QUASIMEME

Laboratory Performance Studies

Round 59

19 October 2009 to 30 January 2010

Exercise Protocols

QUASIMEME Laboratory Performance Studies
Round 59
19 October 2009 to 30 January 2010

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Introduction

Thank you for participating in the 2009 QUASIMEME Laboratory Performance studies.

For this study, you should receive:

- The test materials for the exercises in Round 59 that you have ordered sent to you by courier in the week beginning 19 October 2009.
- The exercise template files, for use with the QUEST[1] data transfer system (Windows 9X, XP, NT or 2000), and the protocol for the studies, to be downloaded from your Quasimeme Sharepoint Site.

There is one exercise template file per laboratory code for each of these studies.

Please check that the contents of your package are correct and that all test materials are intact. If any test materials have been damaged in transit or if the wrong samples have been send, use the form in Annex I of this document to request replacement materials within two weeks after receipt of the samples.

Additional test materials may also be purchased from QUASIMEME.

3.3.2.1 [2]

The concentration range of the test materials is within the guidelines agreed by the QUASIMEME Scientific Assessment Group, September 2008.

3.3.1.4 d

This protocol covers the following studies:

R59 Ex 864	AQ-3	Metals in Seawater
R59 Ex 865	AQ-4	Mercury in Seawater
R59 Ex 866	AQ-11	Chlorophyll-*a* in Seawater
R59 Ex 867	BT-3	Non ortho CBs, PCDDs and PCDFs in biota
R59 Ex 868	BT-7	ASP Shellfish Toxins
R59 Ex 869	DE-10	DSP Shellfish Toxins

3.5.1.2

All data for these studies must be uploaded your Quasimeme Sharepoint Site, no later than 30 January 2010.

3.5.1.3

All other information should be sent to:
QUASIMEME Project Office
Wageningen UR
Alterra CWK
P.O. Box 47
6700 AA Wageningen
The Netherlands

Tel.: +31 (0) 317 48 65 46
Fax: +31 (0) 317 41 90 00
E-mail: quasimeme@wur.nl
Website: http://www.Quasimeme.org

[1] QUEST (QUASIMEME Electronic Storage and Transfer) System. Information on installation and use of this software is given in Annex II.
[2] References to the ILAC G:13 2000 Guidelines for proficiency test providers are given throughout this document.

Exercise 864, AQ-3 (Round 59)
Trace Metals in Seawater QTM145SW, QTM146SW and QTM147SW

Objective

This study covers the determination of trace metals in the seawater test materials QTM145SW and QTM146SW and in the low-salinity seawater test material QTM147SW. The test materials should be analysed and one result for each trace metal in each test material should be reported using the QUEST system with the QUASIMEME Round 59 exercise template file.

Test Materials and Storage

3.5.1.3

The test materials were prepared at Alterra, Wageningen, The Netherlands. The seawater used to prepare the test materials was collected from the North Sea between Belgium and the UK, and was stored in the cold store at 7˚C in 25 litre carboys.

The test materials were prepared in bulk in 50 litre aspirators. The seawater was filtered using a 0.45 μm / 0.2 μm double-membrane filter. The seawater used to prepare test material QTM147SW was diluted with ultra-pure demineralised water, to a salinity of approximately 12 - 18 psu. All test materials are preserved with 2 ml trace metal grade nitric acid per litre of seawater. Test material QTM145SW is unspiked seawater. Test materials QTM146SW and QTM147SW were spiked seawater with aqueous solutions of known trace metal concentration.

Approximately 1 litre of each test material is provided.

Homogeneity of the test materials is assumed, as they were prepared in bulk and thoroughly mixed, before being dispensed into 1 litre polypropylene bottles. The test materials are stable for the purposes of the exercise.

Test materials should be stored ***in a refrigerator at +4^{o} C***, and analysed as soon as possible after receipt. Once the test materials are opened they should be analysed immediately.

Treat all test materials in the same manner as your routine samples.

Determinands and Concentration Ranges

The following trace metals should be determined:

Arsenic	Lead
Boron	Manganese
Cadmium	Nickel
Chromium	Silver
Cobalt	Tin
Copper	Vanadium
Iron	Zinc

Results should be reported for as many of these determinands as possible. Take this opportunity either to develop your methodology or check your performance on the less common determinands.

The concentrations of the trace metals in the spiked test materials are within the ranges mentioned in the Quasimeme brochure 2009 (page 13).

Analysis

3.3.1.1 k; 3.4; 3.5.1.5

Use your normal validated methods and procedures to analyse the test materials. This may include correcting for blanks and for recovery.

Only ***one*** result per determinand per test material is required. The results of each determinand should be expressed on the test material "as received". The concentration of metals should be determined against your own calibration solutions.

If you routinely conduct analyses by more than one technique you may report multiple sets of data. You should inform the QUASIMEME office staff, who will arrange to send you an additional exercise template file. It is not possible to report two sets of data using different methods on the same exercise template.

Some of the elements may present a health hazard, and some are biologically active. Please ensure that your analytical and handling procedures for these materials have been fully assessed for safety and that all specified precautions are taken.

