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EUROPEAN UNION REFERENCE LABORATORY FOR MARINE BIOTOXINS

Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of Lipophilic marine biotoxins in molluscs by LC-MS/MS”

REPORT

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1. Introduction

The Working Group on Liquid Chromatography-Mass Spectrometry (WG on LC-MS) of the European network of National Reference Laboratories (NRL) for Marine Biotoxins has been working since 2005 on the development and validation of a method for the determination of lipophilic marine toxins in molluscs by LC-MS. The WG is coordinated by the EU-Reference Laboratory for Marine Biotoxins (EU-RL-MB, Spain) and NRL members are: Belgium NRL, France NRL, Germany NRL, Ireland NRL, Italy NRL, Sweden NRL, The Netherlands NRL and United Kingdom NRL.

Different studies performed within the framework of the WG on LC-MS activities indicated significant problems for the simultaneous analysis of lipophilic toxins using a single protocol. These problems are related to results dependent upon MS equipment causing, in some cases, poor accuracy in toxin quantification and consequently important differences within the results obtained by different MS instruments, thus bad interlaboratory reproducibility. Reports of all the studies are available at the EU-RL-MB Website: <http://www.aesan.msps.es/en/CRLMB/web/home.shtml>.

According to the difficulties experienced when the same protocol was used for lipophilic toxins analysis, the Group agreed to take the approach of using a protocol with a fixed sample extraction and other open conditions (clean-up, concentration, LC and MS) and fulfilling different agreed quality criteria.

Between June 2008 and March 2009, a prevalidation study (two samples tested by 9 laboratories) was conducted to evaluate the performance of individual laboratories for Okadaic acid (OA) group toxins determination in the prescribed conditions (open protocols with fixed quality criteria). Results obtained in this study allowed the establishment of the intra and inter-laboratory variability in the quantification of this lipophilic toxin group. Thus, a reasonable variability was obtained (RSD_R from 15% to 32%) when using certified reference material for toxin recovery correction for the determination of okadaic acid-group toxin and the need for the refinement of the quality criteria established was identified.

With the experience gained during the prevalidation study, a standard operating procedure and refined quality criteria were defined for OA-group determination. Thus, an interlaboratory validation study was carried out during the last term of 2009 with the aim of determining accuracy, repeatability and between-laboratory reproducibility of the method described in the "EU-Harmonised Standard Operating Procedure for determination of Okadaic Acid-group toxin (OA-group) in molluscs using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)". In the study, 12 laboratories from 10 European countries tested 10 materials (5 of them included as blind duplicates), including 3 different types of molluscs (mussels, cockles and clams) with known concentrations, naturally contaminated at different levels or blanks.

Performance results obtained in the interlaboratory validation study for OA-group toxin determination (OA, DTX1, DTX2 and their esters) are presented in **APPENDIX 1**. Satisfactory precision was obtained for OA and DTX1 determination, with a repeatability relative standard deviation (RSD_r) between 6.7% and 16.4% and a HorRat value < 1.5 . The interlaboratory method reproducibility for DTX2 was higher than expected ($1.5 < \text{HorRat} < 2.0$), probably due to the use of OA recovery for toxin correction (since no DTX2 was available). Recovery was reasonable for OA (71.3%-89.4%) and for DTX1 (114.3%) but it was not possible to study for DTX2 since there is no standard or reference material available.

Since the results obtained in the validation study of OA-group were encouraging, the WG concluded that the EU-Harmonised SOP using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was reliable for OA-group toxins determination and recognised the need for validation extension to all groups of lipophilic toxin included in EU legislation: Azaspiracid group toxin (AZA-group), Pectenotoxin group toxin (PTX-group) and Yessotoxin group toxin (YTX-group).

2. Aim of the Interlaboratory Validation Study

The purpose of this validation study was to determine accuracy, repeatability and between-laboratory reproducibility of the method describes in the “EU-Harmonised Standard Operating Procedure (SOP) for determination of Lipophilic marine biotoxin in molluscs using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)”. The SOP used for the study is provided as **APPENDIX 3**.

The study involved the quantitative determination of free and total Okadaic acid group toxin (OA, DTX1 and DTX2), Pectenotoxin group toxin (PTX1 and PTX2) and Azaspiracid group toxin (AZA1, AZA2 and AZA3).

Although it was not an objective of the validation study, the Standard Operating Procedure is also aimed at the quantification of YTX-group toxin (YTX, homo YTX, 45 OH YTX and 45 OH homo YTX). Some materials included in the validation study contained this group toxin, so participants were asked to voluntarily quantify and report results of this group in the validation test samples, in order to get preliminary information on the performance of the SOP for YTX-group toxin determination.

3. Test materials

Materials including different species of molluscs (mussels, cockles, clams and razor clams) naturally contaminated and fortified at different levels or blanks for the different lipophilic marine biotoxin groups were tested.

For the Interlaboratory Validation Study, participants received in total 14 test samples (7 materials as blind duplicates), a pre-release Reference Material (freeze-dried

mussel tissue), a mix Multi-toxin calibration solution and a Multitoxin spiked extract for matrix correction.

Details on the test materials used are summarised in Table 1.

Table 1. Test materials used for the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of Lipophilic marine biotoxins in molluscs using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)”.

SAMPLE CODE/IDENTIFICATION	SAMPLE DESCRIPTION
DAY 1-EURLMB/VAL/L-01 DAY 1-EURLMB/VAL/L-03	Naturally contaminated (OA and DTX2) and spiked (PTX2, AZA1 and AZA2) raw wedge shell clam homogenate (<i>Donax trunculus</i>)
DAY 1-EURLMB/VAL/L-02 DAY 1-EURLMB/VAL/L-05	Naturally contaminated (OA and DTX2) and spiked (AZA1 and AZA2) raw razor clam homogenate (<i>Ensis acuatus</i>)
DAY 1-EURLMB/VAL/L-04 DAY 1-EURLMB/VAL/L-06	Naturally contaminated (OA, DTX1, AZA1, AZA2, AZA3, YTX and 45 OH YTX) raw mussel homogenate (<i>Mytilus edulis</i>)
DAY 2-EURLMB/VAL/L-07 DAY 2-EURLMB/VAL/L-08	Naturally contaminated (OA and DTX2) and spiked (PTX2, AZA1 and AZA2) raw stripped venus (<i>Chamelea gallina</i>)
DAY 2-EURLMB/VAL/L-09 DAY 2-EURLMB/VAL/L-10	Naturally contaminated (OA, DTX2, AZA1, AZA2, AZA3) cooked mussel homogenate (<i>Mytilus edulis</i>)
DAY 3-EURLMB/VAL/L-11 DAY 3-EURLMB/VAL/L-14	Naturally contaminated (OA and DTX2) and spiked (AZA1 and AZA2) raw cockle homogenate (<i>Cerastoderma edule</i>)
DAY 3-EURLMB/VAL/L-12 DAY 3-EURLMB/VAL/L-13	Naturally contaminated (OA, DTX1, DTX2, AZA1, AZA2, AZA3) cooked mussel homogenate (<i>Mytilus edulis</i>)
NRC RM-FDMT	Non-Certified Freeze-dried Mussel Tissue Containing Multiple Shellfish Toxins (lipophilic toxins included in this study: OA, DTX1, DTX2, PTX2, AZA1, AZA2, AZA3, YTX)
NRC RM-Multi-toxin	Non-Certified Calibration Solution Containing Multiple Lipophilic Shellfish Toxins (OA, DTX1, DTX2, PTX2, AZA1, AZA2, AZA3, YTX)
Spiked extract for matrix correction	Blank mussel extract spiked with NRC RM-Multi-toxin: OA (9.5 ng/ml), DTX1 (11.0 ng/ml), DTX2 (8.4 ng/ml), PTX2 (13.4 ng/ml), AZA1 (5.5 ng/ml), AZA2 (1.8 ng/ml), AZA3 (1.6 ng/ml) and YTX (10.0 ng/ml)

Homogeneity and stability of the test materials were confirmed by LC-MS/MS analysis according to IUPAC and ISO guidelines.

4. Organization of the Interlaboratory Validation Study

4.1 Participants

Participant laboratories were potential end-users of the method since they perform the official control for marine biotoxins. They all were considered experts in the use of

LC-MS/MS methodologies for lipophilic marine biotoxins determination, since they had taken part in previous studies of the WG LC-MS and successfully participated in the EU-RL-MB Proficiency Testing for Lipophilic toxins, using LC-MS/MS methodologies.

Fifteen laboratories from eleven European countries participated in the validation study. They are presented in Table 2.

Table 2. Coordinator and participant laboratories that submitted results for the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure (SOP) for determination of Lipophilic marine biotoxins in molluscs using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)”.

LABORATORY	CONTACT PERSON
Study coordinator: EU-RL-MB, AESAN. Vigo, Spain	María Luisa Rodríguez-Velasco
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NRL France. AFSSA-LERQAP. Maisons Alfort	Sophie Trotereau
NRL Germany. Federal Institute for Risk Assessment, BfR. Berlin	Anja These, Katrin Kapp
NRL Italy. Centro Ricerche Marine. Cesenatico	Silvia Pigozzi
NRL Ireland. Marine Institute. Galway	Sinead O Brien, Connor Duffy
NRL The Netherlands. RIKILT-Institute of Food Safety. Wageningen	Hans van Egmond, Hester van den Top
NRL Sweden. National Food Administration. Uppsala	Aida Z. Zuberovic
NRL United Kingdom. Agri-Food and Biosciences Institute. Belfast	Cowan Higgins
CEFAS. Weymouth, United Kingdom	Steven Morris
IFREMER. Environment, Microbiology and Phycotoxins. Nantes, France	Phillip Hess, Manoella Sibat, Zouher Amzil
IRTA. Tarragona, Spain	Pablo de la Iglesia
L.C.C.RR.PP. Junta de Andalucía. Huelva, Spain	Lourdes Fernández
SCAV. Neuchâtel, Switzerland	José Caperos André Pittet
EU-RL-MB. AESAN. Vigo, Spain	Adriano Villar-González

4.2 Instructions

Participants in the Interlaboratory Validation Study received a Protocol with instructions on how to proceed with samples and analysis.

For the study, participants received:

- By electronic mail: the Standard Operating Procedure and the Protocol for the validation study, together with the “Arrival Form”, the “Reporting Sheet” and a form to report “Method description and Participant’s feedback”.
- By courier: a letter with the Participant Code (in the heading) and the following materials:
 - Bag labelled “DAY 1” (including 6 samples labelled DAY 1-EURLMB/VAL/L-01 to DAY 1-EURLMB/VAL/L-06)
 - Bag labelled “DAY 2” (including 4 samples labelled DAY 2-EURLMB/VAL/L-07 to DAY 2-EURLMB/VAL/L-010)
 - Bag labelled “DAY 3” (including 4 samples labelled DAY 3-EURLMB/VAL/L-011 to DAY 3-EURLMB/VAL/L-014)
 - Ampoule labelled “NRC RM Multitoxin”
 - Bottle with a freeze-dried Mussel labelled “NRC RM FDMT”
 - Vial labelled “MultiToxin spiked extract for matrix correction”

Participants were requested to inspect the packages and make sure the contents were correct and the samples were still frozen and intact. They were asked to fill in the arrival form and return it to the study coordinator as soon as they had checked the completeness of the packages.

All participants received the samples in good condition and frozen, apart from one participant who reported that samples were not frozen on arrival, but cool. Shipment took 1-2 days.

Participants were instructed to perform analysis as soon as possible, to keep the samples frozen (≤ -18 °C) until the day of analysis and to mix them thoroughly before weighing.

4.3 Experiment design and reporting

4.3.1. Sample preparation and analysis

According to the Protocol, analysis must be carried out in three separate days/sequences. Thus, participants were asked to process six samples during the first day (DAY 1-EURLMB/VAL/L-01 to DAY 1-EURLMB/VAL/L-06), four samples p.8

on the second day (DAY 2-EURLMB/VAL/L-07 to DAY 2-EURLMB/VAL/L-010) and four samples the third day (DAY 3-EURLMB/VAL/L-011 to DAY 3-EURLMB/VAL/L-014).

In summary, on each day of analysis, participants had to follow the working plan:

- Reconstitute the pre-release freeze-dried Reference Material NRC RM-FDMT according to the instructions given in the Protocol.
- Perform one extraction of each test sample and the reconstituted RM-FDMT (starting as described in Section 6.2 of the EU-Harmonised-SOP-LIPO-LCMSMS_Version2). Analyse samples and RM-FDMT extracts both, unhydrolysed (Section 6.3) and hydrolysed (Section 6.4).
- Process and analyse an unhydrolysed positive QC (CRM-DSP-Mus-b) as described in Section 8.4 of the EU-Harmonised-SOP-LIPO-LCMSMS_Version2.
- Process and analyse the procedural blank (Blank QC). This Blank QC will be methanol for free OA, PTX, AZA and YTX groups analysis and methanol after hydrolysis, as described in Section 6.4 of the EU-Harmonised-SOP-LIPO-LCMSMS_Version2, for total OA group analysis.
- Follow the sequence injection (calibration curves, samples extracts and QC) as described in Section 8.1 of the EU-Harmonised-SOP-LIPO-LCMSMS_Version2.
- Inject samples, RM-FDMT and positive QC (intermediate calibration standard, spiked extract, CRM-DSP-Mus-b) in duplicate.

4.3.2. Calibration

For this interlaboratory validation study two different approaches for calibration were assessed:

- Using a calibration curve prepared from commercially available individual reference standards for OA, PTX2, AZA1 and YTX (Section 4.3.2 to 4.3.5 of the EU-Harmonised-SOP-LIPO-LCMSMS_Version2), purchased by participants and prepared as described in Section 4.4 of the EU-Harmonised-SOP-LIPO-LCMSMS_Version2.
- Using a calibration curve prepared from the Multi-toxin calibration solution (NRC RM-Multi-toxin) provided for the exercise and containing OA, DTX1, DTX2, PTX2, AZA1, AZA2, AZA3 and YTX. Participants had to prepare this calibration curve by diluting the MultiToxin calibration solution with methanol according to the instructions given in the Protocol.

4.3.3. Quantification

For this interlaboratory validation study two different approaches for quantification were assessed:

- Uncorrected results, after extrapolation from the calibration curves constructed as indicated in 4.3.2 and described in Section 8.3 of the EU-Harmonised-SOP-LIPO-LCMSMS_Version2.
- Corrected results using correction got from the CRM-DSP-Mus-b (recovery correction for the procedure for OA group toxins) and the MultiToxin spiked extract (matrix effects correction) as described in Section 8.4 of the EU-Harmonised-SOP-LIPO-LCMSMS_Version2.

4.3.4. Report of results

Participants in the interlaboratory validation study had not to carry out any calculations for the quantification of the test samples provided for the study. Calibration curves and quantification were automatically calculated in the excel Reporting Sheet provided after filling raw results in.

A summary of the experimental design for the validation study is presented in Table 3.

Table 3. Experimental design of the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of Lipophilic toxins in molluscs by LC-MS/MS”.

DAY 1	DAY 2	DAY 3
<u>FIRST SET CALIBRATION CURVE FROM INDIVIDUAL STANDARDS (4.2.1):</u> One injection each level commencing with the lowest standard concentration to the highest concentration	<u>FIRST SET CALIBRATION CURVE FROM INDIVIDUAL STANDARDS (Section 4.4 of SOP):</u> One injection each level commencing with the lowest standard concentration to the highest concentration	<u>FIRST SET CALIBRATION CURVE FROM INDIVIDUAL STANDARDS (Section 4.4 of SOP):</u> One injection each level commencing with the lowest standard concentration to the highest concentration
<u>PROCEDURAL BLANK (methanol):</u> One injection	<u>PROCEDURAL BLANK (methanol):</u> One injection	<u>PROCEDURAL BLANK (methanol):</u> One injection
<u>FIRST SET CALIBRATION CURVE FROM MULTITOXIN CALIBRATION SOLUTION (4.2.2, table 1):</u> One injection each level commencing with the lowest standard concentration to the highest concentration	<u>FIRST SET CALIBRATION CURVE FROM MULTITOXIN CALIBRATION SOLUTION (4.2.2, table 1):</u> One injection each level commencing with the lowest standard concentration to the highest concentration	<u>FIRST SET CALIBRATION CURVE FROM MULTITOXIN CALIBRATION SOLUTION (4.2.2, table 1):</u> One injection each level commencing with the lowest standard concentration to the highest concentration
<u>SAMPLES AND POSITIVE QC:</u> Duplicate injection DAY 1-EURLMB/VAL/L-01 DAY 1-EURLMB/VAL/L-01-Hyd DAY 1-EURLMB/VAL/L-02 DAY 1-EURLMB/VAL/L-02-Hyd DAY 1-EURLMB/VAL/L-03 DAY 1-EURLMB/VAL/L-03-Hyd DAY 1-EURLMB/VAL/L-04 DAY 1-EURLMB/VAL/L-04-Hyd DAY 1-EURLMB/VAL/L-05 DAY 1-EURLMB/VAL/L-05-Hyd DAY 1-EURLMB/VAL/L-06 DAY 1-EURLMB/VAL/L-06-Hyd CRM-DSP-Mus-b 1/50 CRM-DSP-Mus-b 1/6 MutiToxin Spiked extract NRC RM-FDMT NRC RM-FDMT-Hyd Intermediate individual calibration standard	<u>SAMPLES AND POSITIVE QC:</u> Duplicate injection DAY 2-EURLMB/VAL/L-07 DAY 2-EURLMB/VAL/L-07-Hyd DAY 2-EURLMB/VAL/L-08 DAY 2-EURLMB/VAL/L-08-Hyd DAY 2-EURLMB/VAL/L-09 DAY 2-EURLMB/VAL/L-09-Hyd DAY 2-EURLMB/VAL/L-10 DAY 2-EURLMB/VAL/L-10-Hyd CRM-DSP-Mus-b 1/50 CRM-DSP-Mus-b 1/6 MutiToxin Spiked extract NRC RM-FDMT NRC RM-FDMT-Hyd Intermediate individual calibration standard	<u>SAMPLES AND POSITIVE QC:</u> Duplicate injection DAY 3-EURLMB/VAL/L-11 DAY 3-EURLMB/VAL/L-11-Hyd DAY 3-EURLMB/VAL/L-12 DAY 3-EURLMB/VAL/L-12-Hyd DAY 3-EURLMB/VAL/L-13 DAY 3-EURLMB/VAL/L-13-Hyd DAY 3-EURLMB/VAL/L-14 DAY 3-EURLMB/VAL/L-14-Hyd CRM-DSP-Mus-b 1/50 CRM-DSP-Mus-b 1/6 MutiToxin Spiked extract NRC RM-FDMT NRC RM-FDMT-Hyd Intermediate individual calibration standard
<u>PROCEDURAL BLANK:</u> One injection	<u>PROCEDURAL BLANK:</u> One injection	<u>PROCEDURAL BLANK:</u> One injection
<u>SECOND SET CALIBRATION CURVE FROM INDIVIDUAL STANDARDS (4.2.1):</u> One injection each level commencing with the lowest standard concentration to the highest concentration	<u>SECOND SET CALIBRATION CURVE FROM INDIVIDUAL STANDARDS (Section 4.4 of SOP):</u> One injection each level commencing with the lowest standard concentration to the highest concentration	<u>SECOND SET CALIBRATION CURVE FROM INDIVIDUAL STANDARDS (Section 4.4 of SOP):</u> One injection each level commencing with the lowest standard concentration to the highest concentration
<u>PROCEDURAL BLANK (methanol):</u> One injection	<u>PROCEDURAL BLANK (methanol):</u> One injection	<u>PROCEDURAL BLANK (methanol):</u> One injection
<u>SECOND SET CALIBRATION CURVE FROM MULTITOXIN CALIBRATION SOLUTION (4.2.2, table 1):</u> One injection each level commencing with the lowest standard concentration to the highest concentration	<u>SECOND SET CALIBRATION CURVE FROM MULTITOXIN CALIBRATION SOLUTION (4.2.2, table 1):</u> One injection each level commencing with the lowest standard concentration to the highest concentration	<u>SECOND SET CALIBRATION CURVE FROM MULTITOXIN CALIBRATION SOLUTION (4.2.2, table 1):</u> One injection each level commencing with the lowest standard concentration to the highest concentration

5. Results and discussion

5.1 Methods used for statistical analysis

Statistical analysis of results was carried out following the approach described in the AOAC/IUPAC Harmonised Protocol (2002).

Initial Review of Data

- Results were initially reviewed and only valid data were included in the statistical analysis. Invalid data were assigned when sample result was out of the working range of the calibration curve.

Outliers

Outlier identification was performed by applying two tests on all valid data:

1. **Cochran test** (for blind duplicates samples), to remove laboratories showing significantly greater variability within replicate (within-laboratory) analyses than the other laboratories for a given material. A 1-tail test at a probability value of 2.5% was applied.
2. **Grubbs test** (for blind duplicates and single samples), to remove laboratories with extreme averages. The test was applied in the following order: single value test (2-tail, P = 2.5%); then if no outlier was found, a pair value test was applied (2 values at the highest end, 2 values at the lowest end, and 2 values, one at each end, at an overall P = 2.5%).

Precision

Precision of the method was characterized for 2 circumstances of replication: within-laboratory or repeatability and between-laboratory or reproducibility.

