

# The preparation of biological reference materials for QUASIMEME

Michiel Kotterman

Report number C002/11



# IMARES Wageningen UR

Institute for Marine Resources & Ecosystem Studies

Client:

QUASIMEME Laboratory Performance studies  
Wageningen UR  
Alterra CWK  
P.O. Box 47  
6700 AA Wageningen

Publicatiedatum:

January 10, 2011

**IMARES** is:

- an independent, objective and authoritative institute that provides knowledge necessary for an integrated sustainable protection, exploitation and spatial use of the sea and coastal zones;
- an institute that provides knowledge necessary for an integrated sustainable protection, exploitation and spatial use of the sea and coastal zones;
- a key, proactive player in national and international marine networks (including ICES and EFARO).

P.O. Box 68

1970 AB IJmuiden

Phone: +31 (0)317 48 09 00

Fax: +31 (0)317 48 73 26

E-Mail: [imares@wur.nl](mailto:imares@wur.nl)

[www.imares.wur.nl](http://www.imares.wur.nl)

P.O. Box 77

4400 AB Yerseke

Phone: +31 (0)317 48 09 00

Fax: +31 (0)317 48 73 59

E-Mail: [imares@wur.nl](mailto:imares@wur.nl)

[www.imares.wur.nl](http://www.imares.wur.nl)

P.O. Box 57

1780 AB Den Helder

Phone: +31 (0)317 48 09 00

Fax: +31 (0)223 63 06 87

E-Mail: [imares@wur.nl](mailto:imares@wur.nl)

[www.imares.wur.nl](http://www.imares.wur.nl)

P.O. Box 167

1790 AD Den Burg Texel

Phone: +31 (0)317 48 09 00

Fax: +31 (0)317 48 73 62

E-Mail: [imares@wur.nl](mailto:imares@wur.nl)

[www.imares.wur.nl](http://www.imares.wur.nl)

© 2010 IMARES Wageningen UR

IMARES, institute of Stichting DLO is registered in the Dutch trade record nr. 09098104, BTW nr. NL 806511618

The Management of IMARES is not responsible for resulting damage, as well as for damage resulting from the application of results or research obtained by IMARES, its clients or any claims related to the application of information found within its research. This report has been made on the request of the client and is wholly the client's property. This report may not be reproduced and/or published partially or in its entirety without the express written consent of the client.

A\_4\_3\_2-V11.2

# Contents

- Summary .....4
- 1 Introduction .....5
- 2 Assignment.....6
- 3 Materials and Methods .....7
  - 3.1 Details on the procedure for production of materials .....7
  - 3.2 Analytical determinations .....7
- 4 Results .....8
  - 4.1 Homogeneity tests .....8
  - 4.2 Indicative analyses of contaminants .....8
- 5 Conclusions.....9
- 6 Quality Assurance ..... 10
- 7 Justification..... 11
- Appendix A. Results of Homogeneity testing Turbot liver..... 12
- Appendix B. Results of Homogeneity testing Irish mussels ..... 26

## Summary

Biological materials, consisting of three different batches of mussels; from Den Helder harbour (POPs, TBT), Irish mussels (metals) and Wadden Sea mussels, fortified with highly contaminated mussels from Belgium (POPs), and of one batch of turbot liver (metals) have been prepared for use in QUASIMEME interlaboratory studies for metal and organic contaminant analyses. The homogeneity of the prepared material was tested for metals or POPs, depending on the intended use of the material, and indicative concentrations for selected contaminants were reported.

# 1 Introduction

The QUASIMEME Project Office has requested IMARES Wageningen UR to prepare materials for the use in QUASIMEME interlaboratory studies, for both metal analyses as well as organic pollutants. Based on expertise and additional test analyses, mussels from different origin were used to prepare the test materials. Next to this, a liver material, relatively low in fat, was prepared from turbot caught in the North Sea.

## 2 Assignment

- Approximately 250 aliquots of each homogeneous test material for QUSIMEME laboratory performance studies should be prepared.
- Homogeneity of the prepared material should be tested and reported.
- In the prepared materials for organic contaminants, concentration of contaminants (PCBs, OCPs and BFRs), total lipids and extractable lipids and moisture should be determined and reported as indicative values. Materials to be used for metal analysis were tested for homogeneity by analysing for Cu en Zn, indicative values for Pb, Cd, As, Hg, Ni and Cr were also analysed.

## 3 Materials and Methods

### Mussels

Typically, the meat was removed from the shells after a very short cooking step; mussels were submerged in boiling water for approximately two minutes. The meat from the mussels out of Den Helder Harbour was collected without cooking.

The material was then minced, homogenized, packaged in tins or jars and sterilized.