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Exercise 865, AQ-4 (Round 59)
Mercury in Seawater QTM148SW, QTM149SW and QTM150SW

Objective

This study covers the determination of mercury in the seawater test materials QTM148SW, QTM149SW and QTM150SW. The test materials should be analysed and one result for mercury in each test material should be reported using the QUEST system with the QUASIMEME Round 59 exercise template file.

Test Materials and Storage

3.5.1.3

The test materials were prepared at Alterra, Wageningen, The Netherlands. The seawater used to prepare the test materials was collected from the North Sea between Belgium and the UK, and was stored in the cold store at 7˚C in 25 litre carboys.

The test materials were prepared in bulk in 50 litre aspirators. The seawater was filtered using a 0.45 μm / 0.2 μm double-membrane filter. All test materials are preserved with 2 ml trace metal grade nitric acid per litre of seawater. Test materials QTM148SW, QTM149SW and QTM150SW were spiked with aqueous solutions of known mercury concentration.

Approximately 1 litre of each test material is provided.

Homogeneity of the test materials is assumed, as they were prepared in bulk and thoroughly mixed, before being dispensed into 1 litre glass bottles. The test materials are stable for the purposes of the exercise.

Test materials should be stored ***in a refrigerator at +4^{o} C***, and analysed as soon as possible after receipt. Once the test materials are opened they should be analysed immediately.

Treat all test materials in the same manner as your routine samples.

Determinands and Concentration Ranges

Mercury should be determined in each test material

As a guide, the concentrations of mercury in the spiked test materials are within the range 0.1 – 100 ng/L.

Sample	Indicative value (ng/L)
QTM148SW	0.1 – 10
QTM149SW	0.1 – 10
QTM150SW	1 – 100

Analysis

3.3.1.1 k; 3.4; 3.5.1.5

Use your normal validated methods and procedures to analyse the test materials. This may include correcting for blanks and for recovery.

Only ***one*** result for mercury per test material is required. The results should be expressed on the test material “as received”. The concentration of mercury should be determined against your own calibration solutions.

If you routinely conduct analyses by more than one technique you may report multiple sets of data. You should inform the QUASIMEME office staff, who will arrange to send you an additional exercise template files. It is not possible to report two sets of data using different methods on the same exercise template.

This element presents some health hazard and is biologically active. Please ensure that your analytical and handling procedures for this material have been fully assessed for safety and that all specified precautions are taken.

Exercise 866, AQ-11 (Round 59)
Chlorophyll *a* in Seawater QCH044SW and QCH045SW

Objective

This study covers the determination of chlorophyll *a, b, c* and pheopigments in two filtered seawater residue test materials QCH044SW and QCH045SW. The test materials should be analysed and one result for each determinand in each test material should be reported using the QUEST[1] system with the QUASIMEME Round 59 AQ-11 exercise template file. Method codes should also be reported, to identify any method dependency of the results.

Test Materials and Storage

3.5.1.3

The test materials for the analysis of chlorophyll *a, b, c* and pheopigments were prepared at Alterra, Wageningen the Netherlands.

Test materials QCH044SW and QCH045SW were prepared from cultures of *Chaetocheros* + *Pyramimonas* grown at Wageningen IMARES, Yerseke the Netherlands and sub-sampled onto Whatman GF/F, 47 mm filter papers.

For each test material, the resultant damp filter paper was wrapped in aluminium foil, inserted into a cry vial and immediately 'flash frozen' in liquid nitrogen. The test materials were stored at –80°C until the day of dispatch.

The two test materials differ from each other in respect of their pigment content.

The test materials were homogeneous for the purposes of the LP study.

The filter papers have been shipped on cool packs, and should be stored at –20°C, or a lower temperature, immediately upon receipt, and should be analysed as soon as possible after receipt. The cool packs will thaw slowly during transit, but this does not significantly affect the stability of the material for the purpose of this study, providing the test materials themselves are frozen immediately on receipt.

3.5.1.5

Treat all test materials in the same manner as your routine samples.

[1] QUEST (QUASIMEME Electronic Storage and Transfer) System. Information on installation and use of this software is given in Annex II.

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Determinands and Concentration Ranges

Chlorophyll *a, b, c*, and pheopigments should be determined in each of the two filtered seawater residues, **using the method of your choice. Concentrations need to be calculated based on a filter prepared out of a 1 litre sample.**

Results should be reported for as many of these determinands as possible. Take this opportunity either to develop your methodology or check your performance on the less common determinands.

As a guide, the indicative concentration range of the pigments in the filtered seawater residues is:

Determinant	Units	Indication of Concentration Range
Chlorophyll *a*	µg/l	0.1 - 25
Chlorophyll *b*	µg/l	0.01 - 10
Chlorophyll *c*	µg/l	0.02 – 5
Pheopigments	µg/l	0.02 – 5

Analysis

3.3.1.1 k; 3.4; 3.5.1.5

Use your normal validated methods and procedures to analyse the test materials.

Only **one** result per determinand per test material is required. The results of each determinand should be expressed on the test materials "as received".