The repeatability standard deviation (s_r) was computed as: $s_r = (\text{sum}d_i^2/2L)^{1/2}$, where d_i is the difference between the individual values for the pair in laboratory i and L is the number of laboratories or number of pairs.

The reproducibility standard deviation (s_R) was computed as: $s_R = (1/2(s_d^2 + s_r^2))^{1/2}$, where $s_d^2 = \text{sum}(T_i - \bar{T})^2 / (2(L-1))$, being T_i the sum of the individual values for the pair in laboratory i , \bar{T} the mean of the T_i across all laboratories of pairs, L the number of laboratories or pairs, and s_r^2 is the square of s_r .

In order to facilitate comparison of variabilities for different test materials included in the study, the relative standard deviation (RSD) under repeatability (RSD_r) and reproducibility (RSD_R) conditions were calculated as follows:

$RSD_r (\%) = 100s_r / \text{mean}$; and $RSD_R (\%) = 100s_R / \text{mean}$

HorRat

HorRat value is the ratio of the reproducibility relative standard deviation, expressed as a percent ($RSD_R, \%$) to the predicted reproducibility relative standard deviation, expressed as a percent ($PRSD_R, \%$).

It was calculated as: $\text{HorRat} = RSD_R, \% / PRSD_R, \%$; where $PRSD_R, \% = 2C^{-0.1505}$ and C = the estimated mean concentration expressed as a decimal fraction. In this case, the decimal fraction for each material was computed as: $C = (\text{mean } \mu\text{g/kg})$ multiplied by 10^{-9} .

According to AOAC/IUPAC Harmonised Protocol, the following guidelines were used to evaluate the assay precision:

- $\text{HorRat} \leq 0.5$: method reproducibility may be in question due to lack of study independence, unreported averagingk, or consultations.
- $0.5 < \text{HorRat} \leq 1.5$: method reproducibility as normally would be expected.
- $\text{HorRat} > 1.5$: method reproducibility higher than normally expected: the Study Director should critically look into possible reasons for a “high” HorRat, and discuss this in the report.
- $\text{HorRat} > 2$: method reproducibility is problematic.

Recovery

For percent recovery calculations, the marginal percent recovery was calculated as:

$\% \text{ Recovery} = 100 ((C_f - C_u) / C_A)$, where C_f is the amount found for the fortified concentration, C_u is the amount present originally for the unfortified concentration, and C_A is the amount added for the added concentration.

5.2 Interlaboratory Validation results

The method has been evaluated for the determination of OA-group toxin, AZA-group toxin, PTX-group toxin and YTX-group toxin in four different matrices of molluscs: mussels, cockles, clams and razor clams. A total of seven samples were tested in three days as blind duplicates. 15 out of 16 laboratories registered for the study submitted results for the seven test materials.

According to the study design described in section 4.3, different calibration and quantification approaches were assessed for each toxin:

- Individual calibration, using CRM-individual reference standards from NRC (OA, PTX2, AZA1 and YTX):
 - Uncorrected results: extrapolation from calibration curves
 - Corrected results: extrapolation from calibration curves and matrix correction and/or recovery correction (for OA-group toxins)
- Multitoxin calibration, using the RM-multitoxin reference standard from NRC (OA, DTX1, DTX2, PTX2, AZA1, AZA2, AZA3 and YTX):
 - Uncorrected corrected results: extrapolation from calibration curves
 - Corrected results: extrapolation from calibration curves and matrix correction and/or recovery correction (for OA-group toxins)

Raw data provided by participants for each sample and toxin are presented in **APPENDIX 2**.

5.2.1. OA-group toxin results

Tables 4a, 4b, 4c, 4d, 4e and 4f show, respectively, performance results obtained for OA determination using the different quantification approaches described.

Tables 5a, 5b, 5c, 5d, 5e and 5f show, respectively, performance results obtained for DTX2 determination using the different quantification approaches described.

Tables 6a, 6b, 6c, 6d, 6e and 6f show, respectively, performance results obtained for DTX1 determination using the different quantification approaches described.

Table 4a. Performance characteristics for OA determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
CRM-INDIVIDUAL QUANTITATION UNCORRECTED RESULTS

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	15(0)	184	-	10.8	28.6	1.39
DAY1-EURLMB/VAL/L-02&05	<i>Ensis arcuatus</i> (razor clam)	12(3)	84.6	-	12.4	32.7	1.41
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	13(1)	86.0	-	12.5	31.2	1.35
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	14(1)	182	-	8.34	34.1	1.65
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	15(0)	431	-	4.87	35.5	1.95
DAY3-EURLMB/VAL/L-11&14	<i>Cerastoderma edule</i> (cockle)	15(0)	154	-	22.2	39.7	1.87
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	13(2)	338	-	4.38	32.3	1.72

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 4b. Performance characteristics for OA determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
CRM-INDIVIDUAL QUANTITATION CRM MUS CORRECTION RESULTS

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	14(0)	175	-	10.8	26.1	1.26
DAY1-EURLMB/VAL/L-02&05	<i>Ensis arcuatus</i> (razor clam)	12(2)	83.6	-	19.0	37.3	1.61
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	12(1)	80.0	-	12.7	31.3	1.34
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	12(2)	175	-	9.87	16.3	0.79
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	12(2)	406	-	5.32	13.1	0.71
DAY3-EURLMB/VAL/L-11&14	<i>Cerastoderma edule</i> (cockle)	12(2)	127	-	12.2	36.1	1.65
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	12(2)	311	-	4.07	31.4	1.65

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 4c. Performance characteristics for OA determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
CRM-INDIVIDUAL QUANTITATION MATRIX CORRECTION RESULTS

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	15(0)	143	-	11.0	24.4	1.14
DAY1-EURLMB/VAL/L-02&05	<i>Ensis arcuatus</i> (razor clam)	12(3)	63.3	-	18.8	25.2	1.04
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	13(1)	65.5	-	11.5	18.3	0.76
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	15(0)	154	-	12.9	35.1	1.66
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	13(2)	367	-	5.29	32.0	1.72
DAY3-EURLMB/VAL/L-11&14	<i>Cerastoderma edule</i> (cockle)	13(1)	115	-	15.3	36.0	1.63
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	12(2)	264	-	4.62	31.9	1.63

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 4d. Performance characteristics for OA determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
MULTITOXIN QUANTITATION UNCORRECTED RESULTS

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	15(0)	176	-	10.5	28.9	1.39
DAY1-EURLMB/VAL/L-02&05	<i>Ensis arcuatus</i> (razor clam)	12(3)	82.1	-	12.3	36.9	1.58
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	11(4)	74.2	-	10.5	20.7	0.88
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	14(1)	174	-	7.90	35.3	1.70
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	14(1)	381	-	5.39	25.5	1.38
DAY3-EURLMB/VAL/L-11&14	<i>Cerastoderma edule</i> (cockle)	13(2)	128	-	13.1	34.7	1.59
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	13(2)	303	-	4.13	27.3	1.43

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 4e. Performance characteristics for OA determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
MULTITOXIN QUANTITATION CRM MUS CORRECTION RESULTS

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	14(0)	175	-	10.7	27.4	1.32
DAY1-EURLMB/VAL/L-02&05	<i>Ensis arcuatus</i> (razor clam)	12(2)	87.9	-	16.7	39.8	1.73
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	12(2)	84.1	-	11.3	36.7	1.58
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	12(2)	175	-	9.69	17.7	0.85
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	12(2)	404	-	5.32	12.7	0.69
DAY3-EURLMB/VAL/L-11&14	<i>Cerastoderma edule</i> (cockle)	14(0)	147	-	24.2	51.0	2.39
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	12(2)	306	-	3.99	31.3	1.64

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 4f. Performance characteristics for OA determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
MULTITOXIN QUANTITATION MATRIX CORRECTION RESULTS

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	15(0)	141	-	11.2	24.5	1.14
DAY1-EURLMB/VAL/L-02&05	<i>Ensis arcuatus</i> (razor clam)	12 (3)	64.8	-	17.4	26.9	1.11
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	13(2)	66.6	-	10.6	21.5	0.89
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	14(1)	158	-	9.57	36.3	1.72
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	14(1)	344	-	4.82	30.1	1.60
DAY3-EURLMB/VAL/L-11&14	<i>Cerastoderma edule</i> (cockle)	13(1)	113	-	16.4	34.7	1.56
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	12(2)	253	-	4.39	27.5	1.40

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 5a. Performance characteristics for DTX2 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
CRM-INDIVIDUAL_QUANTITATION_UNCORRECTED RESULTS (against OA calibrant)

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	14(1)	238	-	8.80	27.4	1.38
DAY1-EURLMB/VAL/L-02&05	<i>Ensis arcuatus</i> (razor clam)	11(2)	68.2	-	14.9	23.2	0.97
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	14(0)	80.4	-	19.7	34.4	1.47
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	11(1)	57.7	-	8.03	34.5	1.40
DAY3-EURLMB/VAL/L-11&14	<i>Cerastoderma edule</i> (cockle)	15(0)	104	-	21.9	33.1	1.47
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	14(1)	400	-	6.32	32.3	1.76

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 5b. Performance characteristics for DTX2 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
CRM-INDIVIDUAL_QUANTITATION_CRM MUS CORRECTION RESULTS (against OA calibrant)

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	13(1)	228	-	8.44	25.7	1.29
DAY1-EURLMB/VAL/L-02&05	<i>Ensis arcuatus</i> (razor clam)	12(1)	69.8	-	13.8	33.2	1.39
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	12(2)	73.2	-	10.8	26.1	1.10
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	11(1)	52.7	-	8.25	32.6	1.31
DAY3-EURLMB/VAL/L-11&14	<i>Cerastoderma edule</i> (cockle)	14(0)	98.2	-	24.1	40.7	1.80
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	12(2)	350	-	5.73	27.6	1.47

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 5c. Performance characteristics for DTX2 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
CRM-INDIVIDUAL_QUANTITATION_MATRIX CORRECTION RESULTS (against OA calibrant)

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	15(0)	171	-	12.6	32.9	1.58
DAY1-EURLMB/VAL/L-02&05	<i>Ensis arcuatus</i> (razor clam)	12(1)	51.1	-	11.8	43.0	1.72
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	14(0)	59.6	-	22.4	34.4	1.41
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	11(1)	37.3	-	8.76	32.6	1.24
DAY3-EURLMB/VAL/L-11&14	<i>Cerastoderma edule</i> (cockle)	12(2)	63.1	-	11.9	33.1	1.37
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	13(1)	247	-	6.89	22.4	1.13

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 5d. Performance characteristics for DTX2 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
MULTITOXIN_QUANTITATION_UNCORRECTED RESULTS (against DTX2 calibrant)

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	13(2)	191	-	8.28	20.3	0.99
DAY1-EURLMB/VAL/L-02&05	<i>Ensis arcuatus</i> (razor clam)	13(1)	65.1	-	12.7	34.1	1.41
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	13 (0)	72.2	-	19.1	35.1	1.48
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	12(1)	46.1	-	8.78	39.2	1.54
DAY3-EURLMB/VAL/L-11&14	<i>Cerastoderma edule</i> (cockle)	14(0)	90.1	-	23.6	41.3	1.80
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	15(0)	352	-	7.38	34.5	1.85

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 5e. Performance characteristics for DTX2 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
MULTITOXIN_QUANTITATION_CRM MUS CORRECTION RESULTS (against DTX2 calibrant)

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	13(1)	201	-	7.93	25.0	1.23
DAY1-EURLMB/VAL/L-02&05	<i>Ensis arcuatus</i> (razor clam)	13(1)	64.4	-	12.6	36.4	1.51
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	13(0)	71.6	-	20.5	35.8	1.50
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	12(1)	46.5	-	8.72	44.1	1.74
DAY3-EURLMB/VAL/L-11&14	<i>Cerastoderma edule</i> (cockle)	13(0)	89.7	-	24.1	45.9	2.00
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	14(0)	356	-	7.27	38.4	2.06

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 5f. Performance characteristics for DTX2 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
MULTITOXIN_QUANTITATION_MATRIX CORRECTION RESULTS (against DTX2 calibrant)

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	15(0)	172	-	13.8	37.1	1.78
DAY1-EURLMB/VAL/L-02&05	<i>Ensis arcuatus</i> (razor clam)	13(1)	53.3	-	11.3	36.8	1.48
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	14(0)	57.5	-	22.2	40.1	1.63
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	12(1)	37.5	-	9.40	40.4	1.54
DAY3-EURLMB/VAL/L-11&14	<i>Cerastoderma edule</i> (cockle)	12(2)	63.7	-	11.6	33.8	1.40
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	13(1)	242	-	6.91	20.5	1.04

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 6a. Performance characteristics for DTX1 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
CRM-INDIVIDUAL_QUANTITATION_UNCORRECTED RESULTS (against OA calibrant)

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	14(1)	422	-	5.84	31.0	1.70
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	15(0)	273	-	6.66	39.2	2.01

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 6b. Performance characteristics for DTX1 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
CRM-INDIVIDUAL_QUANTITATION_CRM MUS CORRECTION RESULTS (against OA calibrant)

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	13(1)	312	-	8.86	16.4	0.86
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	12(2)	205	-	6.53	21.5	1.06

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 6c. Performance characteristics for DTX1 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
CRM-INDIVIDUAL_QUANTITATION_MATRIX CORRECTION RESULTS (against OA calibrant)

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	14(1)	249	-	9.36	20.5	1.04
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	12(2)	142	-	7.29	16.1	0.75

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 6d. Performance characteristics for DTX1 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
MULTITOXIN_QUANTITATION_UNCORRECTED RESULTS (against DTX1 calibrant)

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	15(0)	388	-	8.48	27.2	1.48
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	15(0)	244	-	6.40	37.1	1.87

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 6e. Performance characteristics for DTX1 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
MULTITOXIN_QUANTITATION_CRM MUS CORRECTION RESULTS (against DTX1 calibrant)

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	14(1)	301	-	8.36	18.1	0.95
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	14(0)	207	-	6.67	35.2	1.74

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 6f. Performance characteristics for DTX1 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
MULTITOXIN_QUANTITATION_MATRIX CORRECTION RESULTS (against DTX1 calibrant)

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	13(2)	251	-	6.33	21.9	1.11
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	12(2)	141	-	7.60	16.3	0.76

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

5.2.2. AZA-group toxin results

Tables 7a, 7b, 7c and 7d show, respectively, performance results obtained for AZA1 determination using the different quantification approaches described.

Tables 8a, 8b, and 8c show, respectively, performance results obtained for AZA2 determination using the different quantification approaches described.

Tables 9a, 9b, and 9c show, respectively, performance results obtained for AZA3 determination using the different quantification approaches described.

For AZA2 and AZA3, not results evaluation was carried out using the Individual calibration-Matrix correction quantification approach since AZA2 and AZA3 concentrations in the spiked extract for matrix correction was lower than the lowest calibration point for most laboratories.

Table 7a. Performance characteristics for AZA1 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
CRM-INDIVIDUAL QUANTITATION UNCORRECTED RESULTS

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	13(1)	80.4	77.9	11.5	16.9	0.72
DAY1-EURLMB/VAL/L-02&05	<i>Ensis arcuatus</i> (razor clam)	13(1)	47.8	71.8	12.0	23.9	0.94
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	13(1)	347	-	10.2	25.8	1.38
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	13(2)	109	71.4	5.14	19.9	0.89
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	14(1)	231	-	3.85	28.9	1.45
DAY3-EURLMB/VAL/L-11&14	<i>Cerastoderma edule</i> (cockle)	12(2)	40.7	73.7	6.55	14.5	0.56
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	14(1)	434	-	5.43	23.1	1.27

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 7b. Performance characteristics for AZA1 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
CRM-INDIVIDUAL QUANTITATION MATRIX CORRECTION RESULTS

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	14(0)	91.5	88.7	14.8	28.7	1.25
DAY1-EURLMB/VAL/L-02&05	<i>Ensis arcuatus</i> (razor clam)	13(1)	50.5	75.9	12.6	17.8	0.71
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	10(4)	323	-	6.66	10.7	0.57
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	14(1)	113	74.0	5.79	17.9	0.80
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	14(1)	225	-	3.99	15.2	0.76
DAY3-EURLMB/VAL/L-11&14	<i>Cerastoderma edule</i> (cockle)	13(1)	43.5	78.8	11.0	17.3	0.68
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	12(1)	472	-	3.83	12.8	0.71

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 7c. Performance characteristics for AZA1 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
MULTITOXIN_QUANTITATION_UNCORRECTED_RESULTS

Material		N° of labs a(b)	Mean µg/kg	Recovery, %	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	13(1)	82.3	79.7	10.9	14.2	0.61
DAY1-EURLMB/VAL/L-02&05	<i>Ensis arcuatus</i> (razor clam)	14(0)	52.2	74.7	13.7	26.4	1.06
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	13(1)	349	-	9.57	25.4	1.36
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	13(2)	112	73.4	5.11	17.9	0.81
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	14(1)	229	-	3.75	25.6	1.28
DAY3-EURLMB/VAL/L-11&14	<i>Cerastoderma edule</i> (cockle)	13(2)	42.1	76.3	6.38	17.0	0.66
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	14(1)	444	-	5.62	23.5	1.30

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 7d. Performance characteristics for AZA1 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
MULTITOXIN_QUANTITATION_MATRIX_CORRECTION_RESULTS

Material		N° of labs a(b)	Mean µg/kg	Recovery, %	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	13(1)	85.2	82.6	12.4	22.2	0.96
DAY1-EURLMB/VAL/L-02&05	<i>Ensis arcuatus</i> (razor clam)	13(1)	50.6	76.1	10.5	17.2	0.69
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	13(1)	354	-	9.99	23.0	1.25
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	14(1)	111	72.7	6.58	20.4	0.92
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	15(0)	226	-	4.73	21.3	1.06
DAY3-EURLMB/VAL/L-11&14	<i>Cerastoderma edule</i> (cockle)	13(1)	44.1	79.9	11.7	16.6	0.65
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	13(1)	455	-	5.42	23.9	1.33

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 8a. Performance characteristics for AZA2 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
CRM-INDIVIDUAL_QUANTITATION_UNCORRECTED RESULTS (against AZA1 calibrant)

Material		N° of labs a(b)	Mean µg/kg	Recovery ¹ , %	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	10(1)	30.3	138.4	12.1	20.4	0.76
DAY1-EURLMB/VAL/L-02&05	<i>Ensis arcuatus</i> (razor clam)	11(2)	39.5	136.2	6.67	28.6	1.10
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	12(1)	84.4	-	10.2	25.1	1.08
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	11(3)	63.2	117.7	6.91	25.2	1.04
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	13(2)	62.1	-	4.92	28.4	1.17
DAY3-EURLMB/VAL/L-11&14	<i>Cerastoderma edule</i> (cockle)	12(2)	40.0	117.6	9.25	24.2	0.93
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	11(4)	111	-	1.70	29.2	1.31

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

¹Reference concentration from EURLMB analysis (n=20) using a validated and accredited methodology.

Table 8b. Performance characteristics for AZA2 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
MULTITOXIN_QUANTITATION_UNCORRECTED RESULTS (against AZA2 calibrant)

Material		N° of labs a(b)	Mean µg/kg	Recovery ¹ , %	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	12(1)	22.9	104.6	12.6	17.7	0.63
DAY1-EURLMB/VAL/L-02&05	<i>Ensis arcuatus</i> (razor clam)	12(1)	36.0	124.1	7.39	25.6	0.97
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	13(1)	74.8	-	9.22	33.0	1.40
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	11(3)	60.2	112.1	7.71	12.2	0.50
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	12(3)	64.3	-	4.69	16.4	0.68
DAY3-EURLMB/VAL/L-11&14	<i>Cerastoderma edule</i> (cockle)	11(2)	36.9	108.5	7.93	12.2	0.46
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	13(2)	110	-	5.73	23.7	1.06

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

¹Reference concentration from EURLMB analysis (n=20) using a validated and accredited methodology.

Table 8c. Performance characteristics for AZA2 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
MULTITOXIN_QUANTITATION_MATRIX CORRECTION RESULTS (against AZA2 calibrant)

Material		N° of labs a(b)	Mean µg/kg	Recovery ¹ , %	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	13(0)	26.5	121.0	16.7	38.3	1.39
DAY1-EURLMB/VAL/L-02&05	<i>Ensis arcuatus</i> (razor clam)	13(0)	39.2	135.2	12.9	28.0	1.07
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	13(1)	75.7	-	11.0	28.8	1.22
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	14(0)	58.8	109.5	16.1	23.3	0.95
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	15(0)	61.8	-	5.82	20.1	0.83
DAY3-EURLMB/VAL/L-11&14	<i>Cerastoderma edule</i> (cockle)	12(1)	39.6	116.5	11.4	21.0	0.81
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	11(3)	109	-	1.69	23.6	1.06

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

¹Reference concentration from EURLMB analysis (n=20) using a validated and accredited methodology.

