mussel	dcSTX	0.551	0.472	µmol/kg	-0.60
QST284BT	HPLC	Mussel	GTX-2,3	5.160	4.415	µmol/kg	-1.00
QST284BT	HPLC	Mussel	STX	1.290	0.997	µmol/kg	-1.30
<b>QST284BT</b>	<b>HPLC</b>	<b>Mussel</b>	<b>Total toxicity</b>	<b>1830</b>	<b>1533</b>	<b>µgSTXdiHCleq./kg</b>	<b>-1.20</b>
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST285BT	HPLC	Mussel	GTX-2,3	6.330	5.788	µmol/kg	-0.6
QST285BT	HPLC	Mussel	STX	2.030	1.578	µmol/kg	-1.4
<b>QST285BT</b>	<b>HPLC</b>	<b>Mussel</b>	<b>Total toxicity</b>	<b>2247</b>	<b>1880</b>	<b>µgSTXdiHCleq./kg</b>	<b>-1.3</b>
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST286BT	HPLC	Mussel	C1,C2	2.820	2.893	µmol/kg	0.2
QST286BT	HPLC	Mussel	dcSTX	0.342	0.269	µmol/kg	-0.7
QST286BT	HPLC	Mussel	GTX 5	4.080	3.621	µmol/kg	-0.8
QST286BT	HPLC	Mussel	GTX-2,3	0.894	0.756	µmol/kg	-0.8
QST286BT	HPLC	Mussel	STX	0.378	0.282	µmol/kg	-1.0
<b>QST286BT</b>	<b>HPLC</b>	<b>Mussel</b>	<b>Total toxicity</b>	<b>705</b>	<b>617</b>	<b>µgSTXdiHCleq./kg</b>	<b>-0.9</b>

**AFBI Quasimeme Round 2020.2**

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST293BT	HPLC	Mussel	GTX-2,3	2.940	2.444	µmol/kg	-1.10
QST293BT	HPLC	Mussel	STX	1.390	1.135	µmol/kg	-1.10
QST293BT	<b>HPLC</b>	<b>Mussel</b>	<b>Total toxicity</b>	<b>1161</b>	<b>968</b>	<b>µgSTXdiHCleq./kg</b>	<b>-1.30</b>
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST294BT	HPLC	Mussel	dcSTX	1.640	1.342	µmol/kg	-1.1
QST294BT	HPLC	Mussel	STX	0.088	0.064	µmol/kg	-0.4
QST294BT	<b>HPLC</b>	<b>Mussel</b>	<b>Total toxicity</b>	<b>645</b>	<b>524</b>	<b>µgSTXdiHCleq./kg</b>	<b>-1.3</b>

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST295BT	HPLC	Mussel	C1,C2	6.540	6.026	µmol/kg	-0.5
QST295BT	HPLC	Mussel	dcGTX-2,3	0.578	0.456	µmol/kg	-0.6
QST295BT	HPLC	Mussel	dcSTX	0.489	0.424	µmol/kg	-0.6
QST295BT	HPLC	Mussel	GTX 5	7.750	7.057	µmol/kg	-0.6
QST295BT	HPLC	Mussel	GTX-2,3	1.990	1.574	µmol/kg	-1.3
QST295BT	HPLC	Mussel	STX	0.673	0.540	µmol/kg	-1.0
QST295BT	<b>HPLC</b>	<b>Mussel</b>	<b>Total toxicity</b>	<b>1521</b>	<b>1265</b>	<b>µgSTXdiHCleq./kg</b>	<b>-1.3</b>

## Lipophilic 2020 Proficiency Test Summaries

### AFBI EURL 2020

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
EURLMB/20/L/01	LC-MS/MS	Mussel (homogenate)	Free OA	30	26.5	µg/kg	-0.5
EURLMB/20/L/01	LC-MS/MS	Mussel	Free DTX-1	121	121.3	µg/kg	0.0
EURLMB/20/L/01	LC-MS/MS	Mussel	Total OA	59	62.9	µg/kg	0.3
EURLMB/20/L/01	LC-MS/MS	Mussel	Total DTX-1	480	471.0	µg/kg	-0.1
<b>EURLMB/20/L/01</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA group</b>	<b>497</b>	<b>533.9</b>	<b>µg OA eq/kg</b>	<b>0.4</b>
EURLMB/20/L/01	LC-MS/MS	Mussel	AZA1	131	132.1	µg/kg	0.1
EURLMB/20/L/01	LC-MS/MS	Mussel	AZA2	37	34.7	µg/kg	-0.3
EURLMB/20/L/01	LC-MS/MS	Mussel	AZA3	26.78	25.5	µg/kg	-0.2
<b>EURLMB/20/L/01</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total AZA group</b>	<b>228</b>	<b>230.3</b>	<b>µg AZA eq./kg</b>	<b>0.1</b>