In contrast to the mussel material from Den Helder harbour and Ireland, the mussel sample from Belgium consisted a mix of mussels. Due to the fact only small to very small mussels were obtained from the Belgian Harbour, only 6.5 kg of meat could be collected. The amount of mussel meat was increased by addition of 14 kg mussels from the Wadden Sea, resulting in the production of 235 jars of material.

### Turbot liver

Livers were collected by fishermen on the North Sea and stored frozen. After thawing, livers were homogenized, packaged in jars and sterilized identical to the mussel samples.

### 3.1 Details on the procedure for production of materials

The complete volume of meat was minced and homogenized for 60 minutes, after adding 0.02% butylhydroxytoluene (BHT), in a Stephan cutter (Stephan Machines, Almelo, The Netherlands), type UMM/SK25 (made in 1979). The Stefan cutter was cooled with water while running.

For the mussels from Den Helder Harbour:

Coated tins (Eurocan Food, Mechelen, Belgium, volume ca. 75 ml) were filled to the brim with homogenised material using a manual dosing machine (machinenfabrik Engler, Vienna, Switzerland). The tins were sealed by a Lanico TVM 335 sealing machine (Thomassen and Drijver, Deventer, The Netherlands).

The other materials were dispensed into the glass jars (Catalonie Glas verpakkingen BV, 105 ml glass jar, "RTB gold" 48mm lid) and closed.

The liver sample was, due to its fluidity, dispensed into the jars under continuously stirring.

All materials were sterilized in a Muvero-Mat sterilizer (type 90E) for 45 minutes at 122°C (pressure 1.4 bar, heating-time: 90 minutes, cooling time: 20 minutes).

### 3.2 Analytical determinations

Homogeneity and indicative values were determined by IVM, Amsterdam, in case of the materials designed for POP analyses.

In the case of materials designed for metal analyses this analytical work was performed by TNO, Zeist. Homogeneity results have been delivered separately.

## 4 Results

The four materials were produced according to the work plan. In case of the mussel sample, designed for organic pollutant analyses, less than 250 aliquots have been produced. This was due to the fact that only a small amount of polluted Belgian mussel could be collected.

### 4.1 Homogeneity tests

The homogeneity results for material "Den Helder mussels" and "Belgian mussels" were obtained by IVM and not made available to IMARES.

The homogeneity tests of Zn and Cu (performed by TNO) in the material "Irish mussels" and "Turbot liver", both for the use in metal-analyses, are listed in the appendices A and B.

For both materials, no inhomogeneity was observed at the 99% confidence level.

### 4.2 Indicative analyses of contaminants

The results of the indicative analysis of metals for the prepared materials "Irish mussels" and "Turbot liver", analysed in one aliquot, are shown in table 1.

Table 1. Indicative values for metals in the two mussel samples. Data shown are the result of a duplicate analysis in one aliquot.

Compound	unit	Irish mussels		Turbot liver	
		Average	RSD (%)	Average	RSD (%)
Cu	mg/kg	1.77	17.0	2.56	0.6
Zn	mg/kg	25.4	14.6	19.5	1.3
As	mg/kg	1.59	5.3	9.11	0.1
Cd	mg/kg	0.092	9.8	0.029	0.2
Cr	mg/kg	0.442	9.3	0.049	0.1
Hg	mg/kg	0.022	10.4	0.079	1.1
Ni	mg/kg	0.292	4.1	0.022	8.0
Pb	mg/kg	0.394	3.3	0.016	5.2
Se	mg/kg			2.20	0.7



## 5 Conclusions

Four biological reference materials have been prepared. For two of those, the mussel materials for use in metal analysis, it was shown that the between- and within-tin homogeneity was satisfactory for the materials to be used in metal analysis.

Based on the results of both the homogeneity tests and the indicative analysis, the materials are suitable for use in an interlaboratory study.

## 6 Quality Assurance

The production of the materials was performed according to the standard IMARES protocol for the production of test materials.

The suitability of the materials for use in an interlaboratory study was tested by IVM and TNO Nutrition (Zeist).

The TNO laboratory has a valid ISO/IEC 17025 certificate and is accredited for the analyses of metals in fish-matrix.

IMARES utilises an ISO 9001:2008 certified quality management system (certificate number: 57846-2009-AQ-NLD-RvA). This certificate is valid until 15 December 2012. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Environmental Division has NEN-AND-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 27 March 2013 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation.

## 7 Justification

Rapport C002/11

Project Number: 430.51025.01

The scientific quality of this report has been peer reviewed by a colleague scientist and the head of the department of IMARES.