If you routinely conduct analyses by more than one technique you may report multiple sets of data. You should inform the QUASIMEME office staff, who will arrange to send you an additional exercise template file. It is not possible to report two sets of data using different methods on the same exercise template.

Whilst you should use your normal validated methods and procedures to analyse the test materials in this study, previous QUASIMEME development exercises have shown that the best between laboratory agreement was obtained with either the Trichromatic method (Jeffrey and Humphrey 1975) or the Monochromatic method (Lorenzen 1967). For those who wish to use these methods, the detailed "Protocol for the Spectrophotometric Determination of Chlorophyll-*a* and Pheopigments" is provided in the email as "Chlorophyll Spectrophotometric Protocol.pdf". This paper is a condensed version of the original ICES TIMES document No. 31, and contains details of the Trichromatic Method (Jeffery and Humphrey 1975) and the Monochromatic Method with acidification (Lorenzen 1967). This protocol only covers spectrophotometric methods.

Some of the compounds may present a health hazard, and some are biologically active. Please ensure that your analytical and handling procedures for the preparation of these materials have been fully assessed for safety and that all specified precautions are taken.

Exercise 867, BT-3 (Round 59)
Non *ortho* CBs, PCDDs and PCDFs in Biota QPL028BT and QPL029BT in biota

Objective

This study covers the determination of three non-ortho Chlorobiphenyls (CBs), seven polychlorinated dibenzo-p-dioxins (PCDDs), ten polychlorinated dibenzofurans (PCDFs), **total TEQ** and total lipid in two marine biota test materials QPL028BT and QPL029BT. The test materials should be analysed and one result for each determinand in each test material should be reported using the QUEST system with the QUASIMEME Round 59 exercise template file.

Test Materials and Storage

3.5.1.3

The test materials were supplied by Wageningen IMARES, Institute for Marine Resources and Ecosystem Studies, IJmuiden, The Netherlands and the Institute for Environmental Studies, Vrije Universiteit, Amsterdam, The Netherlands.

Test materials QPL028BT is a cod liver homogenate and QPL029BT is a fish homogenate.

The tins contain approximately 70 g of minced sterilized fish material, to which butylhydroxytoluene (BHT) has been added as an antioxidant.

Each batch of material was prepared in bulk. The level of within and between sample homogeneity for the biota was determined. All materials have been shown to be homogeneous at or below the intake mass used by the participants, and stable for the purposes of the test.

The test materials should be stored at room temperature, in a dry place, prior to analysis, and analysed as soon as possible after receipt. Although the tins have a protective coating, moisture can cause corrosion of the surface of the tins. Apart from the moisture, temperatures below $0^{o}C$ will not negatively affect the quality in the material, and we do not expect the contaminants to be affected at these temperatures. Temperatures above $30^{o}C$ may be disadvantageous for the material.

Treat all test materials in the same manner as your routine samples.

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Determinands

The following analytes should be determined:

Non-ortho CBs	
CB 77	3,4,3',4' tetrachlorobiphenyl
CB 126	3,4,5,3',4' pentachlorobiphenyl
CB 169	3,4,5,3',4',5' hexachlorobiphenyl
Polychlorinated dibenzo-p-dioxins (PCDDs)	
2,3,7,8 TCDD	2,3,7,8 Tetrachlorodibenzo-p-dioxin
1,2,3,7,8 PeCDD	1,2,3,7,8 Pentachlorodibenzo-p-dioxin
1,2,3,4,7,8 HxCDD	1,2,3,4,7,8 Hexachlorodibenzo-p-dioxin
1,2,3,6,7,8 HxCDD	1,2,3,6,7,8 Hexachlorodibenzo-p-dioxin
1,2,3,7,8,9 HxCDD	1,2,3,7,8,9 Hexachlorodibenze-p-dioxin
1,2,3,4,6,7,8 HpCDD	1,2,3,4,6,7,8 Heptachlorodibenzo-p-dioxin
OCDD	Octachlorodibenzodioxin
Polychlorinated dibenzofurans (PCDFs)	
2,3,7,8, TCDF	2,3,7,8 Tetrachlorodibenzofuran
1,2,3,7,8, PeCDF	1,2,3,7,8, Pentachlorodibenzofuran
2,3,4,7,8, PeCDF	2,3,4,7,8 Pentachlorodibenzofuran
1,2,3,4,7,8 HxCDF	1,2,3,4,7,8 Hexachlorodibenzofuran
1,2,3,6,7,8 HxCDF	1,2,3,6,7,8 Hexachlorodibenzofuran
2,3,4,6,7,8 HxCDF	2,3,4,6,7,8 Hexachlorodibenzofuran
1,2,3,7,8,9 HxCDF	1,2,3,7,8,9 Hexachlorodibenzofuran
1,2,3,4,6,7,8 HpCDF	1,2,3,4,6,7,8 Pentachlorodibenzofuran
1,2,3,4,7,8,9 HpCDF	1,2,3,4,7,8,9 Pentachlorodibenzofuran
OCDF	Octachlorodibenzofuran
Toxicity	
Total TEQ	**Total Toxicity Equivalents**

In addition total lipid should be determined. If you normally measure **extractable lipid**, there is a field in the template for reporting this measurement. This has been added following the request from a number of participants. However, we would encourage you to also report total lipid.

