Quasimeme Laboratory Performance Studies

Round 63

1 October 2010 to 30 January 2011

Exercise Protocols

Table of Contents

Introduction Round 63 3

AQ-3 Metals in Seawater 4

AQ-4 Mercury in Seawater 6

AQ-11 Chlorophyll-a in Seawater 8

BT-7 ASP Shellfish Toxins 10

DE-10 DSP Shellfish Toxins 12

BT-3 Non ortho CBs, PCDDs and PCDFs in Biota 15

Reporting of Results and Analytical Methods 18

ANNEX 1 Notification of damaged test materials 20

ANNEX 2 Instructions for login into sharepointsite 21

ANNEX 3 Total Lipid Extraction According to Smedes 22

Introduction Round 63

Thank you for participating in the 2010 QUASIMEME Laboratory Performance studies.

The test materials for the exercises in Round63 that you have ordered will be sent to you by courier in the week beginning 11 October 2010. 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 sent, use the form in Annex 1 of this document to request replacement materials within two weeks after receipt of the test materials. Within Round 63 we will also run our postponed Round 60 Exercise 878 (BT-3).

Additional test materials may also be purchased from QUASIMEME.

This protocol covers the following studies :

Round	Exercise	Analysis	
63	906	AQ-3	Metals in Seawater
63	907	AQ-4	Mercury in Seawater
63	908	AQ-11	Chlorophyll-a in Seawater
63	909	BT-7	ASP Shellfish Toxins
63	910	DE-10	DSP Shellfish Toxins
60	878	BT-3	Non ortho CBs, PCDDs and PCDFs in Biota

All data for these studies must be uploaded to your Quasimeme SharePoint Site, using the data submission forms, no later than 30 January 2011

All other information should be sent to: QUASIMEME Project Office

QUASIMEME Project Office Wageningen UR Alterra CWK P.O. Box 47 6700 AA Wageningen The Netherlands	Website: http://www.Quasimeme.org Tel.: +31 (0) 317 48 65 46 Fax: +31 (0) 317 41 90 00 E-mail: Quasimeme@wur.nl

ROUND	63	Exercise 906
AQ-3 Metals in Seawater		
Test materials		

Objective

This study covers the determination of metals in seawater and low salinity seawater test materials.

Test Materials and storage

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 /0.2µm double-membrane filter. Low salinity seawater test material was prepared by diluting the seawater with ultra-pure demineralised water. All test materials are preserved with 2 ml trace metal grade nitric acid per litre of seawater. Spiked test materials were prepared by adding 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°C, and analysed as soon as possible after receipt. Once the test materials are opened they should be analysed immediately.

Code	Description
QTM157SW	Seawater (Salinity > 30 psu) blank
QTM158SW	Seawater (Salinity > 30 psu) spiked
QTM159SW	Low salinity Seawater (Salinity 8 - 20 psu) spiked

Precaution

Some of the substances may presents a health hazard and can be 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.

Determinands and concentration ranges

The following metals should be determined:

Determinand	Unit	Concentration range	Error		AA-EQS
			Const	Prop	
Arsenic	µg/L		0.5	12.5%	
Boron	µg/L		0.4	12.5%	
Cadmium	µg/L		0.005	12.5%	0.08
Chromium	µg/L		0.1	12.5%	
Cobalt	µg/L		0.2	12.5%	
Copper	µg/L		0.2	12.5%	
Iron	µg/L		0.4	12.5%	
Lead	µg/L		0.01	12.5%	7.2
Manganese	µg/L		0.4	12.5%	
Nickel	µg/L		0.2	12.5%	20
Silver	µg/L		0.2	12.5%	
Tin	µg/L		0.2	12.5%	
Vanadium	µg/L		0.2	12.5%	

Zinc	µg/L		0.4	12.5%	

Boron is naturally occurring at higher concentrations.
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

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

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.

Reporting

One result for each determinand in each test material should be reported using the relevant data submission form on your sharepoint site. Methods can also be reported.

It is not possible to report two sets of data using different methods on the same exercise data submission form.

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.

ROUND	63	Exercise 907
AQ-4 Mercury in Seawater		
Test materials		

Objective

This study covers the determination of mercury in the seawater test materials. The test materials should be analysed and one result for mercury in each test material should be reported using the relevant exercise data submission form on your sharepoint site.