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
EURLMB/20/L/02	LC-MS/MS	Mussel (homogenate)	Free OA	24	20.9	µg/kg	-0.6
EURLMB/20/L/02	LC-MS/MS	Mussel	Free DTX-2	22	18.4	µg/kg	-0.6
EURLMB/20/L/02	LC-MS/MS	Mussel	Total OA	187	198.5	µg/kg	0.3
EURLMB/20/L/02	LC-MS/MS	Mussel	Total DTX-2	40	35.2	µg/kg	-0.5
<b>EURLMB/20/L/02</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA group</b>	<b>210.7</b>	<b>219.6</b>	<b>µg OA eq/kg</b>	<b>0.2</b>
EURLMB/20/L/02	LC-MS/MS	Mussel	Homo-YTX	0.26	0.24	mg/kg	-0.5
EURLMB/20/L/02	LC-MS/MS	Mussel	45-OH-Homo-YTX	0.15	0.11	mg/kg	0.2
<b>EURLMB/20/L/02</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total YTX group</b>	<b>0.35</b>	<b>0.38</b>	<b>mg YTX eq./kg</b>	<b>0.3</b>

#### AFBI Quasimeme Round 2020.1

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST281BT	LC-MS/MS	Mussel (homogenate)	AZA-1	1327	1386	µg/kg	0.3
QST281BT	LC-MS/MS	Mussel	AZA-2	341	324	µg/kg	-0.4
QST281BT	LC-MS/MS	Mussel	AZA-3	306	324	µg/kg	0.5
<b>QST281BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total AZA group</b>	<b>2348</b>	<b>2423</b>	<b>µg AZA eq./kg</b>	<b>0.2</b>
QST281BT	LC-MS/MS	Mussel	Free DTX2	261	250	µg/kg	-0.3
QST281BT	LC-MS/MS	Mussel	Free OA	247	230	µg/kg	-0.5
<b>QST281BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Free OA group</b>	<b>424</b>	<b>380</b>	<b>µg OA eq./kg</b>	<b>-0.8</b>
QST281BT	LC-MS/MS	Mussel	Total DTX2	340	350	µg/kg	0.2
QST281BT	LC-MS/MS	Mussel	Total OA	439	497	µg/kg	1
<b>QST281BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA group</b>	<b>657</b>	<b>707</b>	<b>µg OA eq./kg</b>	<b>0.6</b>

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST282BT	LC-MS/MS	Mussel (extract)	AZA-1	91.4	91.7	µg/kg	0
QST282BT	LC-MS/MS	Mussel	AZA-2	19.5	18.8	µg/kg	-0.3
QST282BT	LC-MS/MS	Mussel	AZA-3	38.6	37.4	µg/kg	-0.2
<b>QST282BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total AZA group</b>	<b>183</b>	<b>178</b>	<b>µg AZA eq./kg</b>	<b>-0.2</b>
QST282BT	LC-MS/MS	Mussel	Free DTX2	3.88	2.4	µg/kg	-2.1
QST282BT	LC-MS/MS	Mussel	Free OA	39	36.8	µg/kg	-0.4
<b>QST282BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Free OA group</b>	<b>42.1</b>	<b>38.3</b>	<b>µg OA eq./kg</b>	<b>-0.6</b>
QST282BT	LC-MS/MS	Mussel	Total DTX2	3.82	3.3	µg/kg	-0.9
QST282BT	LC-MS/MS	Mussel	Total OA	44	44	µg/kg	0
<b>QST282BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA group</b>	<b>46.7</b>	<b>46</b>	<b>µg OA eq./kg</b>	<b>-0.1</b>
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST283BT	LC-MS/MS	Mussel (homogenate)	Free DTX1	163	162	µg/kg	-0.1
QST283BT	LC-MS/MS	Mussel	Free OA	26.1	15.9	µg/kg	-2.6
<b>QST283BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Free OA group</b>	<b>192</b>	<b>178</b>	<b>µg OA eq./kg</b>	<b>-0.5</b>
QST283BT	LC-MS/MS	Mussel	Total DTX1	204	245	µg/kg	1.5
QST283BT	LC-MS/MS	Mussel	Total OA	41.6	36	µg/kg	-0.9
<b>QST283BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA group</b>	<b>239</b>	<b>281</b>	<b>µg OA eq./kg</b>	<b>1.2</b>
QST283BT	LC-MS/MS	Mussel	YTX	0.241	0.327	mg/kg	2.1
QST283BT	LC-MS/MS	Mussel	homo-YTX	0.952	1.003	mg/kg	0.4
QST283BT	LC-MS/MS	Mussel	45-OH-YTX	0.16	0.224	mg/kg	1.7
QST283BT	LC-MS/MS	Mussel	45-OH-homo-YTX	0.356	0.408	mg/kg	0.7
<b>QST283BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total YTX group</b>	<b>1.62</b>	<b>1.758</b>	<b>mg YTX eq./kg</b>	<b>0.6</b>