A successful QUASH study has found no significant difference between the Smedes lipid method and the Bligh and Dyer method for total lipid determination. We therefore recommend that wherever possible you use the Smedes lipid method, as it gives better reproducibility and does not involve the use of chlorinated solvents. A copy of the Smedes lipid method protocol is included in Annex III.

Results should be reported for as many of these determinands as possible. Take this opportunity either to develop your methodology or check your performance on the less common determinands.

Analysis

3.3.1.1 k; 3.4; 3.5.1.5

The tins of biota are filled almost to the brim, so should be opened carefully so that no tissue or moisture is spilt. The tins are filled in this way in order to eliminate as much air as possible. This minimizes any degradation of the test materials in transit and storage prior to opening.

The tins of biota should be cooled for a few hours at ca. 4^{o}C prior to opening, to eliminate possible overpressure. Opening the bottom of the tin, instead of the top, may also help to prevent spillage of moisture.

During the sterilisation process moisture may have partly separated from the solid part of the test materials, and this may cause inhomogeneity. Therefore, prior to taking an aliquot for analysis the

following treatment of the test materials is essential. After opening the test material container the complete contents should be thoroughly homogenised. As it is difficult to homogenize the sample in the tin, we recommend transferring the material to a larger container (e.g. glass or plastic beaker) for homogenization and sub-sampling. Aliquots should be taken immediately after homogenizing, to prevent liquid lipids from separating from the solid tissue particles, causing inhomogeneity again.

After sub-sampling the remaining tissue should be immediately transferred to a clean glass jar, sealed and stored at -20^{o}C.

Use your normal validated methods and procedures to analyse the test materials. This may include correcting for blanks and for recovery.

Only ***one*** result per determinand per test material is required. The results of each determinand should be expressed on the test material "as received" i.e. on a wet weight basis. If your normal method is to dry the test material prior to extraction then the dry weight must be determined and the final result calculated as **wet weight** using the dry weight determination. In this case the % dry weight should be reported.

$$\% \text{ dry weight} = 100 - \left[\frac{(\text{wet weight} - \text{dry weight}) * 100}{(\text{wet weight})}\right]$$

The concentrations should be determined against your own calibration solutions.

It should be noted that some of these compounds may present a health hazard and some are biologically active. Please ensure that your analytical and handling procedures for these materials have been fully assessed for safety and that all specified precautions are taken.

If you routinely conduct analyses by more than one technique you may report multiple sets of data. You should inform the QUASIMEME office staff, who will arrange to send you an additional exercise template file. It is not possible to report two sets of data using different methods on the same exercise template.

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Exercise 868, BT-7 (Round 59)
Shellfish Toxins (ASP) Exercise QST077SS, QST078BT and QST079BT

Objective

This study covers the determination of domoic acid and epidomoic acid as a racemic mixture, in a domoic acid standard solution QST077SS and in two tissue samples QST078BT and QST079BT. The test materials should be analysed and the sum of the isomers in each test material should be reported using the QUEST[2] system (Windows 9X, XP, NT or 2000) with the QUASIMEME Round 59 exercise template file, to be downloaded from your Quasimeme Sharepoint Site.

Test Materials and Storage:

3.5.1.3

The test materials were supplied by the Marine Institute, Galway, Republic of Ireland.

- QST077SS is a domoic acid standard solution.
- QST078BT is a Scallop tissue homogenate (adductor muscle) supplied in a plastic vial
- QST079BT is a Mussel tissue homogenate supplied in a plastic vial.

For QST078BT and QST079BT, each vial contains sufficient material for one-shot analysis of domoic and epidomoic acid.

Each batch of material was prepared in bulk. The level of within and between sample homogeneity and stability was determined. All materials have been shown to be homogeneous at or below the intake mass normally used, and stable for the purposes of the test.

Begin the analysis as soon as possible, preferably within 7 days of receipt

The shellfish tissue homogenates (contained in 7ml plastic vials) should be stored at -20°C, or a lower temperature, immediately upon receipt, until analysis.

The Standard solution QST077SS should be stored in the refrigerator at ca 4°C immediately upon receipt, until analysis.

The test materials have been shipped on cool packs. The cool packs will thaw slowly during transit, but this does not significantly affect the stability of the material for the purpose of this study, providing the test materials themselves are stored as directed, immediately on receipt.

[2] QUEST (QUASIMEME Electronic Storage and Transfer) System. Information on installation and use of this software is given in Annex II.

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Determinands

Report the sum of the domoic acid and epidomoic acid as a racemic mixture.

Analysis

3.3.1.1 k; 3.4; 3.5.1.5

It is advisable to analyse the test materials as soon as possible after receipt. Use your normal validated methods and procedures to analyse the test materials. This may include correcting for blanks and for recovery.