Approved: Marion Hoek-van Nieuwenhuizen  
Projectleader

Signature:



Date: January 13, 2011

Approved: Drs. John Schobben  
Head of department

Signature:



Date: January 13, 2011

## Appendix A. Results of Homogeneity testing Turbot liver

### Homogeneity Study - Simple Statistics

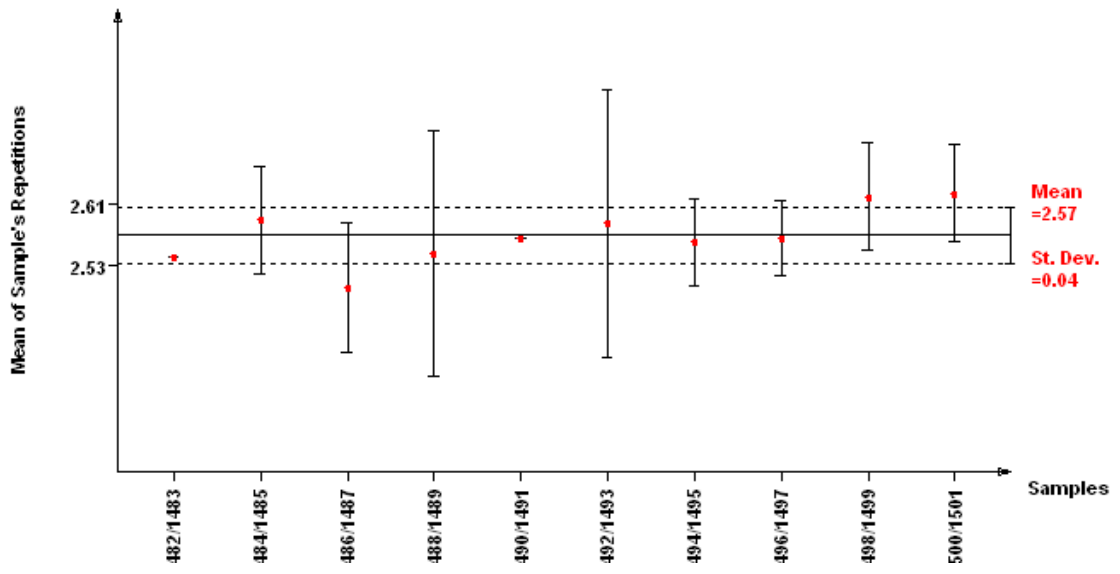
#### Output for : homogeniteit Cu

S/N	Sample ID	Rep. #1	Rep. #2	Rep. #3	Rep. #4	Rep. #5	Rep. #6	Rep. #7	Rep. #8	Rep. #9	Rep. #10	Mean	STDev
1	1482/ 1483	2.54	2.54									2.54	0.00
2	1484/ 1485	2.64	2.54									2.59	0.07
3	1486/ 1487	2.56	2.44									2.50	0.08
4	1488/ 1489	2.43	2.66									2.55	0.16
5	1490/ 1491	2.57	2.56									2.57	0.00
6	1492/ 1493	2.46	2.71									2.59	0.18
7	1494/ 1495	2.6	2.52									2.56	0.06
8	1496/ 1497	2.6	2.53									2.57	0.05
9	1498/ 1499	2.57	2.67									2.62	0.07
10	1500/ 1501	2.67	2.58									2.63	0.06