**AFBI Quasimeme Round 2020.2**

Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST290BT	LC-MS/MS	Mussel (extract)	AZA-1	13.6	13.8	µg/kg	0.1
QST290BT	LC-MS/MS	Mussel	AZA-2	3.92	4.3	µg/kg	0.7
QST290BT	LC-MS/MS	Mussel	AZA-3	3.95	3.6	µg/kg	-0.6
<b>QST290BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total AZA group</b>	<b>25.6</b>	<b>26.5</b>	<b>µg OA eq./kg</b>	<b>0.3</b>
QST290BT	LC-MS/MS	Mussel	Free-DTX2	68.3	63.9	µg/kg	-0.5
QST290BT	LC-MS/MS	Mussel	free-Okadaic-Acid	12	12.8	µg/kg	0.5
<b>QST290BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Free OA group</b>	<b>54</b>	<b>51.2</b>	<b>µg OA eq./kg</b>	<b>-0.4</b>
QST290BT	LC-MS/MS	Mussel	Total-DTX2	74.5	71.1	µg/kg	-0.3
QST290BT	LC-MS/MS	Mussel	Total-Okadaic-Acid	19.6	20.8	µg/kg	0.5
<b>QST290BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA group</b>	<b>65.9</b>	<b>63.4</b>	<b>µg OA eq./kg</b>	<b>-0.3</b>
<b>QST290BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA+PTX group</b>	<b>65.8</b>	<b>63.4</b>	<b>µg OA eq./kg</b>	<b>-0.3</b>
QST290BT	LC-MS/MS	Mussel	YTX	0.033	0.046	mg/kg	0.9
QST290BT	LC-MS/MS	Mussel	homo-YTX	0.308	0.333	mg/kg	0.5
QST290BT	LC-MS/MS	Mussel	45-OH-YTX	0.040	0.041	mg/kg	0.0
QST290BT	LC-MS/MS	Mussel	45-OH-homo-YTX	0.129	0.221	mg/kg	2.8
<b>QST290BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total YTX group</b>	<b>0.441</b>	<b>0.531</b>	<b>mg YTX eq./kg</b>	<b>1.3</b>
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST291BT	LC-MS/MS	Mussel (homogenate)	AZA-1	72.7	59.0	µg/kg	-1.4
QST291BT	LC-MS/MS	Mussel	AZA-2	22.7	23.3	µg/kg	0.2
QST291BT	LC-MS/MS	Mussel	AZA-3	25.5	22.6	µg/kg	-0.9
<b>QST291BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total AZA group</b>	<b>141</b>	<b>133</b>	<b>µg AZA eq./kg</b>	<b>-0.5</b>
QST291BT	LC-MS/MS	Mussel	Free DTX2	519	454	µg/kg	-0.9
QST291BT	LC-MS/MS	Mussel	Free OA	87.4	81.9	µg/kg	-0.4
<b>QST291BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Free OA group</b>	<b>411</b>	<b>355</b>	<b>µg OA eq./kg</b>	<b>-1.0</b>
QST291BT	LC-MS/MS	Mussel	Total DTX2	774	687	µg/kg	-0.8
QST291BT	LC-MS/MS	Mussel	Total OA	210	209	µg/kg	0.0