Table 9a. Performance characteristics for AZA3 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
CRM-INDIVIDUAL_QUANTITATION_UNCORRECTED RESULTS (against AZA1 calibrant)

Material		N° of labs a(b)	Mean µg/kg	Recovery, %	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	8(2)	30.1	-	12.0	42.6	1.57
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	12(3)	95.3	-	13.3	25.4	1.12
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	12(3)	106	-	7.68	20.4	0.91

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 9b. Performance characteristics for AZA3 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
MULTITOXIN_QUANTITATION_UNCORRECTED RESULTS (against AZA3 calibrant)

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	13(1)	22.9	-	10.3	31.4	1.11
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	14(1)	102	-	8.94	30.2	1.34
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	14(1)	114	-	7.09	23.5	1.06

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 9c. Performance characteristics for AZA3 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
MULTITOXIN_QUANTITATION_MATRIX CORRECTION RESULTS (against AZA3 calibrant)

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	13(1)	21.7	-	11.2	26.9	0.94
DAY2-EURLMB/VAL/L-09&10	<i>Mytilus edulis</i> (cooked mussel)	15(0)	97.4	-	9.38	26.3	1.16
DAY3-EURLMB/VAL/L-12&13	<i>Mytilus edulis</i> (cooked mussel)	13(1)	105	-	8.07	19.7	0.88

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

5.2.3. PTX2 results

Tables 10a, 10b, 10c and 10d present, respectively, performance results obtained for PTX2 determination using the different quantification approaches described.

Table 10a. Performance characteristics for PTX2 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
CRM-INDIVIDUAL_QUANTITATION_UNCORRECTED RESULTS

Material		N° of labs a(b)	Mean µg/kg	Recovery ¹ , %	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	13(0)	82.9	114	12.6	32.8	1.41
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	13(0)	77.7	72.8	9.86	34.0	1.45

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

¹Reference concentration from EURLMB analysis (n=20) using a validated and accredited methodology.

Table 10b. Performance characteristics for PTX2 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
CRM-INDIVIDUAL_QUANTITATION_MATRIX CORRECTION RESULTS

Material		N° of labs a(b)	Mean µg/kg	Recovery ¹ , %	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	13(0)	90.4	124	10.5	38.6	1.68
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	12(1)	76.7	71.9	8.52	28.6	1.21

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

¹Reference concentration from EURLMB analysis (n=20) using a validated and accredited methodology.

Table 10c. Performance characteristics for PTX2 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
MULTITOXIN_QUANTITATION_UNCORRECTED RESULTS

Material		N° of labs a(b)	Mean µg/kg	Recovery ¹ , %	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	12(0)	98.9	135	12.1	26.1	1.15
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	12(0)	87.0	81.5	8.53	24.9	1.08

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

¹Reference concentration from EURLMB analysis (n=20) using a validated and accredited methodology.

Table 10d. Performance characteristics for PTX2 determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
MULTITOXIN_QUANTITATION_MATRIX CORRECTION RESULTS

Material		N° of labs a(b)	Mean µg/kg	Recovery ¹ , %	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-01&03	<i>Donax trunculus</i> (wedge shell clam)	12(0)	96.4	132	10.1	30.7	1.35
DAY2-EURLMB/VAL/L-07&08	<i>Chamelea gallina</i> (stripped venus)	11(1)	81.1	76.0	6.70	18.5	0.79

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

¹Reference concentration from EURLMB analysis (n=20) using a validated and accredited methodology.

5.2.4. YTX results

Tables 11a, 11b, 11c and 11d show, respectively, performance results obtained for YTX determination using the different quantification approaches described.

Table 11a. Performance characteristics for YTX determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
CRM-INDIVIDUAL QUANTITATION UNCORRECTED RESULTS

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	11(0)	111	-	9.16	35.2	1.58

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 11b. Performance characteristics for YTX determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
CRM-INDIVIDUAL QUANTITATION MATRIX CORRECTION RESULTS

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	11(0)	104	-	9.19	29.3	1.30

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 11c. Performance characteristics for YTX determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
MULTITOXIN QUANTITATION UNCORRECTED RESULTS

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	12(1)	105	-	9.27	27.7	1.23

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 11d. Performance characteristics for YTX determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.
MULTITOXIN QUANTITATION MATRIX CORRECTION RESULTS

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY1-EURLMB/VAL/L-04&06	<i>Mytilus edulis</i> (raw mussel)	12(0)	101	-	8.82	29.2	1.29

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

5.2.5. NRC RM-FDMT results

Tables 12a, 12b, 12c, 12d, 12e and 12f show performance results obtained for lipophilic marine biotoxins determination in the NRC RM-FDMT using the different quantification approaches studied.

For OA-group toxins determination, only free toxin concentrations in the NRC RM-FDMT were evaluated, since those are the values assigned by the manufacturer in the material. For this group, CRM-Individual calibration was evaluated by quantifying OA, DTX1 and DTX2 against the commercially available OA calibrant solution. On the other hand, the Multitoxin calibration approach was evaluated by using the RM-multitoxin reference standard provided by NRC for this study. In this case, each toxin was quantified against its own calibrant. For each toxin and calibration approach, uncorrected and corrected results (both by using CRM-MUS recovery correction and spiked extract matrix correction) were evaluated.

For AZA-group toxins determination, only CRM-Individual calibration approach was evaluated since the calibration range for AZA1, AZA2 and AZA3 with the Multitoxin calibration approach was far below the content of these toxins in the RM-FDMT. This means that AZA1, AZA2 and AZA3 were quantified against the commercially available AZA1 calibrant solution. Regarding different correction approaches, the spiked extract matrix correction was only evaluated for AZA1 since for AZA2 and AZA3 concentrations in the spiked extract was lower than the lowest calibration point for most laboratories.

For PTX2 and YTX determination, results for both calibration approaches (using CRM-Individual standard and the RM-multitoxin reference standard), uncorrected and applying spiked extract matrix correction were evaluated.

Table 12a. Performance characteristics for lipophilic marine biotoxins determination in the NRC RM-FDMT obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.

CRM-INDIVIDUAL_QUANTITATION_UNCORRECTED RESULTS

FDMT		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
TOXIN	Reference value ⁽¹⁾ (µg/kg)					RSD _R %	HorRat
OA*	230	14(1)	307	134	18.6	31.6	1.65
DTX1*	120	13(2)	163	136	19.1	29.1	1.38
DTX2*	560	14(1)	913	163	13.3	30.2	1.86
AZA1	700	14(0)	609	87.1	11.0	24.5	1.42
AZA2	160	13(2)	151	94.3	13.4	26.7	1.25
AZA3	160	12(3)	150	93.6	15.1	26.6	1.25
PTX2	120	13(0)	98.4	82.0	16.4	35.7	1.57
YTX	420	12(0)	371	88.2	21.0	35.2	1.90

⁽¹⁾Reference concentration assigned by NRC-CNRC Canada to FDMT (they are not final certified values).

*Free toxin concentration

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 12b. Performance characteristics for lipophilic marine biotoxins determination in the NRC RM-FDMT obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.

CRM-INDIVIDUAL_QUANTITATION_CRM MUS CORRECTION RESULTS

FDMT		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
TOXIN	Reference value ⁽¹⁾ (µg/kg)					RSD _R %	HorRat
OA*	230	11(4)	274	119	12.8	15.8	0.81
DTX1*	120	13(2)	135	112	16.5	26.5	1.23
DTX2*	560	13(2)	826	148	17.5	27.6	1.67

⁽¹⁾Reference concentration assigned by NRC-CNRC Canada to FDMT (they are not final certified values).

*Free toxin concentration

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 12c. Performance characteristics for lipophilic marine biotoxins determination in the NRC RM-FDMT obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.

CRM-INDIVIDUAL QUANTITATION MATRIX CORRECTION RESULTS

FDMT		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
TOXIN	Reference value ⁽¹⁾ (µg/kg)					RSD _R %	HorRat
OA*	230	13(2)	236	103	16.1	22.4	1.13
DTX1*	120	14(1)	116	96.3	20.1	29.3	1.32
DTX2*	560	13(2)	596	106	15.9	20.9	1.21
AZA1	700	11(3)	584	83.5	10.5	13.3	0.77
PTX2	120	13(0)	99.3	82.8	12.9	21.8	0.96
YTX	420	11(0)	361	85.9	13.9	34.5	1.85

⁽¹⁾Reference concentration assigned by NRC-CNRC Canada to FDMT (they are not final certified values).

*Free toxin concentration

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 12d. Performance characteristics for lipophilic marine biotoxins determination in the NRC RM-FDMT obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.

MULTITOXIN QUANTITATION UNCORRECTED RESULTS

FDMT		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
TOXIN	Reference value ⁽¹⁾ (µg/kg)					RSD _R %	HorRat
OA*	230	13(2)	287	125	15.7	27.8	1.44
DTX1*	120	13(2)	155	129	18.6	28.6	1.35
DTX2*	560	13(2)	760	136	15.4	26.0	1.56
PTX2	120	13(0)	104	86.8	19.6	39.9	1.77
YTX	420	13(0)	365	87.0	13.1	34.3	1.85

⁽¹⁾Reference concentration assigned by NRC-CNRC Canada to FDMT (they are not final certified values).

*Free toxin concentration

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 12e. Performance characteristics for lipophilic marine biotoxins determination in the NRC RM-FDMT obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.

MULTITOXIN_QUANTITATION_CRM_MUS_CORRECTION_RESULTS

FDMT		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
TOXIN	Reference value ⁽¹⁾ (µg/kg)					RSD _R %	HorRat
OA*	230	11(4)	276	120	11.4	15.5	0.80
DTX1*	120	13(2)	135	113	16.6	27.6	1.28
DTX2*	560	14(1)	725	130	16.6	26.9	1.60

⁽¹⁾Reference concentration assigned by NRC-CNRC Canada to FDMT (they are not final certified values).

*Free toxin concentration

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

Table 12f. Performance characteristics for lipophilic marine biotoxins determination in the NRC RM-FDMT obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS”.

MULTITOXIN_QUANTITATION_MATRIX_CORRECTION_RESULTS

FDMT		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
TOXIN	Reference value ⁽¹⁾ (µg/kg)					RSD _R %	HorRat
OA*	230	12(3)	227	98.5	12.4	16.9	0.84
DTX1*	120	13(2)	116	96.4	16.2	24.9	1.13
DTX2*	560	12(3)	568	101	16.0	18.9	1.09
PTX2	120	13(0)	97.8	81.5	15.8	24.4	1.07
YTX	420	11(1)	344	81.9	9.67	33.2	1.77

⁽¹⁾Reference concentration assigned by NRC-CNRC Canada to FDMT (they are not final certified values).

*Free toxin concentration

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

6. Conclusions

- Acceptable results were obtained for recovery and precision for all toxins analysed, either corrected or uncorrected.
- With regard to reproducibility, HorRat values of less than 1.5 were obtained for the majority of toxins with HorRat values less than 2 obtained for all toxins included in the study.

- For OA-group toxin determination, correction using certified reference material generally improves performance characteristics, both in terms of recovery and precision.
- The use of specific standards for DTX1, DTX2, AZA2 and AZA3 quantification is desirable when commercially available. However, while all toxins are not commercially available, the use of indirect quantification is an optional and reliable tool for lipophilic marine biotoxins quantification.
- The reliability of the “EU-Harmonised Standard Operating Procedure for determination of marine lipophilic biotoxins in molluscs by LC-MS/MS” was shown

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8. Reference Documents

- EU-Harmonised Standard Operating Procedure for determination of Lipophilic marine toxins in molluscs by LC-MS/MS. Version 2. July 2010.
- AOAC Guidelines for collaborative study procedures to validate characteristics of a method of analysis (appendix D). 2002. AOAC International.

APPENDIX 1. Performance results obtained in the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of OA-Group Toxin by LC-MS/MS”

Appendix 1-Table 1. Performance characteristics for **OA** determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of OA-Group Toxin by LC-MS/MS”.

Material		N° of labs a(b)	Mean ⁽¹⁾ µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY 1-VALIDATION OA-001	Mussel	8(0)	39.4	71.3	-	11.9	0.46
DAY 1-VALIDATION OA-002	Mussel	8(0)	70.6	89.4	-	13.9	0.58
DAY 1-VALIDATION OA-003	Mussel	8	<LOQ	-	-	-	-
DAY 1-VALIDATION OA-004	Mussel	8(0)	150.4	84.1	-	17.3	0.81
DAY 1-VALIDATION OA-005	Mussel	8(0)	224.2	83.6	-	15.3	0.76
DAY 2-VALIDATION OA-006&011	Mussel	11(1)	110.6	-	7.9	33.1	1.48
DAY 2-VALIDATION OA-007&010	Mussel	12	ND	-	-	-	-
DAY 2-VALIDATION OA-008&009	Cockle	10(2)	155.2	-	7.5	18.4	0.87
DAY 3-VALIDATION OA-012&015	Mussel	12(0)	202.4	-	7.0	26.3	1.29
DAY 3-VALIDATION OA-013&014	Clam	11(1)	72.2	-	8.8	25.9	1.09

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

⁽¹⁾ Free OA for samples DAY 1-VALIDATION OA-001 to DAY 1-VALIDATION OA-005 and total OA for samples DAY 1-VALIDATION OA-006 to DAY 1-VALIDATION OA-015.

Appendix 1-Table 2. Performance characteristics for **DTX1** determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of OA-Group Toxin by LC-MS/MS”.

Material		N° of labs a(b)	Mean ⁽¹⁾ µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY 1-VALIDATION OA-005	Mussel	6(0)	35.3 ⁽²⁾	114.3	-	17.8	0.67
DAY 2-VALIDATION OA-006&011	Mussel	11(0)	58.0	-	16.4	30.1	1.23

a= Number of laboratories remaining after removal of the number of outliers indicated by (b).

⁽¹⁾ Free DTX1 for sample DAY 1-VALIDATION OA-005 and total DTX1 for sample DAY 1-VALIDATION OA-006&011.

⁽²⁾ Evaluation of performance characteristics was carried out only with six valid results since some participants has not enough sensibility to quantify DTX1 at this level.

Appendix 1-Table 3. Performance characteristics for **DTX2** determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of OA-Group Toxin by LC-MS/MS”.

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY 2-VALIDATION OA-008&009	Cockle	11(0)	131.1	-	7.2	42.9	1.97

a= Number of laboratories remaining after removal of the number of outliers indicated by (b)

Appendix 1-Table 4. Performance characteristics for **OA-group toxin** determination obtained from the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of OA-Group Toxin by LC-MS/MS”.

Material		N° of labs a(b)	Mean µg/kg	Recovery,%	Repeatability RSD _r %	Reproducibility	
Sample Code	Matrix					RSD _R %	HorRat
DAY 2-VALIDATION OA-006&011	Mussel	11(1)	175.5	-	9.1	33.9	1.63
DAY 2-VALIDATION OA-007&010	Mussel	11	ND	-	-	-	-
DAY 2-VALIDATION OA-008&009	Cockle	12(0)	246.1	-	6.7	35.4	1.79
DAY 3-VALIDATION OA-013&014	Clam	11(1)	72.2	-	8.8	25.9	1.09

a= Number of laboratories remaining after removal of the number of outliers indicated by (b)

APPENDIX 2. Raw data provided by participants for the Interlaboratory Validation Study of the “EU-Harmonised Standard Operating Procedure for determination of OA-Group Toxin by LC-MS/MS”

Appendix 2.1. OA-group toxin results

Appendix 2.1-Table 1. Results provided by participants for samples DAY 1-EURLMB/VAL/L-01 and DAY 1-EURLMB/VAL/L-03 (blind duplicates) for OA in µg/kg by CRM-Individual standard quantitation.

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		CRM-INDIVIDUAL_CRM-Mus b	
	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03
A	171.6	152.6	158.3	140.8	159.7	142.0
B	153.5	160.2	109.1	113.9	(1)	(1)
D	140.1	156.8	77.1	86.3	132.2	147.9
E	209.2	222.0	138.7	147.2	215.7	228.9
F	150.3	162.1	96.0	103.5	124.9	134.7
G	145.4	102.4	119.3	84.0	159.8	112.5
H	180.2	175.5	188.3	183.5	191.1	186.2
I	155.6	148.0	159.7	151.9	190.1	180.8
J	138.2	157.6	135.3	154.4	192.3	219.3
K	164.7	187.8	147.7	168.4	144.0	164.2
L	105.7	136.0	120.1	154.6	78.7	101.2
M	243.0	261.0	134.7	144.7	208.9	224.4
N	251.8	249.3	190.9	189.0	233.6	231.3
O	251.4	285.6	151.3	171.8	153.5	174.4
P	297.1	221.4	216.8	161.5	264.5	197.1

⁽¹⁾ Non valid data (OA results out of the calibration range in CRM-Mus b for OA recovery correction)

Appendix 2.1-Table 2. Results provided by participants for samples DAY 1-EURLMB/VAL/L-01 and DAY 1-EURLMB/VAL/L-03 (blind duplicates) for OA in µg/kg by Multitoxin standard quantitation.

QUANTITATION LAB CODE	MULTITOXIN _Uncorrected		MULTITOXIN _Matrix correction		MULTITOXIN _CRM-Mus b	
	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03
A	165.6	148.4	155.1	139.0	163.0	146.1
B	125.7	132.1	107.8	113.2	(1)	(1)
D	116.6	133.7	71.3	81.8	119.7	137.3
E	201.4	213.4	137.7	145.9	217.5	230.5
F	132.3	142.6	96.1	103.6	125.3	135.1
G	132.6	92.8	119.3	83.5	159.1	111.4
H	191.3	186.4	187.7	182.9	192.0	187.1
I	160.0	153.0	153.8	147.1	194.6	186.2
J	191.1	211.1	130.7	144.4	202.5	223.7
K	146.6	169.0	151.0	174.1	139.0	160.3
L	104.9	138.0	120.3	158.2	73.7	97.0
M	232.4	247.8	132.7	141.4	212.0	226.0
N	271.8	269.4	178.9	177.3	234.3	232.2
O	180.9	204.1	148.9	168.0	156.8	176.9
P	277.8	207.0	216.6	161.4	264.4	197.1

⁽¹⁾ Non valid data (OA results out of the calibration range in CRM-Mus b for OA recovery correction)

Appendix 2.1-Table 3. Results provided by participants for samples DAY 1-EURLMB/VAL/L-02 and DAY 1-EURLMB/VAL/L-05 (blind duplicates) for OA in µg/kg by CRM-Individual standard quantitation.

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		CRM-INDIVIDUAL_CRM-Mus b	
	DAY 1- EURLMB/VAL/L- 02	DAY 1- EURLMB/VAL/L- 05	DAY 1- EURLMB/VAL/L- 02	DAY 1- EURLMB/VAL/L- 05	DAY 1- EURLMB/VAL/L- 02	DAY 1- EURLMB/VAL/L- 05
A	46.0	57.6	42.4	53.2	42.8	53.6
B	80.8	91.2	57.5	64.8	(1)	(1)
D	76.6	66.5	42.2	36.6	72.2	62.7
E	80.3	80.7	53.3	53.6	82.8	83.3
F	114.6	123.1	73.2	78.6	95.2	102.4
G	86.2	54.9	70.7	45.0	94.7	60.3
H	151.8	140.5	158.7 ⁽³⁾	146.8 ⁽³⁾	161.0	149.0
I	69.2	72.0	71.0	73.9	84.6	88.0
J	47.5	67.0	46.5	65.6	66.1	93.2
K	91.6	91.9	82.1	82.4	80.1	80.4
L	59.7	82.4	67.8	93.7	44.4	61.3
M	166.7 ⁽²⁾	99.2 ⁽²⁾	92.4	55.0	143.3	85.3
N	269.9 ⁽³⁾	319.2 ⁽³⁾	204.7 ⁽³⁾	242.0 ⁽³⁾	250.4 ⁽³⁾	296.1 ⁽³⁾
O	105.9	91.5	63.8	55.1	64.7	55.9
P	248.9 ⁽²⁾	120.7 ⁽²⁾	181.6 ⁽²⁾	88.1 ⁽²⁾	221.5 ⁽²⁾	107.5 ⁽²⁾

⁽¹⁾ Non valid data (OA results out of the calibration range in CRM-Mus b for OA recovery correction)

⁽²⁾ Cochran outlier

⁽³⁾ Single Grubbs outlier

Appendix 2.1-Table 4. Results provided by participants for samples DAY 1-EURLMB/VAL/L-02 and DAY 1-EURLMB/VAL/L-05 (blind duplicates) for OA in µg/kg by Multitoxin standard quantitation.

QUANTITATION LAB CODE	MULTITOXIN _Uncorrected		MULTITOXIN _Matrix correction		MULTITOXIN _CRM-Mus b	
	DAY 1- EURLMB/VAL/L- 02	DAY 1- EURLMB/VAL/L- 05	DAY 1- EURLMB/VAL/L- 02	DAY 1- EURLMB/VAL/L- 05	DAY 1- EURLMB/VAL/L- 02	DAY 1- EURLMB/VAL/L- 05
A	51.8	62.4	48.6	58.5	51.0	61.4
B	57.4	67.1	49.2	57.5	(1)	(1)
D	51.4	41.1	31.5	25.1	52.8	42.2
E	85.2	85.3	58.2	58.3	92.0	92.2
F	101.0	108.5	73.4	78.9	95.7	102.8
G	77.9	49.0	70.1	44.1	93.5	58.8
H	161.5	149.7	158.5 ⁽³⁾	146.8 ⁽³⁾	162.2	150.2
I	81.0	83.6	77.9	80.4	98.6	101.7
J	97.4	117.5	66.7	80.4	103.3	124.5
K	75.7	76.0	77.9	78.3	71.7	72.0
L	54.7	79.5	62.7	91.2	38.5	55.9
M	167.8 ⁽²⁾	110.5 ⁽²⁾	95.7	63.1	153.0	100.8
N	289.2 ⁽³⁾	336.4 ⁽³⁾	190.3 ⁽³⁾	221.4 ⁽³⁾	249.3 ⁽³⁾	290.0 ⁽³⁾
O	82.4	72.6	67.8	59.8	71.4	62.9
P	232.7 ⁽²⁾	113.0 ⁽²⁾	181.5 ⁽²⁾	88.1 ⁽²⁾	221.5 ⁽²⁾	107.6 ⁽²⁾

⁽¹⁾ Non valid data (OA results out of the calibration range in CRM-Mus b for OA recovery correction)

⁽²⁾ Cochran outlier

⁽³⁾ Single Grubbs outlier

Appendix 2.1-Table 5. Results provided by participants for samples DAY 1-EURLMB/VAL/L-04 and DAY 1-EURLMB/VAL/L-06 (blind duplicates) for OA in µg/kg by CRM-Individual standard quantitation.