*measurement unit : mg/kg*

Mean	2.57
STDev	0.04
CV(%)	1.46

### Homogeniteit Cu



## Homogeneity Study - Simple Statistics

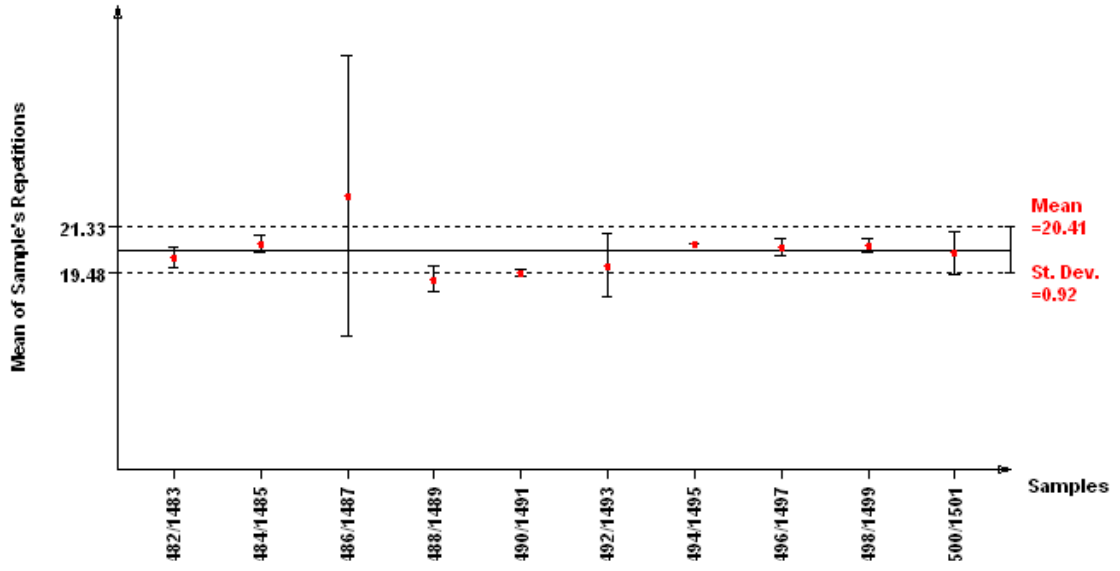
### Output for : Homogeniteit Zn

S/N	Sample ID	Rep. #1	Rep. #2	Rep. #3	Rep. #4	Rep. #5	Rep. #6	Rep. #7	Rep. #8	Rep. #9	Rep. #10	Mean	STDev
1	1482/ 1483	20.4	19.8									20.10	0.42
2	1484/ 1485	20.4	20.9									20.65	0.35
3	1486/ 1487	18.6	26.6									22.60	5.66
4	1488/ 1489	18.9	19.6									19.25	0.49
5	1490/ 1491	19.4	19.6									19.50	0.14
6	1492/ 1493	18.9	20.7									19.80	1.27
7	1494/ 1495	20.7	20.7									20.70	0.00
8	1496/ 1497	20.3	20.8									20.55	0.35
9	1498/ 1499	20.4	20.8									20.60	0.28
10	1500/ 1501	20.9	19.7									20.30	0.85