Test Materials and storage

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 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°C, and analysed as soon as possible after receipt. Once the test materials are opened they should be analysed immediately.

Code	Description
QTM160SW	Seawater (Salinity > 30 psu) unspiked
QTM161SW	Seawater (Salinity > 30 psu) spiked
QTM162SW	Seawater (Salinity > 30 psu) spiked

Precaution

Some of the substances may presents a health hazard and can be 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.

Determinands and concentration ranges

Mercury should be determined in each test material.

Determinand	Unit	Concentration range	Error		AA-EQS
			Const	Prop	
Mercury	ng/L		0.2	12.5%	50

Analysis

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

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 test material is required.

Reporting

One result for each determinand in each test material should be reported using the relevant data submission form on your sharepoint site. Methods can also be reported.

It is not possible to report two sets of data using different methods on the same exercise template.

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.

ROUND	63	Exercise 908
AQ-11	Chlorophyll-a in Seawater	
Test materials		

Objective

This study covers the determination of chlorophyll a, b, c and pheopigments in two filtered seawater residue test materials. The test materials should be analysed One result for each determinand in each test material should be reported using the relevant data submission form on your sharepoint site. Methods can also be reported, to identify any method dependency of the results.

Test Materials and storage

The test materials for the analysis of chlorophyll a, b, c and pheopigments were prepared at Alterra, Wageningen the Netherlands. Test material QCH049SW was prepared from a culture of Chaetocheros + Pyramimonas grown at Wageningen IMARES, Yerseke the Netherlands and sub-sampled onto Whatman GF/F, 47 mm filter papers. Test material QCH 048SW was prepared from an estuarine water sample. For each test material, the resultant damp filter paper was wrapped in aluminium foil, inserted into a numbered cryovial and immediately 'flash frozen' in liquid nitrogen. The test materials were stored at -80°C until the day of dispatch.

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.

Code	Description
QCH048SW	Filtered residues from 1 litre of seawater
QCH049SW	Filtered residues from 1 litre of seawater

Precaution

Some of the substances may presents a health hazard and can be 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.

Determinands and concentration ranges

The following pigments should be determined:

Determinand	Unit	Concentration range	Error		AA-EQS
			Const	Prop	
Chlorophyll-a	µg/L		0.05	12.5%	
Chlorophyll-b	µg/L		0.01	12.5%	
Chlorophyll-c	µg/L		0.01	12.5%	
Pheopigments	µg/L		0.01	12.5%	

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.

Analysis

Treat all test materials in the same manner as your routine samples. 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". Concentrations need to be calculated based on a filter prepared out of a 1 litre sample.

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.

Reporting

One result for each determinand in each test material should be reported using the relevant data submission form on your sharepoint site. Methods can also be reported.

It is not possible to report two sets of data using different methods on the same exercise template.

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.

ROUND	63	Exercise 909
BT-7 ASP Shellfish Toxins		
Test materials		

Objective

This study covers the determination of amnesic shelfish toxins domoic acid and epidomoic acid (as a racemic mixture) in standard solution and shelfish tissue test materials.

Test Materials and storage

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

- QST097SS is a domoic acid standard solution.
- QST098BT is a Oyster tissue homogenate supplied in a plastic vial.
- QST099BT is a Mussel tissue homogenate supplied in a plastic vial.

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 QST097SS 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.

Code	Description
QST097SS	ASP standard solution
QST098BT	Pacific Oyster homogenate
QST099BT	Mussel homogenate

Precaution

Some of the substances may presents a health hazard and can be 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.

Determinands and concentration ranges

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

Determinand	Unit	Concentration range	Error		AA-EQS
			Const	Prop	
Domoic+Epidomoic	mg/kg		0.1	12.5%	

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

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 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.

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 is 0.9853 g/ml at 22°C. The weight of shelfish tissue test materials should be determined prior to analysis.

Reporting

One result for each determinand in each test material should be reported using the relevant data submission form on your sharepoint site. Methods can also be reported.

It is not possible to report two sets of data using different methods on the same exercise template.

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.

ROUND	63	Exercise 910
DE-10	DSP Shellfish Toxins	
Test materials		

Objective

This study covers the determination of diarrhetic toxins in shellfish tissue.