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		CRM-INDIVIDUAL_CRM-Mus b	
	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06
A	44.9	56.5	41.5	52.1	41.8	52.6
B	88.9	85.8	63.2	60.9	(1)	(1)
D	92.4	102.3	50.9	56.3	87.2	96.5
E	102.8	97.0	68.2	64.3	106.0	100.0
F	62.9	88.3	40.2	56.4	52.3	73.4
G	120.5 ⁽²⁾	49.9 ⁽²⁾	98.9 ⁽²⁾	40.9 ⁽²⁾	132.4 ⁽²⁾	54.8 ⁽²⁾
H	73.9	68.3	77.3	71.4	78.4	72.4
I	62.5	78.7	64.2	80.8	76.4	96.2
J	65.2	72.8	63.8	71.3	90.7	101.3
K	82.5	88.4	74.0	79.3	72.1	77.3
L	54.3	64.0	61.7	72.7	40.4	47.6
M	169.4	132.5	93.9	73.5	145.7	113.9
N	(1)	141.4 ⁽¹⁾	(1)	107.2 ⁽¹⁾	(1)	131.2 ⁽¹⁾
O	104.4	114.3	62.8	68.8	63.7	69.8
P	98.2	85.2	71.7	62.2	87.4	75.8

⁽¹⁾ Non valid data (Lab B: OA results out of the calibration range in CRM-Mus b for OA recovery correction; Lab N: no replicated results provided for evaluation)

⁽²⁾ Cochran outlier

Appendix 2.1-Table 6. Results provided by participants for samples DAY 1-EURLMB/VAL/L-04 and DAY 1-EURLMB/VAL/L-06 (blind duplicates) for OA in µg/kg by Multitoxin standard quantitation.

QUANTITATION LAB CODE	MULTITOXIN _Uncorrected		MULTITOXIN _Matrix correction		MULTITOXIN _CRM-Mus b	
	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06
A	50.9	61.3	47.7	57.5	50.1	60.4
B	64.9	62.0	55.7	53.1	(1)	(1)
D	67.7	77.8	41.4	47.6	69.5	79.9
E	105.5	100.1	72.1	68.4	114.0	108.1
F	55.9	78.1	40.6	56.7	52.9	74.0
G	109.6 ⁽²⁾	44.4 ⁽²⁾	98.6 ⁽²⁾	39.9 ⁽²⁾	131.5 ⁽²⁾	53.2 ⁽²⁾
H	80.0	74.0	78.5	72.7	80.3	74.3
I	74.9	89.7	72.0	86.2	91.2	109.1
J	115.7 ⁽⁴⁾	123.6 ⁽⁴⁾	79.1	84.5	122.6	131.0
K	66.8	72.6	68.8	74.8	63.4	68.8
L	48.8	59.4	56.0	68.1	34.3	41.7
M	170.0 ⁽⁴⁾	138.7 ⁽⁴⁾	97.1	79.2	155.1	126.5
N	49.8 ⁽²⁾	165.8 ⁽²⁾	32.8 ⁽²⁾	109.1 ⁽²⁾	42.9 ⁽²⁾	143.0 ⁽²⁾
O	81.3	88.0	66.9	72.4	70.5	76.3
P	92.0	79.8	71.7	62.3	87.6	76.0

⁽¹⁾ Non valid data (OA results out of the calibration range in CRM-Mus b for OA recovery correction)

⁽²⁾ Cochran outlier

⁽⁴⁾ Double Grubbs outlier

Appendix 2.1-Table 7. Results provided by participants for samples DAY 2-EURLMB/VAL/L-07 and DAY 2-EURLMB/VAL/L-08 (blind duplicates) for OA in µg/kg by CRM-Individual standard quantitation.

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		CRM-INDIVIDUAL_CRM-Mus b	
	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08
A	216.1	221.1	154.1	157.6	172.2	176.1
B	60.3 ⁽²⁾	166.5 ⁽²⁾	43.7	120.8	(1)	(1)
D	156.7	160.9	73.9	75.9	142.8	146.5
E	179.8	183.6	141.9	144.9	182.1	185.9
F	242.0	234.0	181.5	175.5	208.3	201.4
G	173.5	138.9	146.5	117.3	197.1	157.8
H	197.0	198.3	188.8	190.0	187.2	188.4
I	172.3	128.1	179.1	133.1	215.8	160.4
J	106.0	124.4	117.2	137.6	139.8	164.1
K	112.8	98.7	257.5	225.3	144.2	126.2
L	61.0	66.7	67.9	74.2	62.3 ⁽⁴⁾	68.2 ⁽⁴⁾
M	175.4	210.7	135.9	163.2	155.0	186.1
N	231.5	233.0	234.9	236.5	271.1 ⁽⁴⁾	272.8 ⁽⁴⁾
O	234.5	268.0	175.4	200.5	144.6	165.3
P	288.3	273.9	197.1	187.3	231.7	220.2

⁽¹⁾ Non valid data (OA results out of the calibration range in CRM-Mus b for OA recovery correction)

⁽⁴⁾ Double Grubbs outlier

Appendix 2.1-Table 8. Results provided by participants for samples DAY 2-EURLMB/VAL/L-07 and DAY 2-EURLMB/VAL/L-08 (blind duplicates) for OA in µg/kg by Multitoxin standard quantitation.

QUANTITATION LAB CODE	MULTITOXIN _Uncorrected		MULTITOXIN _Matrix correction		MULTITOXIN _CRM-Mus b	
	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08
A	161.9	166.9	170.1	175.3	158.0	162.8
B	43.1 ⁽²⁾	147.4 ⁽²⁾	35.3 ⁽²⁾	121.0 ⁽²⁾	(1)	(1)
D	130.2	134.7	65.7	68.0	125.9	130.3
E	179.6	183.1	139.8	142.6	187.2	190.9
F	204.7	198.0	180.7	174.8	208.5	201.6
G	165.7	131.4	147.8	117.2	195.8	155.3
H	211.2	212.6	187.8	189.0	188.6	189.8
I	152.5	112.9	180.2	133.4	215.5	159.5
J	154.2	172.4	117.7	131.6	161.4	180.5
K	111.5	95.5	309.2	265.0	136.5	117.0
L	44.5	50.8	55.8	63.7	45.3 ⁽⁴⁾	51.8 ⁽⁴⁾
M	194.0	227.6	133.3	156.4	162.8	191.1
N	281.5	283.0	200.7	201.7	265.1 ⁽⁴⁾	266.5 ⁽⁴⁾
O	190.2	213.2	163.3	183.0	154.1	172.7
P	262.6	249.7	195.3	185.7	231.8	220.4

⁽¹⁾ Non valid data (OA results out of the calibration range in CRM-Mus b for OA recovery correction)

⁽²⁾ Cochran outlier

⁽⁴⁾ Double Grubbs outlier

Appendix 2.1-Table 9. Results provided by participants for samples DAY 2-EURLMB/VAL/L-09 and DAY 2-EURLMB/VAL/L-10 (blind duplicates) for OA in µg/kg by CRM-Individual standard quantitation.

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		CRM-INDIVIDUAL_CRM-Mus b	
	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10
A	425.5	393.5	303.3	280.5	339.0	313.5
B	369.5	346.1	268.0	251.0	(1)	(1)
D	401.5	419.2	189.4	197.7	365.7	381.8
E	431.1	448.4	340.3	354.0	436.5	454.0
F	585.3	586.9	439.0	440.2	503.7	505.1
G	365.1	305.5	308.3	258.0	414.7	347.0
H	457.4	443.4	438.4	424.9	434.6	421.3
I	366.6	364.8	381.1	379.2	459.2	456.9
J	269.1	262.4	297.7	290.3	354.9	346.2
K	277.6	298.1	633.6	680.5	354.9	381.2
L	156.7	168.1	174.4 ⁽⁴⁾	187.0 ⁽⁴⁾	160.2 ⁽³⁾	171.8 ⁽³⁾
M	453.8	522.6	351.5	404.7	401.0	461.7
N	773.5	760.3	785.1 ⁽⁴⁾	771.6 ⁽⁴⁾	905.8 ⁽³⁾	890.3 ⁽³⁾
O	640.2	601.8	479.0	450.2	394.8	371.1
P	530.9	513.4	363.0	351.0	426.7	412.6

⁽¹⁾ Non valid data (OA results out of the calibration range in CRM-Mus b for OA recovery correction)

⁽³⁾ Single Grubbs outlier

⁽⁴⁾ Double Grubbs outlier

Appendix 2.1-Table 10. Results provided by participants for samples DAY 2-EURLMB/VAL/L-09 and DAY 2-EURLMB/VAL/L-10 (blind duplicates) for OA in µg/kg by Multitoxin standard quantitation.

QUANTITATION LAB CODE	MULTITOXIN _Uncorrected		MULTITOXIN _Matrix correction		MULTITOXIN _CRM-Mus b	
	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10
A	368.6	337.0	387.2	354.0	359.6	328.8
B	346.9	323.8	284.7	265.8	(1)	(1)
D	400.7	420.3	202.4	212.2	387.5	406.4
E	406.0	421.0	316.1	327.9	423.2	438.9
F	492.9	494.3	435.1	436.3	501.9	503.3
G	355.3	296.3	316.9	264.3	419.9	350.2
H	484.8	470.0	431.0	417.8	432.9	419.7
I	326.8	325.1	386.1	384.1	461.7	459.3
J	315.4	308.8	240.6	235.6	330.1	323.2
K	297.5	320.7	825.3 ⁽³⁾	889.7 ⁽³⁾	364.2	392.7
L	149.7	162.2	187.6	203.3	152.6 ⁽³⁾	165.3 ⁽³⁾
M	460.0	525.7	316.0	361.2	386.1	441.3
N	825.6 ⁽³⁾	812.2 ⁽³⁾	588.5	579.0	777.4 ⁽³⁾	764.9 ⁽³⁾
O	468.2	441.8	401.9	379.3	379.3	358.0
P	484.1	468.4	360.1	348.4	427.4	413.6

⁽¹⁾ Non valid data (OA results out of the calibration range in CRM-Mus b for OA recovery correction)

⁽³⁾ Single Grubbs outlier

Appendix 2.1-Table 11. Results provided by participants for samples DAY 3-EURLMB/VAL/L-11 and DAY 3-EURLMB/VAL/L-14 (blind duplicates) for OA in µg/kg by CRM-Individual standard quantitation.

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		CRM-INDIVIDUAL_CRM-Mus b	
	DAY 3- EURLMB/VAL/L- 11	DAY 3- EURLMB/VAL/L- 14	DAY 3- EURLMB/VAL/L- 11	DAY 3- EURLMB/VAL/L- 14	DAY 3- EURLMB/VAL/L- 11	DAY 3- EURLMB/VAL/L- 14
A	131.3	111.5	(1)	(1)	141.5	120.2
B	163.7	155.5	144.7	137.5	(1)	(1)
D	104.4	136.2	62.8	81.9	96.5	125.9
E	150.9	137.6	106.6	97.3	158.7	144.7
F	175.8	163.5	105.6	98.2	152.2	141.5
G	108.5	61.7	99.2	56.4	95.4	54.3
H	172.7	168.9	149.1	145.8	153.2	149.8
I	107.1	110.2	90.1	92.7	114.9	118.2
J	154.5	154.6	195.8	195.9	218.1	218.2
K	115.2	126.7	103.5	113.8	91.6	100.7
L	38.9	70.8	35.2	64.2	29.9	54.6
M	174.5	284.8	92.6	151.0	163.3 ⁽²⁾	266.6 ⁽²⁾
N	207.5	329.1	313.5 ⁽²⁾	497.2 ⁽²⁾	253.4 ⁽²⁾	401.9 ⁽²⁾
O	215.0	201.0	162.8	152.2	141.5	132.3
P	226.4	172.7	148.9	113.6	174.0	132.7

⁽¹⁾ Non valid data (Lab A: OA results for spiked extract no provided for matrix correction; Lab B: OA results out of the calibration range in CRM-Mus b for OA recovery correction)

⁽²⁾ Cochran outlier

Appendix 2.1-Table 12. Results provided by participants for samples DAY 3-EURLMB/VAL/L-11 and DAY 3-EURLMB/VAL/L-14 (blind duplicates) for OA in µg/kg by Multitoxin standard quantitation.

QUANTITATION LAB CODE	MULTITOXIN _Uncorrected		MULTITOXIN _Matrix correction		MULTITOXIN _CRM-Mus b	
	DAY 3- EURLMB/VAL/L- 11	DAY 3- EURLMB/VAL/L- 14	DAY 3- EURLMB/VAL/L- 11	DAY 3- EURLMB/VAL/L- 14	DAY 3- EURLMB/VAL/L- 11	DAY 3- EURLMB/VAL/L- 14
A	123.0	102.1	(1)	(1)	134.4	111.5
B	142.0	135.1	143.8	136.8	(1)	(1)
D	98.4	126.1	65.1	83.5	101.1	129.6
E	148.8	136.4	108.7	99.6	165.6	151.8
F	148.0	137.8	105.7	98.5	153.1	142.5
G	88.9	45.7	97.2	50.0	88.8	45.7
H	173.4	169.7	149.1	145.9	155.8	152.5
I	98.1	100.9	90.0	92.6	114.7	118.0
J	198.2	198.2	175.3	175.4	221.8	221.8
K	87.9	97.8	101.8	113.2	84.9	94.4
L	34.1	73.5	27.4	59.1	22.1	47.6
M	157.1 ⁽²⁾	260.1 ⁽²⁾	91.8	152.0	161.4	267.3
N	255.4 ⁽³⁾	392.5 ⁽³⁾	275.7 ⁽²⁾	423.7 ⁽²⁾	252.2	387.7
O	164.0	154.7	154.9	146.2	150.0	141.5
P	195.6	148.6	149.4	113.5	173.4	131.7

⁽¹⁾ Non valid data (Lab A: OA results for spiked extract no provided for matrix correction; Lab B: OA results out of the calibration range in CRM-Mus b for OA recovery correction)

⁽²⁾ Cochran outlier

⁽³⁾ Single Grubbs outlier

Appendix 2.1-Table 13. Results provided by participants for samples DAY 3-EURLMB/VAL/L-12 and DAY 3-EURLMB/VAL/L-13 (blind duplicates) for OA in µg/kg by CRM-Individual standard quantitation.

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		CRM-INDIVIDUAL_CRM-Mus b	
	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13
A	243.2	263.5	(1)	(1)	262.0	283.8
B	340.7	345.2	301.2	305.1	(1)	(1)
D	311.6	310.8	187.5	187.0	288.1	287.4
E	348.2	359.4	246.0	254.0	366.1	377.9
F	420.7	417.6	252.6	250.7	364.2	361.5
G	164.1 ⁽²⁾	332.9 ⁽²⁾	149.9 ⁽²⁾	304.2 ⁽²⁾	144.3 ⁽²⁾	292.7 ⁽²⁾
H	377.7	353.4	326.0	305.0	335.0	313.5
I	287.6	265.9	242.0	223.8	308.6	285.4
J	293.8	307.6	372.3	389.8	414.8	434.3
K	270.7	287.7	243.1	258.4	215.1	228.7
L	86.1	108.5	78.0	98.4	66.3	83.6
M	467.6	470.8	248.0	249.6	437.7	440.6
N	574.6 ⁽²⁾	679.9 ⁽²⁾	868.2 ⁽³⁾	1027.2 ⁽³⁾	701.7 ⁽³⁾	830.3 ⁽³⁾
O	567.4	512.0	429.7	387.7	373.4	336.9
P	387.2	383.3	254.7	252.1	297.7	294.6

⁽¹⁾ Non valid data (Lab A: OA results for spiked extract no provided for matrix correction; Lab B: OA results out of the calibration range in CRM-Mus b for OA recovery correction)

⁽²⁾ Cochran outlier

⁽³⁾ Single Grubbs outlier

Appendix 2.1-Table 14. Results provided by participants for samples DAY 3-EURLMB/VAL/L-12 and DAY 3-EURLMB/VAL/L-13 (blind duplicates) for OA in µg/kg by Multitoxin standard quantitation.

QUANTITATION LAB CODE	MULTITOXIN _Uncorrected		MULTITOXIN _Matrix correction		MULTITOXIN _CRM-Mus b	
	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13
A	241.4	262.9	(1)	(1)	263.6	287.0
B	290.9	294.6	294.6	298.4	(1)	(1)
D	279.0	278.3	184.6	184.2	286.6	285.9
E	322.7	333.0	235.7	243.2	359.2	370.7
F	351.0	348.4	250.8	248.9	363.0	360.3
G	140.0 ⁽²⁾	295.4 ⁽²⁾	153.1 ⁽²⁾	323.1 ⁽²⁾	139.8 ⁽²⁾	295.1 ⁽²⁾
H	372.1	348.6	319.9	299.7	334.4	313.2
I	264.1	244.1	242.4	224.1	308.8	285.5
J	344.6	359.2	304.9	317.8	385.7	401.9
K	221.6	236.3	256.7	273.7	214.0	228.2
L	92.2	119.9	74.2	96.5	59.8	77.8
M	430.9	433.9	251.8	253.5	442.8	445.8
N	669.3 ⁽²⁾	788.0 ⁽²⁾	722.6 ⁽²⁾	850.7 ⁽²⁾	661.1 ⁽²⁾	778.4 ⁽²⁾
O	395.7	359.3	373.9	339.5	362.0	328.6
P	333.9	330.4	255.0	252.3	296.0	292.9

⁽¹⁾ Non valid data (Lab A: OA results for spiked extract no provided for matrix correction; Lab B: OA results out of the calibration range in CRM-Mus b for OA recovery correction)

⁽²⁾ Cochran outlier

Appendix 2.1-Table 15. Results provided by participants for samples DAY 1-EURLMB/VAL/L-01 and DAY 1-EURLMB/VAL/L-03 (blind duplicates) for DTX2 in µg/kg by CRM-Individual standard quantitation (against OA calibrant).

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		CRM-INDIVIDUAL_CRM-Mus b	
	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03
A	197.5	191.4	183.3	177.7	183.8	178.1
B	164.8	192.2	106.6	124.3	(1)	(1)
D	277.3	330.8	74.8	89.2	261.7	312.1
E	261.2	278.4	166.5	177.5	269.3	287.1
F	224.5	216.5	134.3	129.4	186.6	179.9
G	153.2	156.2	105.0	107.1	168.3	171.6
H	242.1	230.7	233.1	222.1	256.8	244.7
I	192.6	180.3	164.0	153.5	235.4	220.3
J	180.7	203.2	163.6	184.1	251.3	282.7
K	217.7	219.1	165.4	166.5	190.3	191.6
L	184.1	232.7	221.3	279.8	137.0	173.2
M	243.3	284.0	145.8	170.3	209.2	244.2
N	353.2	409.3	237.3	275.1	327.7	379.7
O	331.7	314.6	138.4	131.3	202.6	192.2
P	497.7 ⁽²⁾	353.0 ⁽²⁾	287.2	203.7	443.1 ⁽²⁾	314.2 ⁽²⁾

⁽¹⁾ Non valid data (OA results out of the calibration range in CRM-Mus b for DTX2 recovery correction)⁽²⁾ Cochran outlier

Appendix 2.1-Table 16. Results provided by participants for samples DAY 1-EURLMB/VAL/L-01 and DAY 1-EURLMB/VAL/L-03 (blind duplicates) for DTX2 in µg/kg by Multitoxin standard quantitation (against DTX2 calibrant).

QUANTITATION LAB CODE	MULTITOXIN _Uncorrected		MULTITOXIN _Matrix correction		MULTITOXIN _CRM-Mus b	
	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03
A	149.2	144.8	176.7	171.6	146.9	142.6
B	136.8	161.5	106.7	126.0	(1)	(1)
D	142.7	172.5	73.0	88.3	146.5	177.1
E	236.7	252.0	163.2	173.7	255.7	272.2
F	190.5	183.5	135.2	130.2	180.5	173.9
G	153.0	155.5	105.0	106.7	183.6	186.6
H	212.9	203.0	233.0	222.1	213.8	203.7
I	166.4	156.7	156.4	147.2	202.5	190.7
J	224.6	247.2	147.7	162.6	238.0	262.0
K	180.6	181.9	170.8	172.0	171.2	172.4
L	165.9	215.8	261.3	339.9	116.6	151.7
M	234.8	271.8	142.6	165.0	214.2	247.9
N	325.4 ⁽³⁾	377.8 ⁽³⁾	241.8	280.8	280.5	325.7
O	218.0	207.3	136.7	129.9	189.0	179.7
P	411.4 ⁽²⁾	289.4 ⁽²⁾	289.4	203.5	391.7 ⁽²⁾	275.5 ⁽²⁾

⁽¹⁾ Non valid data (OA results out of the calibration range in CRM-Mus b for DTX2 recovery correction)⁽²⁾ Cochran outlier⁽³⁾ Single Grubbs outlier

Appendix 2.1-Table 17. Results provided by participants for samples DAY 1-EURLMB/VAL/L-02 and DAY 1-EURLMB/VAL/L-05 (blind duplicates) for DTX2 in µg/kg by CRM-Individual standard quantitation (against OA calibrant).