A result per test material is required, for the sum of domoic and epidomoic acid as a racemic mixture. The results of each determinand should be expressed on the test material "as received" i.e. on a wet weight basis. The concentrations should be determined against your own calibration solutions.

If you routinely conduct analyses by more than one technique you may report multiple sets of data. You should inform the QUASIMEME office staff, who will arrange to send you an additional exercise template file. It is not possible to report two sets of data using different methods on the same exercise template. Please use the View Methods function in the QUEST program to report your analytical method for each test material.

QST077SS, QST078BT and QST079BT

Each vial contains sufficient quantity of homogenate for one analysis. The whole transferable contents of each vial should be extracted, and one result reported for the sum of the two isomers. To transfer the contents into a preweighed or tared extraction tube, the vial should be fully defrosted, vortex-mixed and the contents poured into the desired container.

All results should be reported in mg/kg on the basis of wet weight of the test material as provided. The density of the standard solution QST077SS is 0.9853 g/ml at 22°C. For the homogenates QST078BT and QST079BT, the weight of material should be determined prior to analysis.

Exercise 869, DE-10 (Round 59)
Shellfish Toxins (DSP) Development Exercise QST080SS, QST081SS, QST082BT, QST083BT and QST084BT

Objective

This study covers the determination of okadaic acid (OA), dinophysistoxin-1 (DTX1), dinophysistoxin-2 (DTX2) and their esters and azaspiracids, in standard solution QST080SS and QST081SS, one DSP/AZP extract QST082BT, QST083BT is a Scallop tissue homogenate and QST084BT is a mussel tissue homogenate. The test materials should be analysed and the individual analogues as well as the sum of the analogues in each test material should be reported using the QUEST[3] system (Windows 9X, XP, NT or 2000) with the QUASIMEME Round 59 exercise template file.

Test Materials and Storage

3.5.1.3

The test materials were supplied by the Marine Institute, Galway, Republic of Ireland.

- QST080SS is a standard solution of AZA-1 supplied in an amber glass ampoule
- QST081SS is a AZA-2 standard solution
- QST082BT is a DSP/AZP extract
- QST083BT is a Scallop tissue homogenate supplied in a plastic vial
- QST084BT is a mussel tissue homogenate supplied in a plastic vial

For QST083BT and QST084BT, each vial contains sufficient material for one-shot analysis of OA, DTX1, DTX2 and their esters.

Each batch of material was prepared in bulk. The level of within and between sample homogeneity and stability was determined. All materials have been shown to be homogeneous at the intake mass normally used, and stable for the purposes of the test.

Begin the analysis as soon as possible, preferably within 7 days of receipt

All materials (contained either in ampoules or in 7ml plastic vials) should be stored at -20^{o}C, or a lower temperature, immediately upon receipt, until analysis.

The test materials have been shipped on cool packs. The cool packs will thaw slowly during transit, but this does not significantly affect the stability of the material for the purpose of this study, providing the test materials themselves are stored as directed, immediately on receipt.

[3] QUEST (QUASIMEME Electronic Storage and Transfer) System. Information on installation and use of this software is given in Annex II.

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Determinands

a) Methods based on chromatographic separation techniques (e.g. LC-FD, or LC-MS):

For the standard solution QST080SSand QST081SS report concentrations for the azaspiracids AZA1, AZA2 and AZA3 individually and their sum. For the mussel and the scallop homogenated tissues and extract, report concentrations for OA, DTX1, DTX2 as free toxins (pre-hydrolysis), separately and their sum, and for total OA, DTX1 and DTX2 (post-hydrolysis), and the sum of the total toxins post-hydrolysis (hy-OA + hy-DTX1 + hy-DTX2). This means there is no result reported for the ester-forms themselves, only for free toxins and the sum of free toxins plus esters. Also report the azaspiracids AZA1, AZA2 and AZA3 individually and their sum.

b) Methods based on determination of the sum of OA-equivalents present (e.g. PP2a):

For the standard solution, report the sum of OA-equivalents as free toxins (without hydrolysis), and the sum of OA-equivalents post hydrolysis. This means there is no result reported for the esters-forms themselves, only for free toxins and the sum of free toxins plus esters.
If you do not analyse for one of the determinands, eg. DTX-1 or DTX-2, please do not report the sum of OA+DTX-1+DTX-2. Equally if you do not carry out hydrolysis or determination of DTX-1 or DTX-2 post-hydrolysis, please do not report the sum of hydrolysed results.

Please report concentrations of OA, DTX-1, DTX-2 and their esters as well as the TEQ values. Calculating the TEQ values, use the TEF factors used in your own laboratory or use the TEF factors recommended by the EFSA . Report only TEQ values for the azaspiracids AZA1, AZA2 and AZA3.

TEF factors recommended by the EFSA for calculating TEQ values

Determinand	TEF
Free okadaic acid	1.000
Free DTX-1	1.000
Free DTX-2	0.600
Total okadaic acid	1.000
Total DTX-1	1.000
Total DTX-2	0.600
AZA1	1.00
AZA2	1.800
AZA3	1.400

Analysis

3.3.1.1 k; 3.4; 3.5.1.5

It is advisable to analyse the test materials as soon as possible after receipt. Use your normal validated methods and procedures to analyse the test materials. This may include correcting for blanks and for recovery.