Test Materials and storage

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

- standard solutions of AZA were supplied in an amber glass ampoule.
- a DSP/AZP extract was supplied in a glass ampoule
- mussel tissue homogenates supplied in a plastic vial.

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°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.

Code	Description
QST100SS	AZA standard solution
QST101SS	AZA standard solution
QST102BT	DSP-AZP extract
QST103BT	Mussel homogenate
QST104BT	Mussel homogenate

Precaution

Some of the substances may presents a health hazard and can be 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.

Determinands and concentration ranges

Determinands

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

For the standard solution, report concentrations for OA, DTX1, DTX2 as free toxins (without hydrolysis), individual analogues separately and their sum For the standard solution QST070SS report concentrations for the azaspiracids AZA1, AZA2 and AZA3 individually and their sum. For the mussel crude extract and the scallop and oyster tissue, 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.

Determinand	Unit	Concentration range	Error		AA-EQS
			Const	Prop	
AZA-1	µg/kg		0.1	12.5%	
AZA-1 TEQ	TEQ		0.1	12.5%	
AZA-2	µg/kg		0.1	12.5%	
AZA-2 TEQ	TEQ		0.1	12.5%	
AZA-3	µg/kg		0.1	12.5%	
AZA-3 TEQ	TEQ		0.1	12.5%	
AZA-total	µg/kg		0.1	12.5%	
AZA-total TEQ	TEQ		0.1	12.5%	
Free-DTX1	µg/kg		0.1	12.5%	
Free-DTX1 TEQ	TEQ		0.1	12.5%	
Free-DTX2	µg/kg		0.1	12.5%	
Free-DTX2 TEQ	TEQ		0.1	12.5%	
Free-Okadaic-Acid	µg/kg		0.1	12.5%	
Free-Okadaic-Acid TEQ	TEQ		0.1	12.5%	
Total-DTX1	µg/kg		0.1	12.5%	
Total-DTX1 TEQ	TEQ		0.1	12.5%	
Total-DTX2	µg/kg		0.1	12.5%	
Total-DTX2 TEQ	TEQ		0.1	12.5%	
Total-Free-OA+DTX1+DTX2	µg/kg		0.1	12.5%	
Total-Free-OA+DTX1+DTX2 TEQ	TEQ		0.1	12.5%	
Total-hy-OA+DTX1+DTX2	µg/kg		0.1	12.5%	
Total-hy-OA+DTX1+DTX2 TEQ	TEQ		0.1	12.5%	
Total-Okadaic-Acid	µg/kg		0.1	12.5%	
Total-Okadaic-Acid TEQ	TEQ		0.1	12.5%	

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

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 Methods Button on your data submission form to report your analytical method for each test material.

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.

Each vial contains sufficient quantity of extract or homogenate for one analysis. The whole transferable contents of each vial 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 all test materials should be stored in the freezer at ca -20°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 is 0.7918 g/ml (MeOH). For thwe mussel tissues, 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

One result for each determinand in each test material should be reported using the relevant data submission form on your sharepoint site. Methods can also be reported.

It is not possible to report two sets of data using different methods on the same exercise template.

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.

ROUND	60	Exercise 878
BT-3 Non ortho CBs, PCDDs and PCDFs in Biota		
Test materials		QPL030BT, QPL031BT

Objective

This study covers the determination of non-ortho Chlorobiphenyls (CBs), polychlorinated dibenzo-p-dioxins(PCDDs), polychlorinated dibenzofurans (PCDFs), total TEQ and total lipid in two biological tissue test materials.

Test Materials and storage

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.

The tins contain approximately 70g of minced sterilized biological tissue 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°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°C may be disadvantageous for the material.

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

Code	Description
QPL030BT	Fish muscle tissue
QPL031BT	Shellfish tissue

Precaution

The tins with biological tissue 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°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.

Some of the substances may present a health hazard and can be 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.