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		CRM-INDIVIDUAL_CRM-Mus b	
	DAY 1- EURLMB/VAL/L- 02	DAY 1- EURLMB/VAL/L- 05	DAY 1- EURLMB/VAL/L- 02	DAY 1- EURLMB/VAL/L- 05	DAY 1- EURLMB/VAL/L- 02	DAY 1- EURLMB/VAL/L- 05
A	<LOQ	54.8 ⁽¹⁾	<LOQ	50.8 ⁽¹⁾	<LOQ	51.0 ⁽¹⁾
B	⁽¹⁾	72.3 ⁽¹⁾	⁽¹⁾	46.8 ⁽¹⁾	⁽¹⁾	⁽¹⁾
D	85.9	109.9	23.2	29.7	81.0	103.7
E	64.4	65.1	41.1	41.5	66.4	67.1
F	67.7	76.5	40.5	45.7	56.2	63.6
G	70.1	57.9	48.0	39.7	77.0	63.7
H	88.6	81.1	85.3	78.1	94.0	86.0
I	62.5	52.1	53.3	44.4	76.4	63.7
J	38.2	51.2	34.6	46.4	53.1	71.2
K	76.4	66.7	58.1	50.7	66.8	58.4
L	69.4	69.4	83.4	83.4	51.7	51.6
M	74.4	42.3	44.6	25.4	63.9	36.4
N	136.0 ⁽³⁾	125.8 ⁽³⁾	91.4	84.6	126.1	116.7
O	66.6	64.8	27.8	27.0	40.6	39.6
P	202.2 ⁽²⁾	87.2 ⁽²⁾	116.7 ⁽²⁾	50.3 ⁽²⁾	180.0 ⁽²⁾	77.6 ⁽²⁾

⁽¹⁾ Non valid data (Lab A: no replicated results provided for evaluation; Lab B: no replicated results provided for evaluation / OA results out of the calibration range in CRM-Mus b for DTX2 recovery correction)

⁽²⁾ Cochran outlier

⁽³⁾ Single Grubbs outlier

Appendix 2.1-Table 18. Results provided by participants for samples DAY 1-EURLMB/VAL/L-02 and DAY 1-EURLMB/VAL/L-05 (blind duplicates) for DTX2 in µg/kg by Multitoxin standard quantitation (against DTX2 calibrant).

QUANTITATION LAB CODE	MULTITOXIN _Uncorrected		MULTITOXIN _Matrix correction		MULTITOXIN _CRM-Mus b	
	DAY 1- EURLMB/VAL/L- 02	DAY 1- EURLMB/VAL/L- 05	DAY 1- EURLMB/VAL/L- 02	DAY 1- EURLMB/VAL/L- 05	DAY 1- EURLMB/VAL/L- 02	DAY 1- EURLMB/VAL/L- 05
A	34.3	46.8	40.7	55.4	33.8	46.1
B	(1)	53.3 ⁽¹⁾	(1)	41.6 ⁽¹⁾	(1)	(1)
D	35.7	49.2	18.3	25.2	36.7	50.5
E	65.4	65.8	45.1	45.4	70.7	71.1
F	54.2	61.9	38.4	43.9	51.3	58.6
G	85.9	76.1	58.9	52.2	103.0	91.3
H	78.0	71.4	85.4	78.2	78.3	71.7
I	63.8	55.6	60.0	52.2	77.7	67.7
J	81.6	94.7	53.7	62.3	86.5	100.3
K	56.9	48.4	53.8	45.8	53.9	45.9
L	48.3	48.2	76.0	76.0	33.9	33.9
M	81.4	52.3	49.4	31.8	74.3	47.7
N	122.3	112.8	90.9	83.9	105.4	97.3
O	50.9	49.8	31.9	31.2	44.1	43.1
P	166.3 ⁽²⁾	69.4 ⁽²⁾	117.0 ⁽²⁾	48.8 ⁽²⁾	158.4 ⁽²⁾	66.0 ⁽²⁾

⁽¹⁾ Non valid data (no replicated results provided for evaluation / OA results out of the calibration range in CRM-Mus b for DTX2 recovery correction)

⁽²⁾ Cochran outlier

Appendix 2.1-Table 19. Results provided by participants for samples DAY 2-EURLMB/VAL/L-07 and DAY 2-EURLMB/VAL/L-08 (blind duplicates) for DTX2 in µg/kg by CRM-Individual standard quantitation (against OA calibrant).

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		CRM-INDIVIDUAL_CRM-Mus b	
	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08
A	126.8	99.5	79.8	62.6	101.0	79.3
B	68.6 ⁽¹⁾	(1)	45.7 ⁽¹⁾	(1)	(1)	(1)
D	124.0	101.3	34.2	28.0	113.0	92.2
E	69.4	68.7	51.7	51.2	70.3	69.5
F	96.3	80.8	62.6	52.5	82.9	69.5
G	109.8	49.9	92.2	41.9	124.7 ⁽²⁾	56.6 ⁽²⁾
H	90.7	91.3	82.6	83.2	86.2	86.7
I	68.2	72.8	50.1	53.5	85.4	91.2
J	47.4	49.4	53.7	56.1	62.5	65.2
K	47.6	37.6	74.3	58.7	60.9	48.1
L	51.5	47.0	41.0	37.4	52.7	48.0
M	45.6	59.3	41.8	54.3	40.3	52.4
N	117.8	79.4	122.3	82.5	137.9 ⁽²⁾	93.0 ⁽²⁾
O	104.1	93.0	49.8	44.5	64.2	57.3
P	109.9	111.4	63.1	63.9	88.3	89.5

⁽¹⁾ Non valid data (no replicated results provided for evaluation / OA results out of the calibration range in CRM-Mus b for DTX2 recovery correction)

⁽²⁾ Cochran outlier

Appendix 2.1-Table 20. Results provided by participants for samples DAY 2-EURLMB/VAL/L-07 and DAY 2-EURLMB/VAL/L-08 (blind duplicates) for DTX2 in µg/kg by Multitoxin standard quantitation (against DTX2 calibrant).

QUANTITATION LAB CODE	MULTITOXIN _Uncorrected		MULTITOXIN _Matrix correction		MULTITOXIN _CRM-Mus b	
	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08
A	66.1	34.5	61.8	32.3	64.5	33.7
B	55.8 ⁽¹⁾	(1)	44.0 ⁽¹⁾	(1)	(1)	(1)
D	52.5	38.6	26.3	19.3	50.8	37.3
E	78.1	77.7	59.6	59.3	81.4	81.0
F	58.8	45.6	54.0	41.9	59.9	46.5
G	107.9	60.3	94.4	52.8	127.5	71.3
H	80.8	81.3	84.1	84.6	72.2	72.6
I	54.4	58.2	49.1	52.5	76.9	82.3
J	95.2	97.1	72.3	73.8	99.6	101.7
K	<LOQ	<LOQ	48.8	20.3	<LOQ	<LOQ
L	39.4	35.4	37.5	33.7	40.1	36.0
M	64.2	77.8	50.9	61.6	53.9	65.3
N	137.4	105.0	117.0	89.4	129.4	98.9
O	80.1	72.8	54.2	49.3	64.9	59.0
P	88.1	89.2	63.6	64.4	77.7	78.7

⁽¹⁾ Non valid data (no replicated results provided for evaluation / OA results out of the calibration range in CRM-Mus b for DTX2 recovery correction)

Appendix 2.1-Table 21. Results provided by participants for samples DAY 2-EURLMB/VAL/L-09 and DAY 2-EURLMB/VAL/L-10 (blind duplicates) for DTX2 in µg/kg by CRM-Individual standard quantitation (against OA calibrant).

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		CRM-INDIVIDUAL_CRM-Mus b	
	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10
A	96.1	86.0	60.5	54.1	76.6	68.5
B	(1)	(1)	(1)	(1)	(1)	(1)
D	88.4	79.8	24.4	22.0	80.5	72.7
E	45.9	44.0	34.2	32.8	46.5	44.6
F	63.0	69.6	41.0	45.3	54.2	59.9
G	66.8	57.8	56.1	48.6	75.9	65.7
H	58.4	52.6	53.2	48.0	55.5	50.0
I	44.2	48.8	32.5	35.9	55.4	61.2
J	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
K	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
L	33.9	30.1	27.0	24.0	34.7	30.8
M	23.1	31.0	21.2	28.3	20.4	27.4
N	38.2 ⁽²⁾	74.3 ⁽²⁾	39.6 ⁽²⁾	77.2 ⁽²⁾	44.7 ⁽²⁾	87.0 ⁽²⁾
O	62.0	59.1	29.7	28.3	38.3	36.4
P	62.1	67.4	35.6	38.7	49.9	54.2

⁽¹⁾ Non valid data (results no provided for evaluation)⁽²⁾ Cochran outlier

Appendix 2.1-Table 22. Results provided by participants for samples DAY 2-EURLMB/VAL/L-09 and DAY 2-EURLMB/VAL/L-10 (blind duplicates) for DTX2 in µg/kg by Multitoxin standard quantitation (against DTX2 calibrant).

QUANTITATION LAB CODE	MULTITOXIN _Uncorrected		MULTITOXIN _Matrix correction		MULTITOXIN _CRM-Mus b	
	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10
A	30.7	18.9	28.7	17.7	29.9	18.5
B	(1)	(1)	(1)	(1)	(1)	(1)
D	30.7	25.5	15.4	12.8	29.7	24.6
E	59.3	57.1	45.3	43.6	61.8	59.5
F	30.5	36.1	28.1	33.2	31.1	36.8
G	73.8	66.7	64.6	58.3	87.2	78.8
H	53.4	48.6	55.6	50.5	47.7	43.4
I	34.5	38.3	31.1	34.6	48.7	54.2
J	77.2	80.2	58.6	60.9	80.8	83.9
K	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
L	23.8	20.5	22.7	19.5	24.3	20.9
M	42.0	49.7	33.3	39.4	35.3	41.7
N	70.1 ⁽²⁾	100.6 ⁽²⁾	59.7 ⁽²⁾	85.7 ⁽²⁾	66.0 ⁽²⁾	94.8 ⁽²⁾
O	52.7	50.7	35.6	34.3	42.7	41.1
P	50.5	54.8	36.5	39.6	44.6	48.3

⁽¹⁾ Non valid data (results no provided for evaluation)⁽²⁾ Cochran outlier

Appendix 2.1-Table 23. Results provided by participants for samples DAY 3-EURLMB/VAL/L-11 and DAY 3-EURLMB/VAL/L-14 (blind duplicates) for DTX2 in µg/kg by CRM-Individual standard quantitation (against OA calibrant).

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		CRM-INDIVIDUAL_CRM-Mus b	
	DAY 3- EURLMB/VAL/L- 11	DAY 3- EURLMB/VAL/L- 14	DAY 3- EURLMB/VAL/L- 11	DAY 3- EURLMB/VAL/L- 14	DAY 3- EURLMB/VAL/L- 11	DAY 3- EURLMB/VAL/L- 14
A	92.8	78.5	(1)	(1)	100.0	84.6
B	99.6	121.7	74.3	90.8	(1)	(1)
D	92.0	132.1	27.6	39.6	85.1	122.2
E	98.0	91.8	64.8	60.7	103.1	96.6
F	118.3	106.7	61.1	55.1	102.4	92.4
G	62.1	43.6	53.6	37.6	54.6	38.3
H	117.5	117.3	96.6	96.5	104.2	104.1
I	78.2	72.0	61.3	56.5	83.9	77.3
J	94.6	103.3	90.0	98.2	133.6	145.8
K	82.5	92.0	53.9	60.1	65.5	73.1
L	37.7	59.3	28.7	45.1	29.0	45.7
M	94.3	172.7	52.1 ⁽²⁾	95.4 ⁽²⁾	88.3	161.6
N	183.9	114.2	207.0 ⁽²⁾	128.6 ⁽²⁾	224.5	139.5
O	152.1	131.7	63.4	54.9	100.1	86.6
P	147.0	122.9	78.6	65.7	113.0	94.4

⁽¹⁾ Non valid data (Lab A: results for spiked extract no provided for matrix correction; Lab B: OA results out of the calibration range in CRM-Mus b for DTX2 recovery correction)

⁽²⁾ Cochran outlier

Appendix 2.1-Table 24. Results provided by participants for samples DAY 3-EURLMB/VAL/L-11 and DAY 3-EURLMB/VAL/L-14 (blind duplicates) for DTX2 in µg/kg by Multitoxin standard quantitation (against DTX2 calibrant).

QUANTITATION LAB CODE	MULTITOXIN _Uncorrected		MULTITOXIN _Matrix correction		MULTITOXIN _CRM-Mus b	
	DAY 3- EURLMB/VAL/L- 11	DAY 3- EURLMB/VAL/L- 14	DAY 3- EURLMB/VAL/L- 11	DAY 3- EURLMB/VAL/L- 14	DAY 3- EURLMB/VAL/L- 11	DAY 3- EURLMB/VAL/L- 14
A	<LOQ	<LOQ	(1)	(1)	<LOQ	<LOQ
B	81.4	100.1	73.6	90.4	(1)	(1)
D	56.8	76.9	32.1	43.4	58.3	79.0
E	94.8	89.1	68.7	64.5	105.6	99.1
F	101.5	92.4	63.2	57.5	105.0	95.5
G	67.0	52.3	61.5	48.0	66.9	52.2
H	100.2	100.1	98.6	98.5	90.1	90.0
I	58.6	53.3	58.3	53.1	68.5	62.4
J	126.8	135.5	93.4	99.8	141.9	151.7
K	39.0	45.8	43.0	50.6	37.6	44.2
L	31.2	54.2	23.8	41.3	20.2	35.1
M	81.2	159.2	48.3 ⁽²⁾	94.7 ⁽²⁾	83.5	163.6
N	193.0	127.5	188.2 ⁽²⁾	124.3 ⁽²⁾	190.6	125.9
O	108.1	94.2	64.4	56.2	98.9	86.2
P	110.4	92.3	78.7	65.9	97.8	81.8

⁽¹⁾ Non valid data (Lab A: results for spiked extract no provided for matrix correction; Lab B: OA results out of the calibration range in CRM-Mus b for DTX2 recovery correction)

⁽²⁾ Cochran outlier

Appendix 2.1-Table 25. Results provided by participants for samples DAY 3-EURLMB/VAL/L-12 and DAY 3-EURLMB/VAL/L-13 (blind duplicates) for DTX2 in µg/kg by CRM-Individual standard quantitation (against OA calibrant).

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		CRM-INDIVIDUAL_CRM-Mus b	
	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13
A	337.3	320.7	(1)	(1)	363.4	345.6
B	354.1	406.4	264.2	303.1	(1)	(1)
D	539.5 ⁽²⁾	677.6 ⁽²⁾	161.9	203.3	498.9 ⁽²⁾	626.6 ⁽²⁾
E	370.7	395.2	245.0	261.2	389.7	415.5
F	447.6	447.5	231.3	231.3	387.5	387.4
G	320.1	290.4	276.4	250.7	281.5	255.3
H	402.8	399.0	331.2	328.1	357.2	353.9
I	309.3	296.9	242.5	232.8	331.9	318.6
J	340.0	330.0	323.4	313.9	480.0	465.9
K	297.4	314.9	194.2	205.7	236.3	250.2
L	169.4	174.3	128.9	132.7	130.5	134.3
M	511.8	459.8	282.7	254.0	479.0	430.3
N	683.2	657.2	769.3 ⁽³⁾	740.0 ⁽³⁾	834.4 ⁽³⁾	802.6 ⁽³⁾
O	537.7	605.1	224.2	252.3	353.9	398.2
P	542.1	472.0	289.9	252.5	416.7	362.9

⁽¹⁾ Non valid data (Lab A: results for spiked extract no provided for matrix correction; Lab B: OA results out of the calibration range in CRM-Mus b for DTX2 recovery correction)

⁽²⁾ Cochran outlier

⁽³⁾ Single Grubbs outlier

Appendix 2.1-Table 26. Results provided by participants for samples DAY 3-EURLMB/VAL/L-12 and DAY 3-EURLMB/VAL/L-13 (blind duplicates) for DTX2 in µg/kg by Multitoxin standard quantitation (against DTX2 calibrant).

QUANTITATION LAB CODE	MULTITOXIN _Uncorrected		MULTITOXIN _Matrix correction		MULTITOXIN _CRM-Mus b	
	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13
A	520.8	456.9	(1)	(1)	568.7	498.9
B	296.3	340.4	267.8	307.6	(1)	(1)
D	280.7	349.7	158.6	197.6	288.3	359.2
E	324.2	345.2	234.8	250.0	360.8	384.2
F	362.1	362.0	225.3	225.3	374.5	374.4
G	272.4	248.7	250.0	228.3	272.1	248.4
H	324.9	321.9	319.6	316.7	291.9	289.3
I	253.5	243.0	252.2	241.8	296.4	284.2
J	372.6	362.6	274.3	266.9	416.9	405.8
K	193.1	205.7	213.2	227.0	186.5	198.6
L	171.2	176.4	130.5	134.4	111.0	114.4
M	496.6	444.9	295.4	264.6	510.2	457.1
N	663.1	638.6	646.5 ⁽³⁾	622.7 ⁽³⁾	655.0	630.8
O	371.2	417.2	221.2	248.6	339.6	381.6
P	405.9	353.6	289.6	252.3	359.8	313.4

⁽¹⁾ Non valid data (Lab A: results for spiked extract no provided for matrix correction; Lab B: OA results out of the calibration range in CRM-Mus b for DTX2 recovery correction)

⁽³⁾ Single Grubbs outlier

Appendix 2.1-Table 27. Results provided by participants for samples DAY 1-EURLMB/VAL/L-04 and DAY 1-EURLMB/VAL/L-06 (blind duplicates) for DTX1 in µg/kg by CRM-Individual standard quantitation (against OA calibrant).

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		CRM-INDIVIDUAL_CRM-Mus b	
	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06
A	287.5	289.0	267.7	269.2	230.2	231.5
B	591.6	588.3	151.3	150.5	(1)	(1)
D	539.1	596.3	287.7	318.2	285.0	315.2
E	440.3	437.6	242.5	241.0	369.2	366.9
F	231.1	208.6	265.5	239.6	346.0	312.2
G	342.9	318.0	298.7	277.0	316.5	293.5
H	288.0	272.5	335.2	317.2	314.8	297.9
I	309.4	351.1	234.7	266.4	318.5	361.5
J	292.3	345.4	169.4	200.2	304.4	359.7
K	488.8	522.5	238.6	255.0	279.8	299.1
L	446.0	432.5	222.2	215.5	283.6	275.0
M	414.6	491.2	281.2	333.2	332.5	394.0
N	644.3	627.3	529.0 ⁽³⁾	515.0 ⁽³⁾	613.6 ⁽³⁾	597.4 ⁽³⁾
O	400.8 ⁽²⁾	589.9 ⁽²⁾	186.5	274.5	185.3	272.7
P	526.1	508.0	218.0	210.5	385.0	371.7

⁽¹⁾ Non valid data (DTX1 results out of the calibration range in CRM-Mus b for DTX1 recovery correction)

⁽²⁾ Cochran outlier

⁽³⁾ Single Grubbs outlier

Appendix 2.1-Table 28. Results provided by participants for samples DAY 1-EURLMB/VAL/L-04 and DAY 1-EURLMB/VAL/L-06 (blind duplicates) for DTX1 in µg/kg by Multitoxin standard quantitation (against DTX1 calibrant).

QUANTITATION LAB CODE	MULTITOXIN _Uncorrected		MULTITOXIN _Matrix correction		MULTITOXIN _CRM-Mus b	
	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06
A	258.5	259.9	276.9	278.5	229.5	230.8
B	365.3	363.3	151.8	150.9	201.6	200.4
D	514.2	571.5	304.4	338.4	286.7	318.6
E	376.3	373.9	235.4	233.9	363.0	360.7
F	430.3	388.1	266.6	240.5	346.5	312.5
G	329.2	305.7	293.3	272.3	315.3	292.8
H	293.6	276.9	354.1	334.0	314.6	296.7
I	258.4	291.5	226.5	255.6	314.5	354.8
J	344.7	396.9	163.1	187.9	295.0	339.6
K	355.1	380.7	246.8	264.5	281.0	301.3
L	349.4	338.3	229.1	221.8	285.2	276.1
M	412.7	483.2	260.1	304.5	324.8	380.3
N	642.9	625.8	533.5 ⁽³⁾	519.4 ⁽³⁾	616.2 ⁽³⁾	599.9 ⁽³⁾
O	279.0	407.3	184.8 ⁽²⁾	269.8 ⁽²⁾	186.6	272.4
P	494.9	477.5	215.9	208.3	381.2	367.8

⁽²⁾ Cochran outlier⁽³⁾ Single Grubbs outlier

Appendix 2.1-Table 29. Results provided by participants for samples DAY 3-EURLMB/VAL/L-12 and DAY 3-EURLMB/VAL/L-13 (blind duplicates) for DTX1 in µg/kg by CRM-Individual standard quantitation (against OA calibrant).