One determination of each test material are required, for each determinand. The results of each determinand should be expressed on the test material "as received" i.e. on a wet weight basis. The concentrations should be determined against your own calibration solutions.

If you routinely conduct analyses by more than one technique you may report multiple sets of data. You should inform the QUASIMEME office staff, who will arrange to send you an additional exercise template file. It is not possible to report two sets of data using different methods on the same exercise

template. Please use the View Methods function in the QUEST program to report your analytical method for each test material.

QST080SS and QST 081SS

These ampoules contain at least 500 µl, sufficient for 1 injection of a solution into a LC or 1 analyses of the solution by an assay.

QST082BT, QST083BT and QST084BT

Each vial contains sufficient quantity of extract or homogenate for one analysis. The whole transferable contents of each vial of QST083BT and QST084BT should be extracted. To transfer the contents into a preweighed or tared extraction tube, the vial should be fully defrosted, vortex-mixed and the contents poured into the desired container.

Please note – test materials QST080SS, QST081SS, QST082BT, QST083BT and QST084BT should be stored in the freezer at ca –20 ^{o}C or less between analyses.

All results should be reported in µg/kg on the basis of wet weight of the test material as provided. The density of the standard solutions QST080SS and QST081SS is 0.7918 g/ml (MeOH). For QST082BT, QST083BT and QST084BT, the weight of material should be determined prior to analysis.

Please note that if your laboratory does not report on a given analogue, e.g. DTX-1, then your laboratory should not report the sum of toxins, since this will give 2 z-scores out of line and will possibly make data-analysis more difficult for the remaining laboratories which did determine this analyte.

Reporting of Results and Analytical Methods

3.5.1.4

Enter your analytical data and the method codes using the *.qxt files. Instructions for installing the QUEST software and using it to report analytical data and methods are given in Annex II.

Units

The units of measurement are given in the *.qxt template files. Ensure that the concentration of each determinand is reported in the units given. This may differ from your normal units for reporting; it is essential that all data reported are comparable. It is not possible for you to alter the units for reporting in the exercise template using QUEST version2.1.

The precision of the reported results should reflect the level of uncertainty of the measurement in your laboratory.

Reporting Left Censored Values

If the concentration of a determinand is below the detection limit of your method, you may wish to report the value as less than the detection limit. To do this, you should report your detection limit, either as a negative number or preceded by the "less-than" symbol, **<**. I.e. to report a value less than a detection limit of 10, report either "-10" or "<10". The QUEST system will identify either of these formats as left censored (*"less-than"*) values. Left censored values are included in the statistical evaluation of the data, and in the reports.

Method Codes

Method codes are supplied as part of the *.qxt template files. When you use the QUEST program, the exercise template includes your latest method code selection set as default. Report all of the requested method codes. If the method codes in any section do not adequately describe your analytical method, select "Other" from the method code list, and provide additional information on your method, electronically, when you return your data.

3.4.2

Return of Data

Upload all analytical data to the QUASIMEME sharepoint site under data entry in the exercise template *.qxt.. This allows a rapid and accurate transfer of your data and an early report to you. Additional information and comments may be provided as attached files.

Only data submitted using the *.qxt files can be included in the assessment.

Return the results to the QUASIMEME Project Office in Wageningen no later than **30 January 2010**. Data arriving after this deadline may not be entered into the database or appear in the report.

If you have further information on additional methods used or specific ways in which we can improve the data transfer, please inform the QUASIMEME Project Office. Your co-operation is appreciated and will help the assessors in the data analysis and in providing appropriate advice in case of any analytical difficulties.

Please observe the following guidelines, to reduce the need for additional checks, replies and enquires:

- Make sure that you have saved your data, and keep a copy of the data for your own records,

before uploading the files to the Quasimeme sharepoint site.

- **Do not rename the data files** – use the existing file names. The file name contains your laboratory code and the exercise number, which are essential identifiers for returned data.

- Make sure your system is virus free before returning data.

3.5.1.5

Data should only be submitted to the QUASIMEME Project Office when all quality checks have been made. If data are submitted beyond the deadline, they might not be included in the report. Data submitted after the issue of the report will not be included in the report, and these data will also not be included as part of the consensus value. Any certificate prepared with data submitted late will include the statement "Data submitted after report issued". No data will be re-entered into the database after the report is issued. No data will be changed in the database **UNLESS** there is evidence that QUASIMEME or data transfer has caused an error. In such cases QUASIMEME will undertake a quality query to investigate the problem and inform the participant of the outcome of the Query.

The assigned values will be calculated based on the assessment of all data returned, using the Cofino model. The report for each study, including each laboratory's individual assessment and z-scores, will be distributed to participants no later than **30 March 2010**. Background information on the data assessment will be provided with the reports.