*measurement unit : mg/kg*

Mean	20.41
STDev	0.92
CV(%)	4.52

### Homogeniteit Zn



## Homogeneity Study - Nested Design

### Output for : Homogeniteit Zn

#### Data for the Unit: 1482/1483

<b>Sub-Units</b>		
Repetitions	1482/1483/1	1482/1483/2
1	20.4	19.8

*measurement unit : mg/kg*

#### Data for the Unit: 1484/1485

<b>Sub-Units</b>		
Repetitions	1484/1485/1	1484/1485/2
1	20.4	20.9

*measurement unit : mg/kg*

#### Data for the Unit: 1486/1487

<b>Sub-Units</b>		
Repetitions	1486/1487/1	1486/1487/2
1	18.6	26.6

*measurement unit : mg/kg*

#### Data for the Unit: 1488/1489

<b>Sub-Units</b>		
Repetitions	1488/1489/1	1488/1489/2
1	18.9	19.6

*measurement unit : mg/kg*

#### Data for the Unit: 1490/1491

<b>Sub-Units</b>		
Repetitions	1490/1491/1	1490/1491/2
1	19.4	19.6

*measurement unit : mg/kg*

#### Data for the Unit: 1492/1493

<b>Sub-Units</b>		
Repetitions	1492/1493/1	1492/1493/2
1	18.9	20.7

*measurement unit : mg/kg*



**Data for the Unit: 1494/1495**

<b>Sub-Units</b>		
Repetitions	1494/1495/1	1494/1495/2
1	20.7	20.7

*measurement unit : mg/kg*

**Data for the Unit: 1496/1497**

<b>Sub-Units</b>		
Repetitions	1496/1497/1	1496/1497/2
1	20.3	20.8

*measurement unit : mg/kg*

**Data for the Unit: 1498/1499**

<b>Sub-Units</b>		
Repetitions	1498/1499/1	1498/1499/2
1	20.4	20.8

*measurement unit : mg/kg*

**Data for the Unit: 1500/1501**

<b>Sub-Units</b>		
Repetitions	1500/1501/1	1500/1501/2
1	20.9	19.7

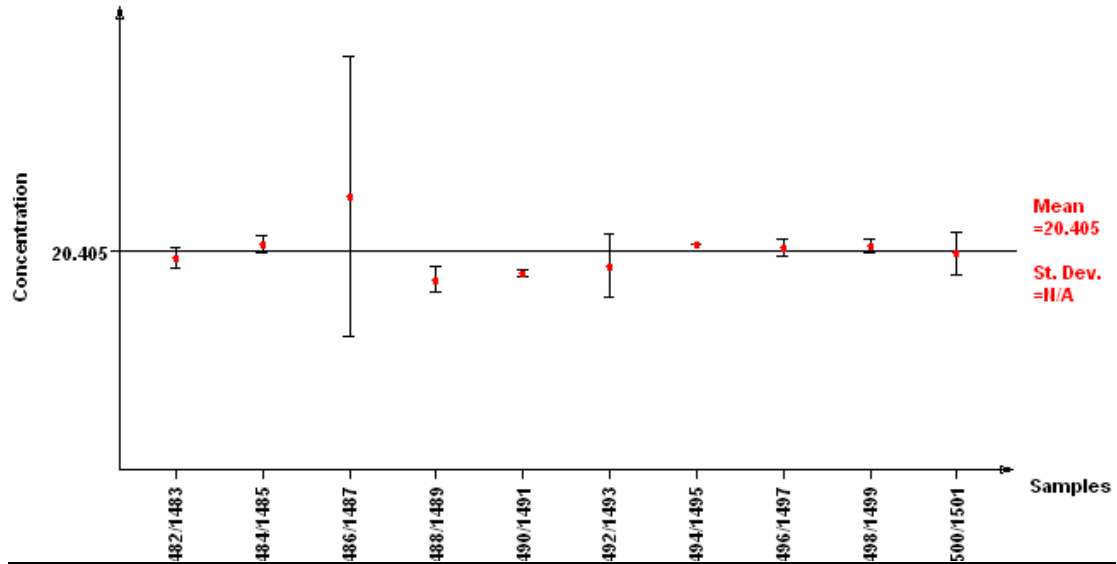
*measurement unit : mg/kg*

<b><u>ANOVA Table</u></b>							
Source of Variation	SS	d.f.	MS	StDev	F	F-crit 95%	F-crit 99%
Between Units	15.295	9	1.699	MSB < MSW	0.484	3.020	4.942
Within Units	35.115	10	3.512	1.874			
Total	50.410	19					

**Snedecor F-Test**

Differences between units statistically significant? (a=95%) :No  
Differences between units statistically significant? (a=99%) :No

### Homogeniteit Zn



## Homogeneity Study - Nested Design

### Output for : Homogeniteit Cu

#### Data for the Unit: 1482/1483

<b>Sub-Units</b>		
Repetitions	1482/1483/1	1482/1483/2
1	2.54	2.54

*measurement unit : mg/kg*

#### Data for the Unit: 1484/1485

<b>Sub-Units</b>		
Repetitions	1484/1485/1	1484/1485/2
1	2.64	2.54

*measurement unit : mg/kg*

#### Data for the Unit: 1486/1487

<b>Sub-Units</b>		
Repetitions	1486/1487/1	1486/1487/2
1	2.56	2.44

*measurement unit : mg/kg*

#### Data for the Unit: 1488/1489

<b>Sub-Units</b>		
Repetitions	1488/1489/1	1488/1489/2
1	2.43	2.66

*measurement unit : mg/kg*

#### Data for the Unit: 1490/1491

<b>Sub-Units</b>		
Repetitions	1490/1491/1	1490/1491/2
1	2.57	2.56

*measurement unit : mg/kg*

#### Data for the Unit: 1492/1493

<b>Sub-Units</b>		
Repetitions	1492/1493/1	1492/1493/2
1	2.46	2.71

*measurement unit : mg/kg*

**Data for the Unit: 1494/1495**

<b>Sub-Units</b>		
Repetitions	1494/1495/1	1494/1495/2
1	2.6	2.52

*measurement unit : mg/kg*

**Data for the Unit: 1496/1497**

<b>Sub-Units</b>		
Repetitions	1496/1497/1	1496/1497/2
1	2.6	2.53

*measurement unit : mg/kg*

**Data for the Unit: 1498/1499**

<b>Sub-Units</b>		
Repetitions	1498/1499/1	1498/1499/2
1	2.57	2.67

*measurement unit : mg/kg*

**Data for the Unit: 1500/1501**

<b>Sub-Units</b>		
Repetitions	1500/1501/1	1500/1501/2
1	2.67	2.58

*measurement unit : mg/kg*

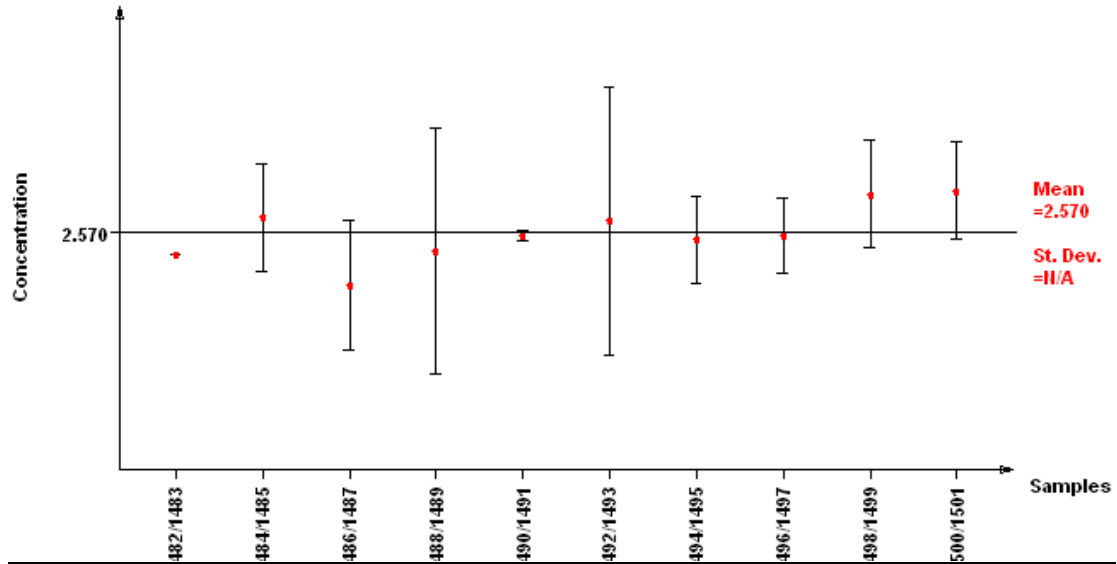
<b><u>ANOVA Table</u></b>							
Source of Variation	SS	d.f.	MS	StDev	F	F-crit 95%	F-crit 99%
Between Units	0.025	9	0.003	MSB < MSW	0.334	3.020	4.942
Within Units	0.085	10	0.008	0.092			
Total	0.110	19					