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		CRM-INDIVIDUAL_CRM-Mus b	
	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13
A	172.2	196.3	(1)	(1)	194.1	221.3
B	479.5	492.9	125.0	128.5	(1)	(1)
D	289.6	325.8	180.8	203.4	220.7	248.3
E	266.3	281.6	159.3	168.5	234.5	248.0
F	142.9	150.0	145.6	152.8	216.0	226.7
G	205.1	188.0	154.4	141.6	161.2	147.8
H	230.4	229.7	216.0 ⁽³⁾	215.3 ⁽³⁾	220.1	219.4
I	186.5	190.6	135.3	138.2	174.8	178.6
J	255.2	221.4	150.4	130.5	270.6	234.7
K	259.2	293.7	131.2	148.7	139.7	158.3
L	113.7	88.9	124.2	97.1	80.1 ⁽⁴⁾	62.7 ⁽⁴⁾
M	379.3	358.3	152.2	143.8	283.6	267.9
N	455.4	408.1	466.5 ⁽³⁾	418.0 ⁽³⁾	419.0 ⁽⁴⁾	375.5 ⁽⁴⁾
O	333.6	358.6	120.0	129.0	145.4	156.2
P	330.9	299.4	126.2	114.2	183.6	166.1

⁽¹⁾ Non valid data (Lab A: results for spiked extract no provided for matrix correction; Lab B: Non valid data (DTX1 results out of the calibration range in CRM-Mus b for DTX1 recovery correction)

⁽³⁾ Single Grubbs outlier

⁽⁴⁾ Double Grubbs outlier

Appendix 2.1-Table 30. Results provided by participants for samples DAY 3-EURLMB/VAL/L-12 and DAY 3-EURLMB/VAL/L-13 (blind duplicates) for DTX1 in µg/kg by Multitoxin standard quantitation (against DTX1 calibrant).

QUANTITATION LAB CODE	MULTITOXIN _Uncorrected		MULTITOXIN _Matrix correction		MULTITOXIN _CRM-Mus b	
	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13
A	168.6	191.6	(1)	(1)	195.4	222.0
B	289.3	297.3	125.2	128.7	(1)	(1)
D	238.5	267.2	178.6	200.1	221.8	248.5
E	234.0	247.0	158.7	167.5	237.4	250.5
F	283.7	297.4	145.3	152.3	217.1	227.5
G	172.7	160.2	152.1	141.0	168.9	156.7
H	219.6	218.9	219.1 ⁽³⁾	218.3 ⁽³⁾	216.4	215.7
I	162.0	165.5	135.3	138.2	176.6	180.4
J	290.8	258.6	148.1	131.7	267.8	238.2
K	166.6	190.7	130.7	149.6	134.4	153.9
L	86.7	62.9	121.1	87.9	68.0	49.4
M	367.1	346.8	152.3	143.9	283.7	268.0
N	474.9	432.4	350.5 ⁽³⁾	319.1 ⁽³⁾	384.8	350.3
O	248.1	265.9	120.6	129.3	147.6	158.3
P	266.4	241.3	126.3	114.4	184.1	166.8

⁽¹⁾ Non valid data (Lab A: results for spiked extract no provided for matrix correction; Lab B: DTX1 results out of the calibration range in CRM-Mus b for DTX1 recovery correction)

⁽³⁾ Single Grubbs outlier

Appendix 2.2. AZA-group toxins results

Appendix 2.2-Table 1. Results provided by participants for samples DAY 1-EURLMB/VAL/L-01 and DAY 1-EURLMB/VAL/L-03 (blind duplicates) for AZA1 in µg/kg.

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		MULTITOXIN_Uncorrected		MULTITOXIN_Matrix correction	
	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03
A	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
B	46.8	61.3	106.3	139.4	57.9	72.0	88.3	109.7
D	92.1	92.5	72.6	72.9	85.9	86.3	76.6	77.0
E	91.2	75.8	79.9	66.4	83.1	68.3	81.9	67.3
F	96.7	81.0	106.0	88.8	96.9	80.3	111.7	92.6
G	60.2	89.7	90.6	135.1	65.8	92.6	81.6	114.8
H	72.8	69.6	72.8	69.6	74.6	71.4	72.4	69.3
I	87.8	89.9	77.5	79.4	82.8	84.9	78.5	80.4
J	80.7	81.6	73.4	74.3	97.7	98.5	66.0	66.5
K	92.1	91.7	74.4	74.1	86.8	86.3	75.4	75.1
L	85.3	107.1	92.0	115.6	88.1	110.5	91.8	115.2
M	78.5	84.7	54.4	58.8	79.5	86.6	54.4	59.2
N	166.6 ⁽²⁾	136.9 ⁽²⁾	158.6	130.4	161.5 ⁽³⁾	130.7 ⁽³⁾	183.1 ⁽³⁾	148.2 ⁽³⁾
O	72.0	61.9	98.2	84.3	78.8	68.0	96.2	83.0
P	72.5	74.8	106.2	109.5	76.8	79.3	114.0	117.8

⁽¹⁾ Non valid data (results no provided for evaluation)

⁽²⁾ Cochran outlier

⁽³⁾ Single Grubbs outlier

Appendix 2.2-Table 2. Results provided by participants for samples DAY 1-EURLMB/VAL/L-02 and DAY 1-EURLMB/VAL/L-05 (blind duplicates) for AZA1 in µg/kg.

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		MULTITOXIN_Uncorrected		MULTITOXIN_Matrix correction	
	DAY 1- EURLMB/VAL/L- 02	DAY 1- EURLMB/VAL/L- 05	DAY 1- EURLMB/VAL/L- 02	DAY 1- EURLMB/VAL/L- 05	DAY 1- EURLMB/VAL/L- 02	DAY 1- EURLMB/VAL/L- 05	DAY 1- EURLMB/VAL/L- 02	DAY 1- EURLMB/VAL/L- 05
A	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
B	22.8	27.0	51.8	61.4	34.7	38.8	52.9	59.1
D	59.2	55.6	46.6	43.8	50.1	46.2	44.7	41.2
E	45.9	53.0	40.2	46.5	39.1	46.4	38.5	45.8
F	59.2	57.0	64.9	62.6	57.3	55.0	66.0	63.4
G	41.6	26.5	62.7	39.9	49.0	35.3	60.7	43.7
H	50.4	47.7	50.4	47.7	52.0	49.3	50.5	47.8
I	54.4	52.8	48.0	46.6	50.4	48.8	47.7	46.2
J	37.3	49.9	34.0	45.5	62.9	73.0	42.5	49.3
K	58.4	57.4	47.2	46.3	53.8	52.8	46.8	45.9
L	57.6	53.6	62.1	57.8	59.6	55.5	62.1	57.8
M	49.0	66.4	34.0	46.0	46.4	66.0	31.7	45.1
N	81.6 ⁽³⁾	104.9 ⁽³⁾	77.7 ⁽³⁾	99.9 ⁽³⁾	73.3	97.4	83.0 ⁽³⁾	110.5 ⁽³⁾
O	40.6	47.0	55.4	64.0	45.4	52.1	55.3	63.6
P	35.4	37.8	51.9	55.3	34.6	37.3	51.4	55.3

⁽¹⁾ Non valid data (results no provided for evaluation)⁽³⁾ Single Grubbs outlier

Appendix 2.2-Table 3. Results provided by participants for samples DAY 1-EURLMB/VAL/L-04 and DAY 1-EURLMB/VAL/L-06 (blind duplicates) for AZA1 in µg/kg.

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		MULTITOXIN_Uncorrected		MULTITOXIN_Matrix correction	
	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06
A	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
B	296.7	290.3	674.9 ⁽⁴⁾	660.4 ⁽⁴⁾	299.8	293.7	456.8	447.4
D	372.8	375.3	293.8	295.8	391.1	393.8	349.0	351.4
E	412.6	333.3	361.8	292.2	390.8	314.6	385.0	309.8
F	344.3	332.3	377.7	364.5	358.7	346.0	413.3	398.6
G	325.4	216.2	490.0 ⁽²⁾	325.5 ⁽²⁾	306.4	207.3	379.7	256.9
H	327.8	325.1	327.9	325.1	331.4	328.6	321.8	319.1
I	387.2	395.6	341.9	349.2	373.8	381.9	354.3	362.0
J	378.9	431.3	345.0	392.8	337.0	379.1	227.6	256.1
K	410.5	384.8	331.7	310.9	397.9	372.8	345.9	324.1
L	465.5	552.6	502.0 ⁽³⁾	596.0 ⁽³⁾	478.9	568.4	499.2	592.6
M	443.6	390.9	307.7	271.1	481.0	430.4	328.8	294.2
N	732.3 ⁽³⁾	712.3 ⁽³⁾	697.4 ⁽⁴⁾	678.3 ⁽⁴⁾	749.1 ⁽³⁾	728.3 ⁽³⁾	849.1 ⁽³⁾	825.6 ⁽³⁾
O	219.6	217.3	299.3	296.3	235.9	233.5	287.9	285.0
P	200.5	192.8	293.6	282.3	222.3	213.5	330.0	317.0

⁽¹⁾ Non valid data (results no provided for evaluation)

⁽²⁾ Cochran outlier

⁽³⁾ Single Grubbs outlier

⁽⁴⁾ Double Grubbs outlier

Appendix 2.2-Table 4. Results provided by participants for samples DAY 2-EURLMB/VAL/L-07 and DAY 2-EURLMB/VAL/L-08 (blind duplicates) for AZA1 in µg/kg.

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		MULTITOXIN_Uncorrected		MULTITOXIN_Matrix correction	
	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08
A	324.0 ⁽²⁾	374.1 ⁽²⁾	71.3	82.3	209.1 ⁽²⁾	251.5 ⁽²⁾	78.6	94.5
B	102.0	119.0	129.2	150.8	98.7	116.7	147.2	174.0
D	119.3	115.4	112.0	108.2	126.1	121.5	122.7	118.3
E	119.9	117.6	109.5	107.4	115.2	115.2	95.1	95.1
F	129.2	121.0	145.3	136.0	133.4	124.7	150.2	140.4
G	72.6	62.2	86.8	74.4	77.8	67.9	81.6	71.2
H	99.2	100.7	107.4	109.1	103.9	105.4	101.5	102.9
I	121.4	132.2	116.0	126.4	116.7	126.9	113.5	123.4
J	139.1	131.9	131.0	124.2	140.9	135.2	100.2	96.1
K	131.0	134.8	103.9	106.9	125.6	129.4	110.2	113.6
L	113.4	111.4	113.2	111.2	108.9	106.9	114.5	112.4
M	109.7	118.9	96.2	104.3	125.0	135.2	94.1	101.7
N	240.8 ⁽²⁾	143.1 ⁽²⁾	219.4 ⁽²⁾	130.4 ⁽²⁾	231.2 ⁽²⁾	142.6 ⁽²⁾	188.4 ⁽²⁾	116.1 ⁽²⁾
O	82.8	75.5	116.9	106.5	92.1	84.0	116.7	106.4
P	80.8	82.5	136.3	139.1	85.7	87.3	119.6	121.8

⁽²⁾ Cochran outlier

Appendix 2.2-Table 5. Results provided by participants for samples DAY 2-EURLMB/VAL/L-09 and DAY 2-EURLMB/VAL/L-10 (blind duplicates) for AZA1 in µg/kg.

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		MULTITOXIN_Uncorrected		MULTITOXIN_Matrix correction	
	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10
A	721.6 ⁽²⁾	821.7 ⁽²⁾	158.8	180.8	545.7 ⁽²⁾	630.5 ⁽²⁾	205.1	236.9
B	212.6	232.5	269.4	294.6	215.4	236.4	321.3	352.6
D	237.8	243.7	223.1	228.6	261.8	268.5	255.0	261.5
E	256.2	249.9	233.9	228.2	224.8	219.8	185.6	181.5
F	221.0	212.9	248.4	239.3	230.0	221.5	259.0	249.4
G	188.0	184.3	224.7	220.4	187.7	184.2	196.7	193.0
H	222.2	225.5	240.6	244.1	224.7	228.0	219.5	222.7
I	258.4	263.7	247.0	252.1	245.7	250.7	239.0	243.8
J	285.0	301.2	268.4	283.6	255.7	268.4	181.8	190.9
K	263.4	264.0	208.8	209.3	260.6	261.2	228.6	229.2
L	179.6	165.8	179.3	165.5	173.0	159.6	181.9	167.8
M	221.6	210.5	194.3	184.6	248.7	236.4	187.2	177.9
N	384.0	412.9	349.9 ⁽³⁾	376.2 ⁽³⁾	361.2	387.5	294.2	315.6
O	146.0	160.3	206.1	226.2	162.3	178.1	205.6	225.7
P	126.5	127.7	213.4	215.3	129.6	130.7	180.9	182.4

⁽²⁾ Cochran outlier⁽³⁾ Single Grubbs outlier

Appendix 2.2-Table 6. Results provided by participants for samples DAY 3-EURLMB/VAL/L-11 and DAY 3-EURLMB/VAL/L-14 (blind duplicates) for AZA1 in µg/kg.

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		MULTITOXIN_Uncorrected		MULTITOXIN_Matrix correction	
	DAY 3- EURLMB/VAL/L- 11	DAY 3- EURLMB/VAL/L- 14	DAY 3- EURLMB/VAL/L- 11	DAY 3- EURLMB/VAL/L- 14	DAY 3- EURLMB/VAL/L- 11	DAY 3- EURLMB/VAL/L- 14	DAY 3- EURLMB/VAL/L- 11	DAY 3- EURLMB/VAL/L- 14
A	<LOQ	<LOQ	(1)	(1)	44.7	43.1	(1)	(1)
B	33.5	38.1	46.6	53.1	31.9	36.8	45.9	52.9
D	40.2	37.6	38.7	36.2	39.0	36.4	38.7	36.1
E	48.7	46.8	41.5	39.9	46.8	45.3	43.3	41.9
F	47.4	42.3	52.7	47.0	42.1	36.6	52.2	45.4
G	38.2	33.7	50.7	44.7	45.4	40.8	51.2	46.0
H	43.5	43.9	45.8	46.3	46.2	46.6	46.6	47.0
I	40.5	41.3	40.5	41.3	39.0	39.8	39.5	40.3
J	33.8	34.3	33.8	34.3	59.5	59.8	43.1	43.3
K	53.2	52.2	41.6	40.8	47.0	46.0	40.0	39.1
L	31.6 ⁽²⁾	49.2 ⁽²⁾	29.2	45.5	33.3 ⁽²⁾	53.0 ⁽²⁾	28.4	45.2
M	36.6	35.6	37.1	36.1	36.0	34.9	36.4	35.3
N	86.2 ⁽³⁾	91.8 ⁽³⁾	160.2 ⁽³⁾	170.5 ⁽³⁾	104.9 ⁽³⁾	110.4 ⁽³⁾	119.7 ⁽³⁾	126.0 ⁽³⁾
O	42.3	33.1	64.6	50.5	38.8	29.1	66.3	49.7
P	41.1	39.1	48.0	45.6	43.2	41.2	48.3	46.0

⁽¹⁾ Non valid data (results for spiked extract no provided for matrix correction)⁽²⁾ Cochran outlier⁽³⁾ Single Grubbs outlier

Appendix 2.2-Table 7. Results provided by participants for samples DAY 3-EURLMB/VAL/L-12 and DAY 3-EURLMB/VAL/L-13 (blind duplicates) for AZA1 in µg/kg.

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		MULTITOXIN_Uncorrected		MULTITOXIN_Matrix correction	
	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13
A	387.6	425.5	(1)	(1)	561.3	612.3	(1)	(1)
B	362.6	370.7	504.6	515.9	376.8	385.3	541.8	554.0
D	390.6	442.9	375.9	426.3	379.9	430.9	376.5	427.0
E	567.2	550.8	483.3	469.4	468.7	455.2	433.6	421.1
F	524.0	526.2	582.3	584.8	547.1	549.4	678.6	681.5
G	172.5	248.9	228.9 ⁽²⁾	330.3 ⁽²⁾	184.4	263.5	208.0	297.2
H	468.7	459.7	493.9	484.5	451.7	443.2	455.8	447.2
I	468.4	494.2	467.9	493.7	490.4	517.6	496.5	524.0
J	521.7	495.3	521.3	494.9	438.1	417.6	317.3	302.4
K	517.2	505.1	404.7	395.2	524.9	512.4	446.6	436.0
L	489.8	540.7	452.4	499.5	544.3	601.1	464.4	512.8
M	359.4	387.9	364.2	393.2	372.4	402.1	376.9	407.0
N	913.9 ⁽³⁾	988.9 ⁽³⁾	1697.9 ⁽¹⁾	1837.3 ⁽¹⁾	933.3 ⁽³⁾	1008.4 ⁽³⁾	1064.7 ⁽³⁾	1150.3 ⁽³⁾
O	291.6	278.7	445.0	425.3	300.2	286.7	513.2	490.1
P	446.7	446.3	520.8	520.3	452.3	451.9	505.7	505.3

⁽¹⁾ Non valid data (Lab A: results for spiked extract no provided for matrix correction; Lab N: results out of the calibration range)

⁽²⁾ Cochran outlier

⁽³⁾ Single Grubbs outlier

Appendix 2.2-Table 8. Results provided by participants for samples DAY 1-EURLMB/VAL/L-01 and DAY 1-EURLMB/VAL/L-03 (blind duplicates) for AZA2 in µg/kg.

QUANTITATION LAB CODE	CRM-INDIVIDUAL(AZA1)_Uncorrected		MULTITOXIN(AZA2)_Uncorrected		MULTITOXIN(AZA2)_Matrix correct.	
	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03
A	(1)	(1)	(1)	(1)	(1)	(1)
B	<LOQ	<LOQ	15.8	20.0	22.9	28.9
D	33.9	33.9	19.3	19.4	16.7	16.7
E	33.0	28.2	24.3	20.2	22.2	18.4
F	36.2	32.9	25.8	23.0	29.8	26.5
G	46.4	33.5	21.2	16.2	50.1	38.3
H	<LOQ	<LOQ	19.8	19.1	21.1	20.3
I	30.2	29.7	25.0	24.6	22.5	22.1
J	19.7	20.4	27.8	28.3	22.2	22.6
K	34.3	34.0	23.1	22.9	20.2	19.9
L	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
M	25.8	29.3	21.4	25.9	19.8	24.0
N	124.6 ⁽²⁾	94.4 ⁽²⁾	55.1 ⁽³⁾	39.2 ⁽³⁾	52.2	37.1
O	25.1	27.3	23.4	26.2	36.2	40.4
P	22.7	29.9	23.4	33.6	15.2	21.9

⁽¹⁾ Non valid data (results no provided for evaluation)⁽²⁾ Cochran outlier⁽³⁾ Single Grubbs outlier

Appendix 2.2-Table 9. Results provided by participants for samples DAY 1-EURLMB/VAL/L-02 and DAY 1-EURLMB/VAL/L-05 (blind duplicates) for AZA2 in µg/kg.

QUANTITATION LAB CODE	CRM-INDIVIDUAL(AZA1)_Uncorrected		MULTITOXIN(AZA2)_Uncorrected		MULTITOXIN(AZA2)_Matrix correct.	
	DAY 1- EURLMB/VAL/L- 02	DAY 1- EURLMB/VAL/L- 05	DAY 1- EURLMB/VAL/L- 02	DAY 1- EURLMB/VAL/L- 05	DAY 1- EURLMB/VAL/L- 02	DAY 1- EURLMB/VAL/L- 05
A	(1)	(1)	(1)	(1)	(1)	(1)
B	11.2	13.2	27.0	28.6	39.0	41.4
D	44.0	46.5	28.3	30.6	24.4	26.4
E	39.4	44.5	29.2	33.8	26.6	30.9
F	51.2	48.5	39.0	36.7	45.0	42.2
G	48.7 ⁽²⁾	27.9 ⁽²⁾	22.1	14.0	52.2	33.1
H	31.4	30.5	37.3	36.3	39.7	38.6
I	41.1	44.6	35.7	39.1	32.1	35.2
J	28.0	37.7	34.0	41.1	27.1	32.8
K	53.1	52.6	38.2	37.8	33.3	32.9
L	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
M	41.9	42.8	42.1	43.3	39.0	40.1
N	120.8 ⁽²⁾	150.4 ⁽²⁾	53.1 ⁽²⁾	68.7 ⁽²⁾	50.3	65.1
O	38.5	37.2	40.0	38.4	61.8	59.3
P	45.7	44.8	55.9	54.7	36.4	35.6

⁽¹⁾ Non valid data (results no provided for evaluation)⁽²⁾ Cochran outlier⁽³⁾ Single Grubbs outlier

Appendix 2.2-Table 10. Results provided by participants for samples DAY 1-EURLMB/VAL/L-04 and DAY 1-EURLMB/VAL/L-06 (blind duplicates) for AZA2 in µg/kg.