Determinands and concentration ranges

The following analytes should be determined:

Determinand	Unit	Concentration range		Error		AA-EQS
		Fish muscle tissue	Shellfish tissue	Const	Prop	
1,2,3,4,6,7,8-HpCDD	ng/kg	0.05—5	0.05—5	0.02	12.5%	
1,2,3,4,6,7,8-HpCDF	ng/kg	0.02—0.5	0.02—0.5	0.02	12.5%	
1,2,3,4,7,8,9-HpCDF	ng/kg			0.02	12.5%	
1,2,3,4,7,8-HxCDD	ng/kg	0.02—0.2	0.02—0.2	0.02	12.5%	
1,2,3,4,7,8-HxCDF	ng/kg	0.01—0.2	0.01—0.2	0.02	12.5%	
1,2,3,6,7,8-HxCDD	ng/kg	0.03—1	0.03—1	0.02	12.5%	
1,2,3,6,7,8-HxCDF	ng/kg	0.01—0.2	0.01—0.2	0.02	12.5%	
1,2,3,7,8,9-HxCDD	ng/kg	0.02—0.5	0.02—0.5	0.02	12.5%	
1,2,3,7,8,9-HxCDF	ng/kg			0.02	12.5%	
1,2,3,7,8-PeCDD	ng/kg	0.02—1	0.02—1	0.02	12.5%	
1,2,3,7,8-PeCDF	ng/kg	0.03—1	0.03—1	0.02	12.5%	
2,3,4,6,7,8-HxCDF	ng/kg	0.01—0.5	0.01—0.5	0.02	12.5%	
2,3,4,7,8-PeCDF	ng/kg	0.05—3	0.05—3	0.02	12.5%	
2,3,7,8-TCDD	ng/kg	0.01—0.5	0.01—0.5	0.02	12.5%	
2,3,7,8-TCDF	ng/kg	0.03—5	0.03—5	0.02	12.5%	
CB126	ng/kg	1—20	1—20	0.02	12.5%	
CB169	ng/kg	0.2—5	0.2—5	0.02	12.5%	
CB77	ng/kg	10—100	10—100	0.02	12.5%	
Extractable-Lipid	%			0.1	12.5%	
OCDD	ng/kg	0.05—5	0.05—5	0.02	12.5%	
OCDF	ng/kg	0.02—0.5	0.02—0.5	0.02	12.5%	
Total-Lipid	%			0.1	12.5%	
Total-TEQ(Dr.CALUX)	ng/kg			0.1	12.5%	

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

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 homogenise the sample in the tin, we recommend transferring the material to a larger container for homogenisation and sub-sampling. Aliquots should be taken immediately after homogenising, 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°C.

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

The concentrations should be determined against your own calibration solutions.

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.

% dry weight = 100 - [(wet weight - dry weight) ∗ 100]/(wet weight)

Reporting

One result for each determinand in each test material should be reported using the relevant data submission form on your sharepoint site. Methods can also be reported.

It is not possible to report two sets of data using different methods on the same exercise template.

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.

Reporting of Results and Analytical Methods

Units

The units of measurement are given in the data submission forms. 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 data submission forms.

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 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 data submission forms. 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.

Return of Data

Upload all analytical data to the QUASIMEME SharePoint site only with the data submission forms. 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 data submission forms can be included in the assessment. Return the results to the QUASIMEME Project Office in Wageningen no later than 30 January 2011. 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:

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 2011. Background information on the data assessment will be provided with the reports.

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.

ANNEX 1 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)317 486 546

E-mail : QUASIMEME@wur.nl

ANNEX 2 Instructions for login into sharepointsite

Login to http://www.quasimeme.org

Select sharepointsite

Username: wur\x……. (your specific logincode e.g. xcrum012)

Password: your specific password in capitals

Ask the Quasimeme project office when the login information is unknown

Select the correct year

Select the correct round

Select the correct exercise

Enter your results and method information into the data submission form

Lower than results will be automatically transferred into – values.

Click on the save button to store your data into the database

ANNEX 3 Total Lipid Extraction According to Smedes

This method is based on research carried out by Foppe Smedes.

See : Determination of total lipid using non-chlorinated solvents
Smedes, F., Analyst 124 (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[1]
- 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 :

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

- Mix with Ultra Turrax for another minute.
- Separate the phases by centrifugation[2].
- 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[3].

[1] 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.
[2] Some tissues, like liver extracts, form an emulsion which can be prevented by replacing the water by 1 M HClO4 to denature the proteins. The addition of NaCl may also help.
[3] 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.

- 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.