<b>QST291BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA group</b>	<b>680</b>	<b>621</b>	<b>µg OA eq./kg</b>	<b>-0.6</b>
<b>QST291BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA+PTX group</b>	<b>689</b>	<b>621</b>	<b>µg OA eq./kg</b>	<b>-0.7</b>
Sample ID	Method	Matrix	Determinand	Assigned Value	Reported value	Units	Z-Score
QST292BT	LC-MS/MS	Mussel (homogenate)	Free DTX1	156	136	µg/kg	-1.0
QST292BT	LC-MS/MS	Mussel	Free OA	76.2	73.3	µg/kg	-0.3
<b>QST292BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Free OA group</b>	<b>234</b>	<b>210</b>	<b>µg OA eq./kg</b>	<b>-0.8</b>
QST292BT	LC-MS/MS	Mussel	Total DTX1	215	189	µg/kg	-0.9
QST292BT	LC-MS/MS	Mussel	Total OA	355	373	µg/kg	0.4
<b>QST292BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA group</b>	<b>573</b>	<b>562</b>	<b>µg OA eq./kg</b>	<b>-0.1</b>
<b>QST292BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total OA+PTX group</b>	<b>573</b>	<b>562</b>	<b>µg OA eq./kg</b>	<b>-0.1</b>
QST292BT	LC-MS/MS	Mussel	YTX	0.147	0.172	mg/kg	0.9
QST292BT	LC-MS/MS	Mussel	45-OH-YTX	0.065	0.130	mg/kg	3.3
<b>QST292BT</b>	<b>LC-MS/MS</b>	<b>Mussel</b>	<b>Total YTX group</b>	<b>0.215</b>	<b>0.302</b>	<b>mg YTX eq./kg</b>	<b>2.2</b>

## Tetrodotoxin development exercise

### AFBI Quasimeme Round 2020.2

Sample ID	Sample description	Assigned value	Reported value	Units	z-score
QTT006SS	Solvent standard	37.3	40.5	µg/L	0.66
QTT007BT	Oyster homogenate	-	<10	µg/kg	-
QTT008BT	Oyster homogenate	40.1	46	µg/kg	0.72

**Phytoplankton - IPI exercise was cancelled in 2020 due to COVID. There are no other available PTs.**

(UK-NRL has been employing greater in-house control measures. No performance issues based on UKAS audits completed over the accounting period).

**Appendix 2 – UKNRL 2020-21 annual work programme**



Agri-Food & Biosciences Institute

VETERINARY SCIENCES DIVISION

Chemical Surveillance and Immunodiagnostics  
Branch

Work Programme  
UK National Reference Laboratory  
For Marine Biotoxins

2020-21

Contact:

Email [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

**e-WG:** Electronic working group

**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 2020-2021 will be drafted and submitted to the Competent Authority for comment in May 2021.

### NRL Standard Operating Procedures

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

### Proficiency tests 2020

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 2019, the UK-NRL was again 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 2019-20, with results of these analyses being sent to the UK-NRL for assessment of OCL performance.

In March 2020, the UK-NRL was notified of a delay to the 2020 EURL-MB PT schemes (ASP, PSP, LT) as a result of the COVID-19 pandemic. Providing that this activity is rescheduled and an invitation received in 2020, the UK-NRL will participate and request additional materials to help with inter-comparison at internal UK level. OCL results are to be submitted to the UK-NRL for retrospective assessment of performance, pending receipt of the final EURL PT reports issued to NRLs.

In 2020/21, the UK-NRL will participate in further proposed method assessment exercises for TTX in shellfish (see additional work activities).

## Meetings

*EURL – NRLs Workshop 2020:* The NRL will seek advice from FSA prior to offering to participate in any Workshop (government EU Transition related restrictions may apply). Note: this may be conducted electronically in 2020 due to COVID-19 restrictions.

*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 2020-21, to consider new data on TEFs, implementation and application of new technology, methods or modifications to existing methods, emerging toxin issues or measurement uncertainty. The NRL will seek advice from FSA prior to offering to participate.

*EURL working Group on Phytoplankton:* The majority of this work has been conducted electronically over the past three years, with the NRL co-ordinating UK responses and input from UK experts. A final EURL guideline document has now been circulated for comment to OCLs. The NRL will continue to represent the UK CA as required.

*EURL working Group on ‘Guideline document for marine biotoxins’:* The NRL will participate in this e-WG and co-ordinate UK responses (OCL and CA) as required. This activity commenced in 2020 at the EURL, within a restricted WG. The EURL propose that draft documents prepared through the restricted WG will be circulated to NRLs and CA contacts in member states (members of the electronic WG) for comment.

*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 2020. The first in June 2020, and a second in October/November 2020.

## **Additional work activities 2020-21**

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

The UK-NRL will participate in a method assessment exercise for TTX analysis by LC-MS/MS (Quasimeme PT 2020-21)

The UK-NRL will undertake the single lab validation of a Tetrodotoxin (TTX) LC-MS/MS based screening method in house (pending results of successful method assessment exercises and considering FSA requirements).

In 2019/20 the UK-NRL extended the scope of the routine Lipophilic marine toxins method applied in official control to include Pinnatoxin screening (based on parent analogue PnTX-G). The UK-NRL has recommended that both OCLs do this, to gather data on this emerging toxin class in parallel with the OCL programme. In 2020/21 the UK-NRL will continue to monitor NI samples for PnTX-G (including archived samples if appropriate).