**Snedecor F-Test**

Differences between units statistically significant? (a=95%) :No

Differences between units statistically significant? (a=99%) :No

### Homogeniteit Cu



## Homogeneity Study - Repeatability

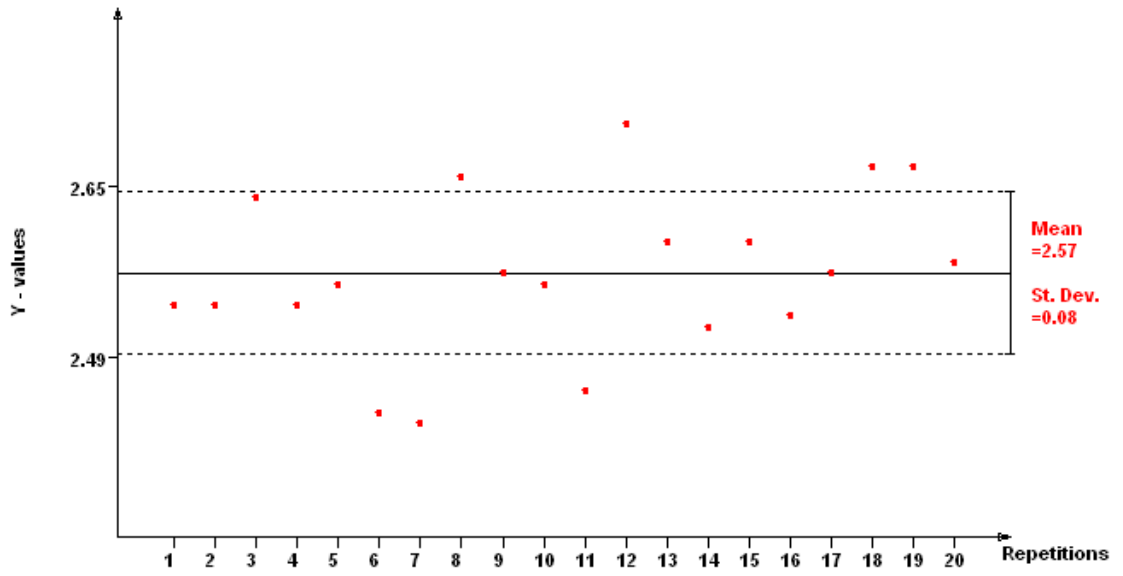
### Output for : Herhaalbaarheid Cu

S/N	Sample Value
1	2.54
2	2.54
3	2.64
4	2.54
5	2.56
6	2.44
7	2.43
8	2.66
9	2.57
10	2.56
11	2.46
12	2.71
13	2.6
14	2.52
15	2.6
16	2.53
17	2.57
18	2.67
19	2.67
20	2.58