3.3.1.1 i; 3.5.1.6

3.9

Collusion and Falsification of Results

QUASIMEME accepts that most participants operate with professional integrity and that data returned as part of the LP studies are correct and are submitted without interference or collusion. However, in some circumstances, data or information may be influenced by, for example, (i) repeated analyses and submitting mean data, or (ii) collaboration with colleagues undertaking the same study.

QUASIMEME checks for evidence of collusion and confirm to all participants that such activity is contrary to professional scientific conduct and will only nullify the benefits of the LP studies to accreditation bodies and analysts alike.

QUASIMEME reserves the right to withdraw participation of any institute who, in the opinion of the Scientific Assessment Group, has submitted data following collusion or falsification.

This statement is made as a formal requirement for accreditation for Laboratory Performance Studies under G13: 2000 3.9.

QUASIMEME Laboratory Performance Studies
Round 59
19 October 2009 to 30 January 2010

ANNEX I - Notification of damaged test materials.

You do not need to notify QUASIMEME if the test materials arrived in good condition

Laboratory Code: .....

Damaged container number: .....

Loss of weight container number: .....

I request a new test material for:…… Because:

Date: .....

Signature:.....

Name of participant:.....

Name and address of institute: .....

.....

.....

Telephone number:.....

Fax number:.....

Return this form to:

QUASIMEME Project Office
Wageningen UR
Alterra CWK
P.O. Box 47
6700 AA Wageningen
The Netherlands
Fax No: +31(0)317 486546
E-mail: QUASIMEME@wur.nl

ANNEX II - Instructions for Installing and Using QUEST Version 2.1

(QUASIMEME ELECTRONIC STORAGE AND TRANSFER SYSTEM)

QUEST provides a Windows environment for the transfer of data for the QUASIMEME Laboratory Performance studies.

Installing QUEST 2.1

Version 2.1 of the QUEST program (for Windows 9x, NT4, 2000 or XP) has been provided to all participants. Either you received It on the Quasimeme CD (before 2007) or it can be downloaded from the QUASIMEME website. If you are not able to download the program from the website, please contact the QUASIMEME Project Office.

QUEST 2.1 from website:
On the SharePoint site you can find an instruction (General documents / Instruction Quest from website) how to download and install QUEST on your computer. It also gives you the instruction how to download QUEST files (templates) and upload your results from and onto the SharePoint site.

QUEST 2.1 provided on the QUASIMEME CD. To install QUEST 2.1 please follow these instructions:

QUEST 2.1 on CD
Copy the files from the QUEST directory on the CD to an empty temporary directory on the hard drive of your computer (e.g. *c:\temp2*).

Select <Start>, <Run>, <Browse> then select *setup.exe* from the temporary directory containing the QUEST 2.1 files and click <OK> to install QUEST 2.1. Follow the instructions on screen during the installation.

The QUEST program will install in the *C:\Program Files* directory unless you have specified another directory during the setup procedure.

Using QUEST 2.1

Use QUEST to open your exercise template and enter your data. All of the exercises for the same laboratory are provided in one e-mail message. The files are arranged in directories using the variable laboratory code for the round and exercise numbers as unique identifiers e.g. *AD479.639*

The file names to be used with the QUEST program are structured:
<exercise number><variable laboratory code>.qxt, e.g. *639AD479.qxt*

To start the QUEST Program, select <Start> <Programs><Quest>. The QUEST Logo screen will appear while the program is loading and then, initially, an empty data sheet will appear. Once the program has been used to save data, the last data sheet will normally appear by default.

It is also possible to configure the computer to start the QUEST program from the exercise file. To do this, open the *Windows Explorer* and go to the directory containing the Exercise Template files. The file names for use with QUEST are structured: <exercise number><variable laboratory code>.qxt, e.g. *639AD479.qxt*

Double click on any **.qxt* file to bring up the *Open With* dialogue box. Click on the button marked *Other...* , and select *quest.exe* from the *C:\Program Files\ QUEST* directory. QUEST will be added to the program list. Check the box marked *"Always use this program to open this file"*. Click <OK>. This

sets the *Windows Explorer* to open QUEST by double clicking on any file with the extension <*qxt*>. Copies of the Exercise Template directories and files can be made if multiple users wish to enter data for different exercises.

The **.qxt* files can be used from floppy disk or copied into a directory of your choice on the hard drive and used from there.

Please note:
If you are running Windows NT4 with User and Administrator privilege access you should install the QUEST program with administrator access but only use the QUEST program as a user.

Operating Procedures in QUEST 2.1

QUEST has been developed using the standard Windows nomenclature.

File menu
The file menu contains the standard Windows file options. This can be selected by clicking on File or <Alt>+F

Open (<Ctrl>+O) opens the file by bringing up the Explorer Directory structure. To open the file, highlight the required file, double click on the Open button using the mouse, or use the keyboard shortcut <Ctrl>+O.

Save (<Ctrl>+S) saves the open file to its directory. Once the file has been saved to the hard drive, it is possible to also save the data to disk. A dialogue box asks *"Files saved to disk. Do you want to save a copy in A?"*. If "yes" then insert a disk in the A:\ drive and click on the "yes" button or press <Enter>. If you do not want a copy on disk, click on the "no" button.