QUANTITATION LAB CODE	CRM-INDIVIDUAL(AZA1)_Uncorrected		MULTITOXIN(AZA2)_Uncorrected		MULTITOXIN(AZA2)_Matrix correct.	
	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06
A	(1)	(1)	(1)	(1)	(1)	(1)
B	60.5	55.7	66.9	63.0	96.6	91.0
D	92.6	93.3	71.6	72.2	61.7	62.3
E	117.9	90.7	95.6	72.5	87.2	66.1
F	108.3	103.3	89.3	84.9	102.8	97.8
G	52.8	77.7	23.7	33.4	55.9	78.8
H	65.3	64.6	74.3	73.5	79.0	78.2
I	98.4	98.2	91.6	91.4	82.4	82.2
J	68.8	77.6	64.1	70.6	51.2	56.4
K	118.6	120.0	91.0	92.1	79.3	80.3
L	<LOQ	<LOQ	30.5	37.1	21.1	25.7
M	88.5	75.8	102.4	86.0	94.8	79.6
N	410.9 ⁽³⁾	368.2 ⁽³⁾	206.1 ⁽³⁾	183.6 ⁽³⁾	195.4 ⁽³⁾	174.0 ⁽³⁾
O	53.7	65.6	58.8	73.5	90.7	113.4
P	88.5	89.7	116.4	118.2	75.7	76.9

⁽¹⁾ Non valid data (results no provided for evaluation)⁽³⁾ Single Grubbs outlier

Appendix 2.2-Table 11. Results provided by participants for samples DAY 2-EURLMB/VAL/L-07 and DAY 2-EURLMB/VAL/L-08 (blind duplicates) for AZA2 in µg/kg.

QUANTITATION LAB CODE	CRM-INDIVIDUAL(AZA1)_Uncorrected		MULTITOXIN(AZA2)_Uncorrected		MULTITOXIN(AZA2)_Matrix correct.	
	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08
A	311.0 ⁽²⁾	329.0 ⁽²⁾	106.4 ⁽⁴⁾	114.8 ⁽⁴⁾	43.3	46.8
B	54.7	65.2	47.6	56.8	64.8	77.3
D	72.4	73.9	62.3	63.7	54.5	55.7
E	64.3	63.4	55.7	56.2	44.5	44.8
F	79.4	77.3	66.4	64.6	69.8	68.0
G	38.5 ⁽³⁾	72.3 ⁽³⁾	17.4 ⁽³⁾	31.7 ⁽³⁾	32.8	59.9
H	41.3	44.2	51.9	55.0	53.5	56.7
I	65.2	68.9	62.4	65.9	52.6	55.7
J	71.4	65.0	65.9	61.2	50.5	46.9
K	93.0	94.4	66.0	67.2	59.2	60.2
L	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
M	39.7	45.5	52.6	60.1	65.6	75.0
N	330.2 ⁽²⁾	197.9 ⁽²⁾	128.9 ⁽⁴⁾	78.6 ⁽⁴⁾	92.0	56.1
O	48.8	44.9	52.3	48.0	84.2	77.2
P	51.5	65.3	61.3	77.4	44.3	55.9

⁽²⁾ Cochran outlier

⁽³⁾ Single Grubbs outlier

⁽⁴⁾ Double Grubbs outlier

Appendix 2.2-Table 12. Results provided by participants for samples DAY 2-EURLMB/VAL/L-09 and DAY 2-EURLMB/VAL/L-10 (blind duplicates) for AZA2 in µg/kg.

QUANTITATION LAB CODE	CRM-INDIVIDUAL(AZA1)_Uncorrected		MULTITOXIN(AZA2)_Uncorrected		MULTITOXIN(AZA2)_Matrix correct.	
	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10
A	385.7 ⁽³⁾	369.3 ⁽³⁾	141.6 ⁽⁴⁾	133.9 ⁽⁴⁾	57.7	54.5
B	61.2	68.5	53.3	59.7	72.5	81.3
D	77.8	76.2	67.3	65.9	58.8	57.6
E	72.3	70.9	61.6	60.6	49.1	48.3
F	73.9	72.3	61.6	60.3	64.8	63.4
G	38.7	46.8	17.4 ⁽⁴⁾	20.9 ⁽⁴⁾	32.9	39.4
H	48.2	49.0	59.3	60.3	61.1	62.1
I	71.1	72.2	68.0	69.1	57.4	58.3
J	66.0	71.7	61.9	66.1	47.5	50.7
K	98.6	98.3	70.7	70.4	63.4	63.1
L	42.8	39.0	89.8	81.9	83.3	76.0
M	41.8	41.5	55.4	55.0	69.0	68.6
N	252.7 ⁽²⁾	290.9 ⁽²⁾	99.4 ⁽³⁾	114.0 ⁽³⁾	70.9	81.3
O	45.5	41.9	48.6	44.6	78.2	71.7
P	60.8	68.1	72.1	80.7	52.1	58.3

⁽²⁾ Cochran outlier⁽³⁾ Single Grubbs outlier⁽⁴⁾ Double Grubbs outlier

Appendix 2.2-Table 13. Results provided by participants for samples DAY 3-EURLMB/VAL/L-11 and DAY 3-EURLMB/VAL/L-14 (blind duplicates) for AZA2 in µg/kg.

QUANTITATION LAB CODE	CRM-INDIVIDUAL(AZA1)_Uncorrected		MULTITOXIN(AZA2)_Uncorrected		MULTITOXIN(AZA2)_Matrix correct.	
	DAY 3- EURLMB/VAL/L- 11	DAY 3- EURLMB/VAL/L- 14	DAY 3- EURLMB/VAL/L- 11	DAY 3- EURLMB/VAL/L- 14	DAY 3- EURLMB/VAL/L- 11	DAY 3- EURLMB/VAL/L- 14
A	174.2 ⁽³⁾	197.0 ⁽³⁾	56.9 ⁽³⁾	62.9 ⁽³⁾	(1)	(1)
B	28.5	36.7	26.3	33.8	45.1	58.0
D	43.6	48.4	36.4	41.3	30.4	34.5
E	50.5	48.9	40.0	38.8	35.1	34.1
F	51.6	43.3	39.6	32.1	49.0	39.6
G	43.0	33.0	<LOQ	<LOQ	47.1	36.2
H	34.9	35.6	42.5	43.2	45.4	46.2
I	40.5	39.0	40.4	38.7	41.4	39.6
J	32.8	31.6	38.5	37.6	32.1	31.4
K	60.2	59.7	40.0	39.6	30.4	30.1
L	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
M	26.2	27.1	29.6	30.7	35.2	36.6
N	191.4 ⁽²⁾	-2.8 ⁽²⁾	83.5 ⁽²⁾	1.3 ⁽²⁾	88.1 ⁽²⁾	1.4 ⁽²⁾
O	43.2	35.7	38.1	31.7	57.4	47.7
P	33.7	32.1	37.5	35.7	34.4	32.8

⁽¹⁾ Non valid data (results for spiked extract no provided for matrix correction)⁽²⁾ Cochran outlier⁽³⁾ Single Grubbs outlier

Appendix 2.2-Table 14. Results provided by participants for samples DAY 3-EURLMB/VAL/L-12 and DAY 3-EURLMB/VAL/L-13 (blind duplicates) for AZA2 in µg/kg.

QUANTITATION LAB CODE	CRM-INDIVIDUAL(AZA1)_Uncorrected		MULTITOXIN(AZA2)_Uncorrected		MULTITOXIN(AZA2)_Matrix correct.	
	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13
A	972.9 ⁽³⁾	955.4 ⁽³⁾	269.1 ⁽³⁾	264.4 ⁽³⁾	(1)	(1)
B	109.1	110.7	99.5	101.0	170.9	173.4
D	138.1	138.0	132.5	132.4	110.6	110.6
E	166.4	161.7	124.3	120.8	109.2	106.1
F	164.4 ⁽²⁾	140.1 ⁽²⁾	142.2	120.1	175.9 ⁽²⁾	148.5 ⁽²⁾
G	105.4 ⁽²⁾	152.6 ⁽²⁾	48.1	69.6	114.8 ⁽²⁾	166.0 ⁽²⁾
H	98.9	98.0	109.0	108.0	116.4	115.4
I	112.5	112.3	120.4	120.1	123.4	123.1
J	117.4	119.2	99.5	100.8	83.1	84.2
K	151.2	153.4	115.1	116.9	87.4	88.8
L	56.2	57.2	150.2	152.5	72.3	73.4
M	80.0	77.5	96.1	93.1	114.5	110.8
N	557.2 ⁽²⁾	4.6 ⁽²⁾	238.3 ⁽²⁾	4.5 ⁽²⁾	251.4 ⁽²⁾	4.7 ⁽²⁾
O	80.1	78.5	69.5	68.1	104.6	102.5
P	113.3	107.5	127.3	120.8	116.8	110.8

⁽¹⁾ Non valid data (results for spiked extract no provided for matrix correction)⁽²⁾ Cochran outlier⁽³⁾ Single Grubbs outlier

Appendix 2.2-Table 15. Results provided by participants for samples DAY 1-EURLMB/VAL/L-04 and DAY 1-EURLMB/VAL/L-06 (blind duplicates) for AZA3 in µg/kg.

QUANTITATION LAB CODE	CRM-INDIVIDUAL(AZA1)_Uncorrected		MULTITOXIN(AZA3)_Uncorrected		MULTITOXIN(AZA3)_Matrix correct.	
	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06
A	(1)	(1)	(1)	(1)	(1)	(1)
B	<LOQ	<LOQ	15.4	15.1	21.4	21.0
D	44.6	41.4	38.4	34.7	31.1	28.1
E	43.6	36.1	36.7	28.8	35.4	27.8
F	25.0	23.5	16.7	15.3	20.5	18.8
G	34.6	23.3	19.4	13.6	26.5	18.6
H	<LOQ	<LOQ	18.8	19.1	17.3	17.6
I	<LOQ	<LOQ	20.3	23.1	18.0	20.5
J	8.5	11.5	22.7	25.9	15.0	17.1
K	46.5	47.9	23.0	23.7	19.2	19.8
L	107.6 ⁽⁴⁾	123.9 ⁽⁴⁾	104.8 ⁽²⁾	120.0 ⁽²⁾	101.7 ⁽²⁾	116.4 ⁽²⁾
M	<LOQ	<LOQ	21.5	18.4	17.2	14.7
N	101.1 ⁽⁴⁾	95.7 ⁽⁴⁾	34.1	32.1	32.4	30.5
O	28.1	26.8	20.7	19.6	18.6	17.6
P	20.2	19.8	19.0	18.5	19.9	19.4

⁽¹⁾ Non valid data (results no provided for evaluation)⁽²⁾ Cochran outlier⁽⁴⁾ Double Grubbs outlier

Appendix 2.2-Table 16. Results provided by participants for samples DAY 2-EURLMB/VAL/L-09 and DAY 2-EURLMB/VAL/L-10 (blind duplicates) for AZA3 in µg/kg.

QUANTITATION LAB CODE	CRM-INDIVIDUAL(AZA1)_Uncorrected		MULTITOXIN(AZA3)_Uncorrected		MULTITOXIN(AZA3)_Matrix correct.	
	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10	DAY 2- EURLMB/VAL/L- 09	DAY 2- EURLMB/VAL/L- 10
A	230.4 ⁽⁴⁾	237.2 ⁽⁴⁾	513.5 ⁽³⁾	539.0 ⁽³⁾	80.6	84.6
B	93.1	96.2	79.4	82.1	123.1	127.2
D	123.2	124.3	157.1	158.5	122.4	123.6
E	114.2	116.1	97.7	99.2	77.7	78.9
F	111.1	103.3	103.6	96.2	114.7	106.5
G	133.0	77.7	75.8	43.8	84.0	48.5
H	62.9	59.2	107.7	101.8	98.6	93.3
I	81.3	84.0	120.8	125.0	116.3	120.4
J	102.1	107.3	115.6	120.7	81.5	85.1
K	213.9 ⁽⁴⁾	210.2 ⁽⁴⁾	110.4	108.3	98.6	96.7
L	52.5	51.1	44.5	43.3	51.2	49.9
M	96.1	122.1	110.7	140.5	104.3	132.3
N	450.6 ⁽³⁾	507.5 ⁽³⁾	130.6	147.1	132.7	149.4
O	108.9	114.8	81.7	86.0	81.1	85.4
P	76.9	76.6	90.0	89.7	87.2	86.9

⁽³⁾ Single Grubbs outlier⁽⁴⁾ Double Grubbs outlier

Appendix 2.2-Table 17. Results provided by participants for samples DAY 3-EURLMB/VAL/L-12 and DAY 3-EURLMB/VAL/L-13 (blind duplicates) for AZA3 in µg/kg.

QUANTITATION LAB CODE	CRM-INDIVIDUAL(AZA1)_Uncorrected		MULTITOXIN(AZA3)_Uncorrected		MULTITOXIN(AZA3)_Matrix correct.	
	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13	DAY 3- EURLMB/VAL/L- 12	DAY 3- EURLMB/VAL/L- 13
A	288.7 ⁽³⁾	301.6 ⁽³⁾	445.9 ⁽³⁾	463.9 ⁽³⁾	(1)	(1)
B	99.1	90.7	96.0	88.0	136.3	124.9
D	108.7	130.9	135.4	164.4	116.2	141.1
E	127.5	126.5	109.4	108.5	92.9	92.1
F	137.6	116.6	128.8	107.9	156.9	131.5
G	132.4	112.5	79.2	66.9	118.1	99.8
H	69.0	66.9	116.3	113.1	111.2	108.2
I	87.4	81.9	120.0	112.2	111.2	103.9
J	89.5	82.8	101.0	94.6	71.6	67.1
K	223.9 ⁽³⁾	221.7 ⁽³⁾	114.7	113.5	95.6	94.6
L	119.4	121.0	115.5	116.9	90.2	91.3
M	118.2	128.2	118.9	129.1	90.8	98.5
N	595.1 ⁽³⁾	632.2 ⁽³⁾	173.6	184.2	225.9 ⁽³⁾	239.7 ⁽³⁾
O	112.8	113.2	86.4	86.7	94.5	94.9
P	85.4	84.3	105.1	103.6	103.8	102.4

⁽¹⁾ Non valid data (results for spiked extract no provided for matrix correction)⁽³⁾ Single Grubbs outlier

Appendix 2.3. PTX2 results

Appendix 2.3-Table 1. Results provided by participants for samples DAY 1-EURLMB/VAL/L-01 and DAY 1-EURLMB/VAL/L-03 (blind duplicates) for PTX2 in µg/kg.

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		MULTITOXIN_Uncorrected		MULTITOXIN_Matrix correction	
	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03	DAY 1- EURLMB/VAL/L- 01	DAY 1- EURLMB/VAL/L- 03
A	100.9	93.6	148.3	137.6	112.7	105.0	147.5	137.4
B	91.3	105.8	90.8	105.2	99.5	113.6	94.1	107.4
D	81.3	109.3	54.5	73.3	99.7	133.2	55.3	73.9
E	132.2	126.1	93.9	89.6	145.6	139.3	95.3	91.2
F	66.4	62.9	75.3	71.3	64.0	60.2	71.0	66.8
G	77.1	79.5	153.5	158.2	84.0	86.5	152.9	157.6
H	72.4	68.8	78.2	74.4	80.0	75.9	77.2	73.3
I	77.5	83.0	69.8	74.8	101.5	107.0	78.9	83.2
J	*	*	*	*	*	*	*	*
K	65.6	63.6	106.0	102.8	60.4	58.1	100.6	96.7
L	25.5	28.1	27.7	30.4	23.8 ⁽¹⁾	26.9 ⁽¹⁾	22.1 ⁽¹⁾	25.0 ⁽¹⁾
M	59.0	77.1	53.7	70.2	90.8	110.6	65.1	79.3
N	*	*	*	*	*	*	*	*
O	136.4	100.0	134.1	98.3	142.6	103.5	134.1	97.4
P	86.8	85.1	90.1	88.3	101.8	99.7	89.3	87.5

*Not results provided for PTX2

⁽¹⁾ Non valid data (quantified below the calibration working range)

Appendix 2.3-Table 2. Results provided by participants for samples DAY 2-EURLMB/VAL/L-07 and DAY 2-EURLMB/VAL/L-08 (blind duplicates) for PTX2 in µg/kg.

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		MULTITOXIN_Uncorrected		MULTITOXIN_Matrix correction	
	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08	DAY 2- EURLMB/VAL/L- 07	DAY 2- EURLMB/VAL/L- 08
A	110.4	102.7	101.2	94.2	106.7	98.9	99.8	92.5
B	100.5	106.8	95.4	101.4	96.1	102.6	93.4	99.7
D	68.0	71.0	60.4	63.1	72.1	75.6	57.5	60.2
E	115.2	120.6	70.8	74.1	112.4	117.9	72.9	76.5
F	64.9	65.3	69.0	69.4	70.3	70.8	69.2	69.6
G	65.2	45.0	126.6 ⁽²⁾	87.3 ⁽²⁾	76.5	54.6	127.2 ⁽²⁾	90.8 ⁽²⁾
H	70.1	67.4	84.2	81.0	81.1	78.0	85.1	81.9
I	90.0	96.0	79.2	84.5	106.8	113.2	82.5	87.4
J	*	*	*	*	*	*	*	*
K	56.5	57.0	91.1	91.9	44.9	45.5	79.9	80.9
L	22.6	42.4	21.8	41.0	-0.8 ⁽¹⁾	25.6 ⁽¹⁾	-0.7 ⁽¹⁾	22.3 ⁽¹⁾
M	55.5	72.4	54.6	71.2	71.2	90.5	58.4	74.2
N	*	*	*	*	*	*	*	*
O	103.9	116.6	103.2	115.9	95.5	108.8	100.0	114.0
P	62.4	72.1	56.9	65.8	94.4	104.2	70.5	77.8

* Not results provided for PTX2

⁽¹⁾ Non valid data (quantified below the calibration working range)⁽²⁾ Cochran outlier

Appendix 2.4. YTX results

Appendix 2.4-Table 1. Results provided by participants for samples DAY 1-EURLMB/VAL/L-04 and DAY 1-EURLMB/VAL/L-06 (blind duplicates) for YTX in µg/kg.

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected		CRM-INDIVIDUAL_Matrix correction		MULTITOXIN_Uncorrected		MULTITOXIN_Matrix correction	
	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06	DAY 1- EURLMB/VAL/L- 04	DAY 1- EURLMB/VAL/L- 06
A	296.2 ⁽¹⁾	290.2 ⁽¹⁾	141.4 ⁽¹⁾	138.5 ⁽¹⁾	138.0	129.2	1500.7 ⁽¹⁾	1404.7 ⁽¹⁾
B	105.2	94.3	107.2	96.0	97.5	87.8	106.8	96.2
D	93.9	84.2	67.5	60.5	54.8	44.6	53.5	44.0
E	166.7	142.4	110.0	94.0	143.2	123.9	109.6	94.9
F	95.5	96.4	94.3	95.2	100.5	101.4	94.8	95.6
G	83.6	62.7	111.1	83.3	101.6	75.5	111.4	82.8
H	129.1	122.9	108.0	102.8	146.8	140.0	107.6	102.6
I	122.0	143.8	95.5	112.5	115.8	133.9	96.0	111.0
J	*	*	*	*	120.1	110.0	104.0	95.3
K	103.4	83.0	92.4	74.2	128.5	109.3	94.1	80.1
L	69.2	64.8	163.7	153.2	86.9	82.2	150.1	141.8
M	194.9	195.8	157.4	158.1	259.6 ⁽²⁾	260.8 ⁽²⁾	156.6	157.3
N	*	*	*	*	*	*	*	*
O	130.4 ⁽¹⁾	111.6 ⁽¹⁾	281.9 ⁽¹⁾	241.3 ⁽¹⁾	178.7 ⁽¹⁾	163.7 ⁽¹⁾	160.5 ⁽¹⁾	147.0 ⁽¹⁾
P	94.8	86.5	79.2	72.3	77.4	68.6	74.6	66.1

*Not results provided for YTX

⁽¹⁾ Non valid data (Lab A: quantified below the calibration working range; Lab O: quantitation calibration curve $R^2 < 0.7$)

⁽²⁾ Single Grubbs outlier

Appendix 2.5. NRC RM-FDMT results

Appendix 2.5-Table 1. Results provided by participants for NRC RM-FDMT for OA in µg/kg (reconstituted wet tissue) by CRM-Individual standard quantitation.