*measurement unit : mg/kg*

Mean	2.57
STDev	0.08
CV(%)	2.96

### Herhaalbaarheid Cu



## Homogeneity Study - Repeatability

### Output for : Herhaalbaarheid Zn

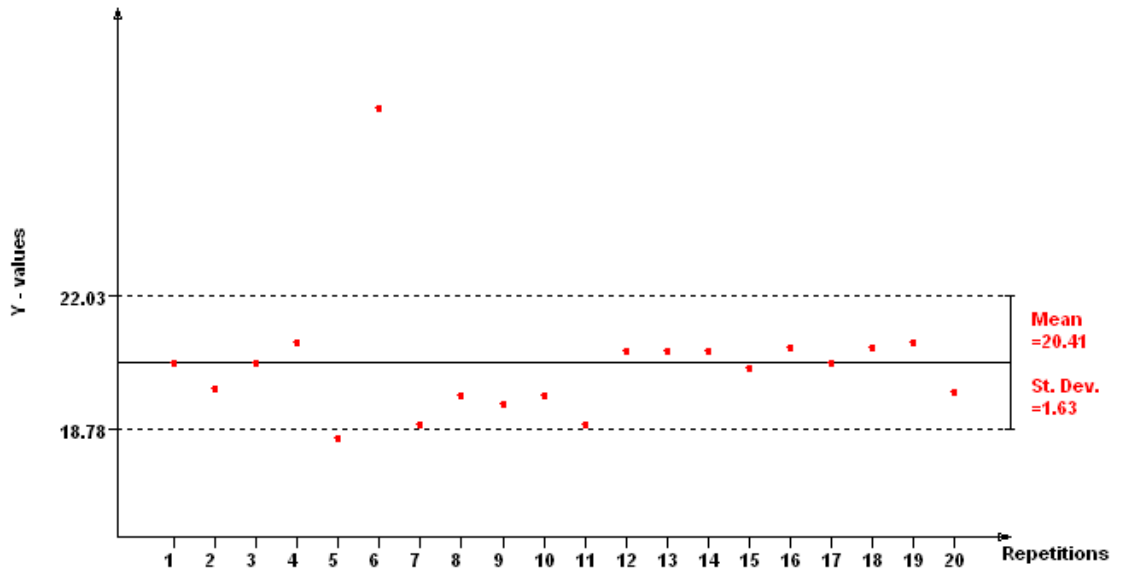
S/N	Sample Value
1	20.4
2	19.8
3	20.4
4	20.9
5	18.6
6	26.6
7	18.9
8	19.6
9	19.4
10	19.6
11	18.9
12	20.7
13	20.7
14	20.7
15	20.3
16	20.8
17	20.4
18	20.8
19	20.9
20	19.7

*measurement unit : mg/kg*

Mean	20.41
STDev	1.63
CV(%)	7.98



### Herhaalbaarheid Zn



## Appendix B. Results of Homogeneity testing Irish mussels

### Homogeneity Study - Simple Statistics

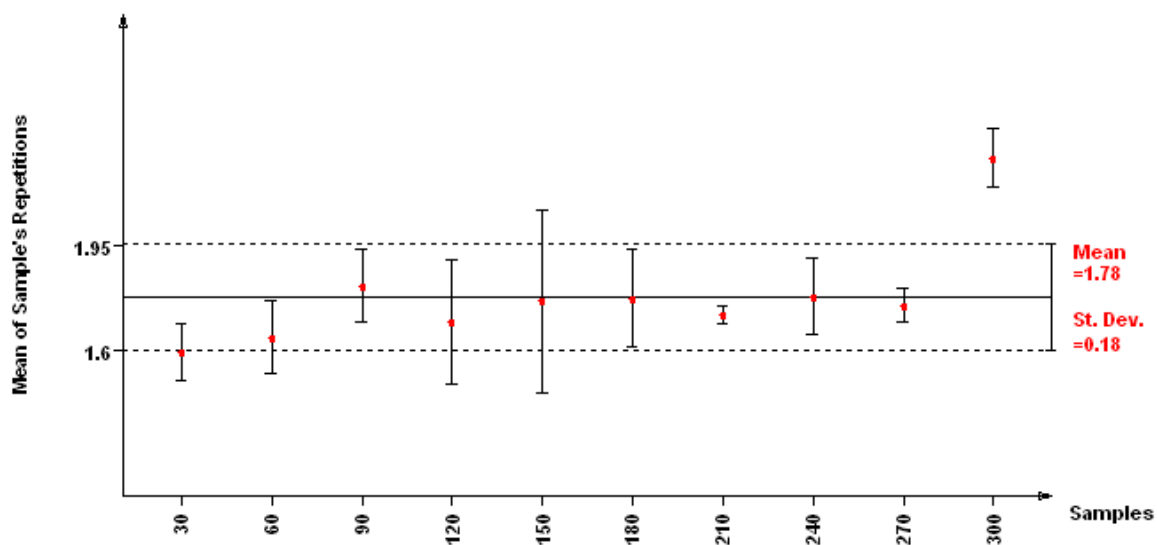
#### Output for : Simple statistics Cu

S/N	Sample ID	Rep.# 1	Rep. #2	Rep. #3	Rep. #4	Rep. #5	Rep. #6	Rep. #7	Rep. #8	Rep. #9	Rep. #10	Mean	STDev
1	30	1.53	1.66									1.60	0.09
2	60	1.56	1.73									1.65	0.12
3	90	1.73	1.9									1.82	0.12
4	120	1.55	1.84									1.70	0.21
5	150	1.98	1.55									1.77	0.30
6	180	1.66	1.89									1.78	0.16
7	210	1.74	1.7									1.72	0.03
8	240	1.69	1.87									1.78	0.13
9	270	1.71	1.79									1.75	0.06
10	300	2.17	2.31									2.24	0.10