The ***default*** option will always save the file to the ***root*** directory and not in the exercise directory on the A:\ drive. To save the data to the Exercise directory use the normal Windows save options.

Save As... Brings up the *Save as...* dialogue box. Select the required directory, enter the file name, if you require a different name, and click on the "Save" button or type <Alt>+S.

Please do NOT use **Save as** to *change* the file name of the Exercise Template files that you return to the Project Office. The file name contains your laboratory code and the exercise number, which are essential identifiers for returned data.

Close (<Ctrl>+W) closes the file. If the file has been edited a dialogue box will appear. *"Do you want to save the edits you have made?"* Click on "Yes" or press <Enter> to save the data. Click on "No" to close the file without saving the data. "Cancel" allows you to return to the file without it closing.

Print (<Ctrl>+P). The print option takes the default setting of the Windows system. In the Data view a print out of the whole data table is provided. In the Methods view a printout of the Methods Codes is obtained for the determinand which is highlighted only. This saves replicate printouts for other determinands, which, in many cases, will have the same codes.

Edit
The edit functions of Cut (<Ctrl>+X), Copy (<Ctrl>+C) and Paste (<Ctrl>+V) are similar to the standard Windows functions. In the View Values window, cut, copy and paste can only be done one cell at a time. Although the window gives the appearance of a spreadsheet, it is still a database entry.

Paste to All is only available in the Methods View and allows the highlighted method to be copied to all

other determinands in that specific exercise.

View
The upper Window bar contains the Title of the Exercise.
In the Values View the lower Windows bar contains the data value in the highlighted cell, the directory of the file that is opened, and the current time.
In the Methods view the lower Windows bar contains the determinand/matrix name of the methods that are currently highlighted.

Values. The values window contains the analytical data. Column 1 lists the matrices, column 2 the determinands and column 3 the units for each of the determinands. These first three columns are not available for edit.

The value columns provide cells with the required number of observation for each determinand. For many of the routine measurements this will be a single column. Data may be entered into any of these cells. To enter the data highlight the required cell by clicking on the cell and enter the value.

To blank the cell, highlight the cell and press <delete>.

All the data are held in memory. To save the data to hard disc, use the Save or Save As... facility.

If the file has been edited and you try to exit the program, you will be asked if you want to save the edits that you have made. This is a standard function when closing any file after editing the data.

ANNEX III - Total Lipid Extraction According to Smedes

This method is based on research carried out by Foppe Smedes. [Smedes, F., *The Analyst*, **124**, (11), (1999), 1711-1718.]

Instruments and Chemicals

- Balance with a precision of 0.1 mg
- Ultra Turrax
- Centrifuge capable of holding 100 ml tubes or glass jar at a speed of 2000 rpm [6]
- Heated waterbath with condensers.
- Evaporation flasks in suitable shape and size
- Pipettes
- Deionised water
- Isopropanol
- Cyclohexane
- Solution of 13%(w/w) isopropanol in cyclohexane.

Procedure

- Carry out a dry-weight determination on a representative portion of the test material to be analyses.
- Take a portion of wet test material, which does not contain more than 1g lipid or 8g of water.
- Weigh the test material with known moisture content in a 100ml centrifuge tube or appropriate glass jar.
- Add 18ml isopropanol and 20ml cyclohexane.
- Mix with Ultra Turrax for two minutes.
- Add W ml of water.

  W is calculated by:

$$W = 22 - \frac{sampleintake\,(g) * moisturecontent\,(\%)}{100}$$

- Mix with Ultra Turrax for another minute
- Separate the phases by centrifugation [7].

6 When a centrifuge is not used, the phases may take time to separate and the interface is less sharp, which can result in a low recovery of the organic phase. A check should be made to determine whether > 80% of the organic phase has been recovered (18 ml). A third extraction is recommended in the case of a lower recovery.

- Transfer as much as possible of the organic phase to an evaporation flask (by small pipette).

  Filtration is optional but makes the method more robust [8].

- Add 20 ml cyclohexane containing 13%(w/w) isopropanol and mix for one minute by Ultra Turrax.

- Centrifuge.

- Transfer the upper phase to the flask containing the first extract and evaporate the solvent.

- Quantitavely transfer the residue to a weighed wide-mouth cup by using a few ml of the cyclohexane/isopropanol mixture or diethylether.

- Evaporate in a moderately warm place to dryness (do not boil). The temperature used should be 5 – 10 ° C below the boiling point of the the washing solvent. Evaporation may be assisted by a stream of nitrogen.

- Further dry the residue for one hour at 105°C.

- Weigh the residue and calculate the lipid content from the intake.

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7 Some tissues, like liver extracts, form an emulsion which can be prevented by replacing the water by 1 M $HClO_4$ to denature the proteins. The addition of NaCl may also help.

8 In some cases the organic phase may contain some tissue particles when using the B & D Method. This also depends on the mixing method used (e.g. ultra sonic). When this occurs the extract should be filtered by passing the extract through a glass column plugged with ca 2cm of cottonwool which has previously been extracted with solvent.