QUANTITATION	CRM-INDIVIDUAL_Uncorrected			CRM-INDIVIDUAL_CRM correction			CRM-INDIVIDUAL_Matrix correction		
	LAB CODE	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2
A	269.1	288.7	268.2	250.4	230.0	288.9	248.2	205.8	*
B	2652.2 ⁽³⁾	2838.8 ⁽³⁾	2880.9 ⁽³⁾	210.8	245.0	217.7	1885.1 ⁽²⁾	2059.3 ⁽²⁾	2546.5 ⁽²⁾
D	267.0	310.2	284.7	251.9	282.6	263.2	147.0	146.4	171.3
E	401.1	281.7	301.5	413.6	285.2	317.0	266.1	222.4	213.0
F	394.4	303.3	325.7	327.8	261.0	281.9	251.9	227.5	195.5
G	297.5	335.9	189.3	326.9 ⁽²⁾	381.5 ⁽²⁾	166.5 ⁽²⁾	244.1	283.6	173.0
H	222.3	230.2	274.3	235.8	218.8	243.3	232.4	220.7	236.7
I	234.6	235.1	248.2	286.7	294.5	266.4	240.8	244.4	208.9
J	233.7	168.3	247.7	325.2	222.0	349.7	228.9	186.1	314.0
K	310.1	184.9	321.8	271.2	236.5	255.8	278.1	422.1	289.0
L	139.1	175.8	212.4	103.5 ⁽⁴⁾	179.7 ⁽⁴⁾	163.6 ⁽⁴⁾	158.1	195.6	192.6
M	549.4	375.1	512.0	472.4 ⁽⁴⁾	331.4 ⁽⁴⁾	479.2 ⁽⁴⁾	304.6	290.5	271.5
N	283.7	312.5	490.1	263.2 ⁽²⁾	366.0 ⁽²⁾	598.5 ⁽²⁾	215.1 ⁽²⁾	317.2 ⁽²⁾	740.4 ⁽²⁾
O	396.2	494.0	440.6	243.2	303.2	270.4	207.2	258.4	230.5
P	354.9	365.2	369.1	315.9	293.5	283.8	258.9	249.7	242.8

*Not results provided for spiked extract

⁽²⁾ Cochran outlier

⁽³⁾ Single Grubbs outlier

⁽⁴⁾ Double Grubbs outlier

Appendix 2.5-Table 2. Results provided by participants for NRC RM-FDMT for OA in µg/kg (reconstituted wet tissue) by Multitoxin standard quantitation.

QUANTITATION LAB CODE	Multitoxin_Uncorrected			Multitoxin_CRM correction			Multitoxin_Matrix correction		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
A	251.8	243.8	271.0	247.9	237.9	295.9	235.9	256.1	*
B	2346.7 ⁽³⁾	2659.6 ⁽³⁾	2457.6 ⁽³⁾	199.5	235.1	220.4	2011.4 ⁽²⁾	2182.9 ⁽²⁾	2488.9 ⁽²⁾
D	252.0	308.5	254.1	258.9	298.3	261.0	154.3	155.8	168.1
E	371.6	282.8	290.2	401.4	294.8	323.0	254.0	220.2	212.0
F	346.9	258.5	275.4	328.7	263.2	284.8	252.1	228.2	196.8
G	273.4	327.6	165.5	328.0 ⁽²⁾	387.1 ⁽²⁾	165.3 ⁽²⁾	246.0	292.2	181.0
H	234.5	244.6	269.7	235.4	218.4	242.4	230.1	217.4	231.9
I	228.6	209.2	227.9	278.1	295.6	266.5	219.8	247.2	209.2
J	280.0	205.9	289.1	296.7	215.5	323.5	191.5	157.1	255.8
K	290.4	196.1	267.8	275.3	240.1	258.6	299.1 ⁽²⁾	544.0 ⁽²⁾	310.3 ⁽²⁾
L	143.5	175.2	250.7	100.8 ⁽⁴⁾	178.6 ⁽⁴⁾	162.6 ⁽⁴⁾	164.5	219.6	201.7
M	508.2	379.5	468.8	463.5 ⁽⁴⁾	318.6 ⁽⁴⁾	481.6 ⁽⁴⁾	290.1	260.7	273.9
N	296.4 ⁽²⁾	353.0 ⁽²⁾	569.7 ⁽²⁾	255.5 ⁽²⁾	332.4 ⁽²⁾	562.7 ⁽²⁾	195.1 ⁽²⁾	251.7 ⁽²⁾	615.1 ⁽²⁾
O	350.6	421.7	398.7	277.2	333.3	315.1	225.6	271.3	256.5
P	332.5	342.8	313.0	316.6	302.6	277.4	259.3	255.0	239.0

*Not results provided for spiked extract

⁽²⁾ Cochran outlier

⁽³⁾ Single Grubbs outlier

⁽⁴⁾ Double Grubbs outlier

Appendix 2.5-Table 3. Results provided by participants for NRC RM-FDMT for DTX2 in µg/kg (reconstituted wet tissue) by CRM-Individual standard quantitation (against OA calibrant).

QUANTITATION	CRM-INDIVIDUAL_Uncorrected			CRM-INDIVIDUAL_CRM correction			CRM-INDIVIDUAL_Matrix correction		
	LAB CODE	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2
A	714.8	743.6	782.0	665.1	592.3	842.5	663.5	468.0	*
B	6594.9 ⁽³⁾	6380.4 ⁽³⁾	6378.5 ⁽³⁾	524.1 ⁽⁴⁾	550.6 ⁽⁴⁾	482.0 ⁽⁴⁾	4266.1 ⁽²⁾	4256.4 ⁽²⁾	4758.0 ⁽²⁾
D	1549.2	1364.6	1713.3	1461.6 ⁽⁴⁾	1242.8 ⁽⁴⁾	1584.3 ⁽⁴⁾	418.0	376.7	514.1
E	928.7	730.9	796.5	957.6	740.0	837.4	592.1	545.0	526.4
F	981.7	854.1	773.3	815.9	735.1	669.4	587.1	555.3	399.6
G	678.9	835.0	748.3	745.9	948.4	658.0	465.4	701.2	646.2
H	655.8	691.0	798.5	695.6	656.6	708.3	631.4	629.7	656.7
I	757.8	632.3	741.8	926.1	792.0	796.1	645.4	464.9	581.7
J	758.5	547.3	866.4	1055.2	721.8	1223.2	687.0	620.7	824.1
K	928.0	608.1	935.3	811.4	777.5	743.3	705.1	948.7	610.9
L	535.0	542.8	794.5	398.2	554.9	612.2	643.2	432.1	604.8
M	1201.3	917.9	1307.7	1032.9	811.0	1223.9	720.1	840.4	722.5
N	1093.9	1185.6	1252.0	1014.7	1388.4	1529.0	735.0 ⁽²⁾	1231.5 ⁽²⁾	1409.7 ⁽²⁾
O	1210.1	1108.6	1182.7	742.7	680.4	725.9	545.8	500.0	533.4
P	1061.9	914.7	941.1	945.2	735.2	723.5	612.8	525.1	503.3

*Not results provided for spiked extract

⁽²⁾ Cochran outlier⁽³⁾ Single Grubbs outlier⁽⁴⁾ Double Grubbs outlier

Appendix 2.5-Table 4. Results provided by participants for NRC RM-FDMT for DTX2 in µg/kg (reconstituted wet tissue) by Multitoxin standard quantitation (against DTX2 calibrant).

QUANTITATION LAB CODE	Multitoxin_Uncorrected			Multitoxin_CRM correction			Multitoxin_Matrix correction		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
A	518.9 ⁽²⁾	793.6 ⁽²⁾	2391.5 ⁽²⁾	510.8 ⁽²⁾	774.2 ⁽²⁾	2611.4 ⁽²⁾	614.6	742.7	*
B	5858.2 ⁽³⁾	5522.3 ⁽³⁾	5363.8 ⁽³⁾	497.9	488.0	481.0	4572.2 ⁽³⁾	4355.5 ⁽³⁾	4846.3 ⁽³⁾
D	855.2	816.9	865.7	878.3	789.9	889.2	437.8	409.4	489.1
E	815.3	648.0	689.1	880.8	675.5	767.0	562.1	494.7	499.1
F	842.2	670.8	630.9	798.0	683.0	652.6	597.6	616.5	392.6
G	572.1	679.6	609.6	686.3	803.2	608.9	392.5	594.6	559.5
H	576.6	589.0	633.8	578.8	526.0	569.5	630.8	612.9	623.5
I	609.5	523.8	619.8	741.5	740.0	724.7	572.5	472.4	616.6
J	795.7	562.0	893.4	843.1	588.2	999.8	523.4	426.7	657.7
K	804.8	437.0	654.8	763.0	535.1	632.3	761.3 ⁽²⁾	1665.9 ⁽²⁾	722.7 ⁽²⁾
L	530.5	474.1	837.4	372.8	483.2	543.1	835.4	451.3	638.2
M	1113.0	938.5	1280.9	1015.2	787.8	1316.0	675.7	743.5	762.0
N	1018.5	1033.2	1194.6	878.0	973.0	1180.0	757.1 ⁽³⁾	880.0 ⁽³⁾	1164.7 ⁽³⁾
O	873.9	798.1	846.2	690.8	630.9	668.9	557.3	509.0	539.7
P	878.9	724.7	705.0	836.7	639.8	624.9	618.2	523.7	503.0

*Not results provided for spiked extract

⁽²⁾ Cochran outlier⁽³⁾ Single Grubbs outlier

Appendix 2.5-Table 5. Results provided by participants for NRC RM-FDMT for DTX1 in µg/kg (reconstituted wet tissue) by CRM-Individual standard quantitation (against OA calibrant).

QUANTITATION	CRM-INDIVIDUAL_Uncorrected			CRM-INDIVIDUAL_CRM correction			CRM-INDIVIDUAL_Matrix correction		
	LAB CODE	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2
A	135.8	173.4	143.1	108.8	178.4	161.4	126.5	111.5	*
B	2922.4 ⁽³⁾	2979.5 ⁽³⁾	2952.5 ⁽³⁾	1001.6 ⁽²⁾	1159.3 ⁽²⁾	846.1 ⁽²⁾	747.7 ⁽³⁾	780.5 ⁽³⁾	770.0 ⁽³⁾
D	189.8	239.4	167.3	100.3	102.1	127.5	101.3	128.9	104.4
E	176.2	165.0	186.7	147.7	135.7	164.4	97.0	108.7	111.7
F	148.5	102.9	92.4	222.2	201.6	139.7	170.5	124.4	94.1
G	124.9	181.0	123.1	115.3	152.8	96.7	108.8	156.2	92.7
H	104.4	119.5	130.5	114.1	129.9	124.6	121.5	125.9	122.3
I	182.9	148.9	146.7	188.4	131.7	137.5	138.8	101.5	106.4
J	125.0	101.6	135.3	130.2	118.6	143.4	72.5	77.7	79.8
K	222.3	115.6	208.1	127.3	106.9	112.2	108.5	103.5	105.3
L	119.6	107.5	138.0	76.1	96.9	97.2	59.6	98.0	150.7
M	190.0 ⁽²⁾	160.5 ⁽²⁾	448.6 ⁽²⁾	152.4 ⁽²⁾	176.3 ⁽²⁾	335.4 ⁽²⁾	128.9	126.4	180.1
N	191.6	199.7	209.7	182.5	206.5	193.0	157.3	183.6	214.8
O	215.5	213.5	314.3	99.6	106.3	136.9	100.3	114.8	113.1
P	152.1	183.6	207.4	111.3	107.9	115.1	63.0	66.8	79.1

* Not results provided for spiked extract

⁽²⁾ Cochran outlier⁽³⁾ Single Grubbs outlier

Appendix 2.5-Table 6. Results provided by participants for NRC RM-FDMT for DTX1 in µg/kg (reconstituted wet tissue) by Multitoxin standard quantitation (against DTX1 calibrant).

QUANTITATION LAB CODE	Multitoxin_Uncorrected			Multitoxin_CRM correction			Multitoxin_Matrix correction		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
A	119.2	144.1	140.0	105.8	173.0	162.3	127.7	111.9	*
B	1772.8 ⁽²⁾	1975.3 ⁽²⁾	1790.0 ⁽²⁾	978.0 ⁽²⁾	1129.6 ⁽²⁾	856.7 ⁽²⁾	736.5 ⁽³⁾	766.9 ⁽³⁾	774.9 ⁽³⁾
D	169.5	228.2	139.8	94.5	92.0	130.0	100.3	133.9	104.7
E	156.6	155.0	165.3	151.1	142.5	167.6	98.0	109.7	112.1
F	276.1	185.6	184.8	222.3	201.6	141.4	171.1	124.7	94.6
G	122.2	161.2	108.0	117.0	158.3	105.6	108.9	147.5	95.1
H	101.9	124.3	123.5	109.2	132.6	121.7	122.9	124.9	123.2
I	155.5	109.1	127.6	189.3	131.5	139.0	136.4	101.4	106.5
J	168.6	143.2	167.2	144.3	137.7	154.0	79.8	86.1	85.2
K	156.0	104.0	133.8	123.4	105.9	107.9	108.4	103.3	105.0
L	84.7	74.4	114.5	69.1	89.6	89.8	55.5 ⁽²⁾	96.3 ⁽²⁾	160.0 ⁽²⁾
M	200.1 ⁽²⁾	175.5 ⁽²⁾	435.0 ⁽²⁾	157.5 ⁽²⁾	177.5 ⁽²⁾	336.2 ⁽²⁾	126.1	125.4	180.5
N	190.2	220.1	241.0	182.3	206.0	195.3	157.9	165.4	177.9
O	151.9	171.6	232.4	101.6	101.5	138.3	100.6	115.3	113.0
P	132.7	170.7	176.5	102.2	112.4	122.0	57.9	69.6	83.7

*Not results provided for spiked extract

⁽²⁾ Cochran outlier⁽³⁾ Single Grubbs outlier

Appendix 2.5-Table 7. Results provided by participants for NRC RM-FDMT for AZA1 in µg/kg (reconstituted wet tissue).

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected			CRM-INDIVIDUAL_Matrix correction		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
A	*	2805.6 ⁽¹⁾	586.7 ⁽¹⁾	*	617.4 ⁽¹⁾	*
B	440.8	418.1	395.0	1002.7 ⁽²⁾	529.7 ⁽²⁾	549.7 ⁽²⁾
D	556.2	641.9	577.4	438.4	602.3	555.7
E	527.7	740.6	730.3	462.7	676.3	622.2
F	635.4	574.6	439.7	697.0	646.0	488.6
G	326.3	438.3	330.5	491.3	523.9	438.5
H	546.9	518.3	564.8	547.0	561.2	595.3
I	665.6	679.5	599.8	587.6	649.6	599.2
J	628.5	660.3	623.3	572.4	621.7	622.8
K	699.9	650.1	658.4	565.4	515.4	515.1
L	674.8	648.9	768.9	727.8	647.8	710.3
M	717.6	666.1	558.2	497.8	584.2	565.7
N	811.6	1040.5	993.1	772.9 ⁽²⁾	948.0 ⁽²⁾	1845.0 ⁽²⁾
O	525.0	491.1	484.2	683.4	639.3	630.2
P	694.5	598.8	651.6	1016.9 ⁽³⁾	1009.9 ⁽³⁾	759.7 ⁽³⁾

*Results not provided for evaluation

⁽¹⁾ Non valid data (Lab A: result out of the calibration range, not considered for evaluation)

⁽²⁾ Cochran outlier

⁽³⁾ Single Grubbs outlier

Appendix 2.5-Table 8. Results provided by participants for NRC RM-FDMT for AZA2 and AZA3 in µg/kg (reconstituted wet tissue) by CRM-Individual standard quantitation (against AZA-1 calibrant).

TOXIN/QUANTITATION	AZA2/CRM-INDIVIDUAL_Uncorrected			AZA3/CRM-INDIVIDUAL_Uncorrected		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
A	*	1017.7 ⁽²⁾	1419.9 ⁽²⁾		305.2 ⁽⁴⁾	251.4 ⁽⁴⁾
B	108.9	119.7	115.9	121.7	129.9	122.9
D	210.1	167.9	159.7	194.5	159.7	146.9
E	161.9	204.6	229.5	157.2	175.1	196.7
F	199.7	178.9	134.8	182.9	158.3	129.3
G	164.6	218.4	167.5	121.4	162.8	129.8
H	127.5	120.1	128.2	98.7	88.4	98.3
I	146.1	148.6	132.9	123.5	123.0	122.7
J	159.1	163.5	149.9	133.7	145.0	134.9
K	226.4	188.2	221.2	325.8 ⁽⁴⁾	314.7 ⁽⁴⁾	341.9 ⁽⁴⁾
L	90.3	97.5	108.5	178.2	170.1	101.0
M	112.1	70.1	111.8	160.8	117.5	206.4
N	507.9 ⁽²⁾	758.7 ⁽²⁾	696.0 ⁽²⁾	542.2 ⁽²⁾	814.0 ⁽²⁾	795.2 ⁽²⁾
O	142.8	148.0	140.0	245.6	235.3	229.3
P	138.6	124.0	144.3	134.7	124.0	131.5

*Results not provided for evaluation

⁽²⁾ Cochran outlier⁽⁴⁾ Double Grubbs outlier

Appendix 2.5-Table 9. Results provided by participants for NRC RM-FDMT for PTX2 in µg/kg (reconstituted wet tissue).

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected			CRM-INDIVIDUAL_Matrix correction			MULTITOXIN_Uncorrected			MULTITOXIN_Matrix correction		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
A	86.2	84.1	<LOQ	126.7	77.1	*	97.1	79.8	67.6	127.1	74.6	*
B	83.3	80.7	73.8	82.8	76.6	75.5	91.7	75.8	71.7	86.7	73.6	70.7
D	142.8	112.3	97.6	95.8	99.8	70.4	173.4	123.5	124.4	96.2	98.4	71.8
E	160.3	174.4	152.3	113.9	107.1	106.9	175.5	166.8	154.2	114.9	108.3	107.8
F	98.3	104.3	89.3	111.4	110.9	98.4	98.9	112.9	53.7	109.7	111.0	60.3
G	42.2	46.7	45.0	84.0	90.7	97.4	46.9	56.5	48.8	85.4	93.9	97.1
H	76.0	71.5	67.7	82.1	85.9	73.7	84.1	82.6	73.6	81.2	86.8	71.6
I	115.7	119.0	91.1	104.2	104.8	86.8	139.4	137.9	103.1	108.5	106.5	88.9
J	*	*	*	*	*	*	*	*	*	*	*	*
K	77.9	78.4	80.6	125.8	126.4	123.0	74.6	69.9	78.1	124.2	124.4	121.0
L	<LOQ	64.0	74.2	<LOQ	61.8	58.0	<LOQ	54.3	73.2	<LOQ	47.3	50.3
M	153.4	113.7	167.9	139.7	111.8	140.8	193.7	137.7	170.9	138.9	112.9	141.5
N	*	*	*	*	*	*	*	*	*	*	*	*
O	137.8	97.0	72.4	135.4	96.3	108.8	144.0	88.3	69.1	135.5	92.5	107.6
P	100.6	91.6	116.1	104.4	83.6	93.8	118.4	123.9	123.9	103.9	92.5	95.3

*Not results provided for PTX2

⁽¹⁾ Non valid data (quantified below the calibration working range)

Appendix 2.5-Table 9. Results provided by participants for NRC RM-FDMT for YTX in µg/kg (reconstituted wet tissue).

QUANTITATION LAB CODE	CRM-INDIVIDUAL_Uncorrected			CRM-INDIVIDUAL_Matrix correction			MULTITOXIN_Uncorrected			MULTITOXIN_Matrix correction		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
A	673.5	493.1	267.1	321.4 ⁽¹⁾	211.3 ⁽¹⁾	*	699.0 ⁽¹⁾	415.5	430.2	*	*	*
B	277.1	269.9	260.9	282.3	281.9	276.5	248.8	249.8	229.1	272.6	301.3	274.6
D	238.3	246.7	230.9	171.3	229.4	220.3	207.0	205.7	211.8	201.9	221.3	269.8
E	489.0	433.8	458.2	322.7	348.8	354.6	406.3	340.2	434.9	311.0	315.0	341.4
F	490.3	377.7	301.2	484.4	396.9	312.5	498.9	350.3	310.8	470.4	405.3	311.7
G	218.2	184.0	187.3	290.0	293.4	269.5	269.2	221.4	225.8	295.2	288.6	271.0
H	532.0	527.8	592.9	444.9	393.3	464.2	586.5	593.6	579.9	429.9	433.0	412.3
I	481.0	534.4	337.8	376.4	529.9	532.5	412.7	473.7	390.1	342.0	461.5	777.6
J	*	*	*	*	*	*	301.7	333.2	309.0	261.3	275.4	243.3
K	415.4	460.0	388.7	371.1	338.8	422.8	421.9	452.5	360.8	309.1	371.5	407.0
L	315.8	257.8	386.9	747.0	584.6	8920.4	352.5	268.8	458.6	608.6	589.7	628.6
M	452.1	367.6	474.1	365.1	365.9	420.7	599.0	516.3	523.6	361.3	393.7	397.5
N	*	*	*	*	*	*	*	*	*	*	*	*
O	186.7 ⁽¹⁾	393.1 ⁽¹⁾	178.6 ⁽¹⁾	403.7 ⁽¹⁾	933.6 ⁽¹⁾	181.5 ⁽¹⁾	223.8 ⁽¹⁾	363.1 ⁽¹⁾	270.2 ⁽¹⁾	200.9 ⁽¹⁾	504.4 ⁽¹⁾	161.3 ⁽¹⁾
P	248.6	215.4	251.6	207.9	213.4	230.6	240.7	218.9	232.2	232.1	206.9	237.8

*Not results provided for YTX

⁽¹⁾ Non valid data (Lab A: quantified out of the calibration working range; Lab O: quantitation calibration curve $R^2 < 0.9$)⁽²⁾ Single Grubbs outlier

APPENDIX 3. Standard Operating Procedure used for the validation study

(see separate pdf file)