*measurement unit : mg/kg*

Mean	1.78
STDev	0.18
CV(%)	9.87

#### Graph Simple Statistics Cu



## Homogeneity Study - Simple Statistics

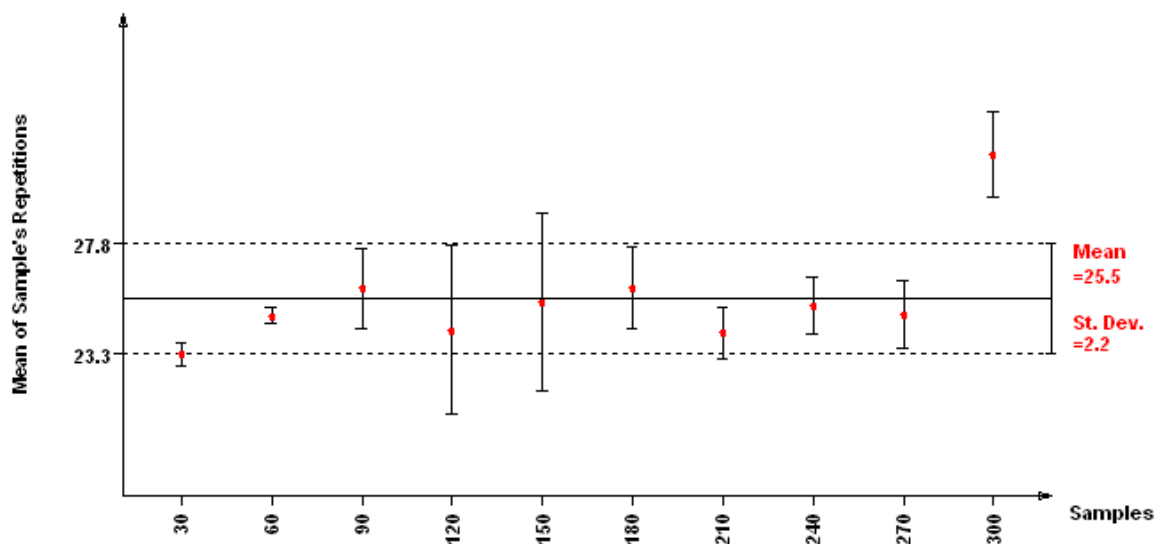
### Output for : Simple statistics Zn

S/N	Sample ID	Rep. #1	Rep. #2	Rep. #3	Rep. #4	Rep. #5	Rep. #6	Rep. #7	Rep. #8	Rep. #9	Rep. #10	Mean	STD ev
1	30	22.9	23.6									23.3	0.5
2	60	24.6	25.1									24.9	0.4
3	90	24.8	27.1									26.0	1.6
4	120	21.8	26.7									24.3	3.5
5	150	28	22.8									25.4	3.7
6	180	24.8	27.2									26.0	1.7
7	210	24.9	23.4									24.2	1.1
8	240	24.4	26.1									25.3	1.2
9	270	23.9	25.9									24.9	1.4
10	300	30.2	32.7									31.5	1.8

*measurement unit : mg/kg*

Mean	25.5
STDev	2.2
CV(%)	8.8

### Graph Simple Statistics Zn



## Homogeneity Study - Nested Design

### Output for : Nested Designs Cu

#### Data for the Unit: 30

##### **Sub-Units**

Repetitions	30/1	30/2
1	1.53	1.66

*measurement unit : mg/kg*

#### Data for the Unit: 60

##### **Sub-Units**

Repetitions	60/1	60/2
1	1.56	1.73

*measurement unit : mg/kg*

#### Data for the Unit: 90

##### **Sub-Units**

Repetitions	90/1	90/2
1	1.73	1.9

*measurement unit : mg/kg*

#### Data for the Unit: 120

##### **Sub-Units**

Repetitions	120/1	120/2
1	1.55	1.84

*measurement unit : mg/kg*

#### Data for the Unit: 150

##### **Sub-Units**

Repetitions	150/1	150/2
1	1.98	1.55

*measurement unit : mg/kg*

#### Data for the Unit: 180

##### **Sub-Units**

Repetitions	180/1	180/2
1	1.66	1.89

*measurement unit : mg/kg*

**Data for the Unit: 210**

***Sub-Units***

Repetitions	210/1	210/2
1	1.74	1.7

*measurement unit : mg/kg*

**Data for the Unit: 240**

***Sub-Units***

Repetitions	240/1	240/2
1	1.69	1.87

*measurement unit : mg/kg*

**Data for the Unit: 270**

***Sub-Units***

Repetitions	270/1	270/2
1	1.71	1.79

*measurement unit : mg/kg*

**Data for the Unit: 300**

***Sub-Units***

Repetitions	300/1	300/2
1	2.17	2.31

*measurement unit : mg/kg*

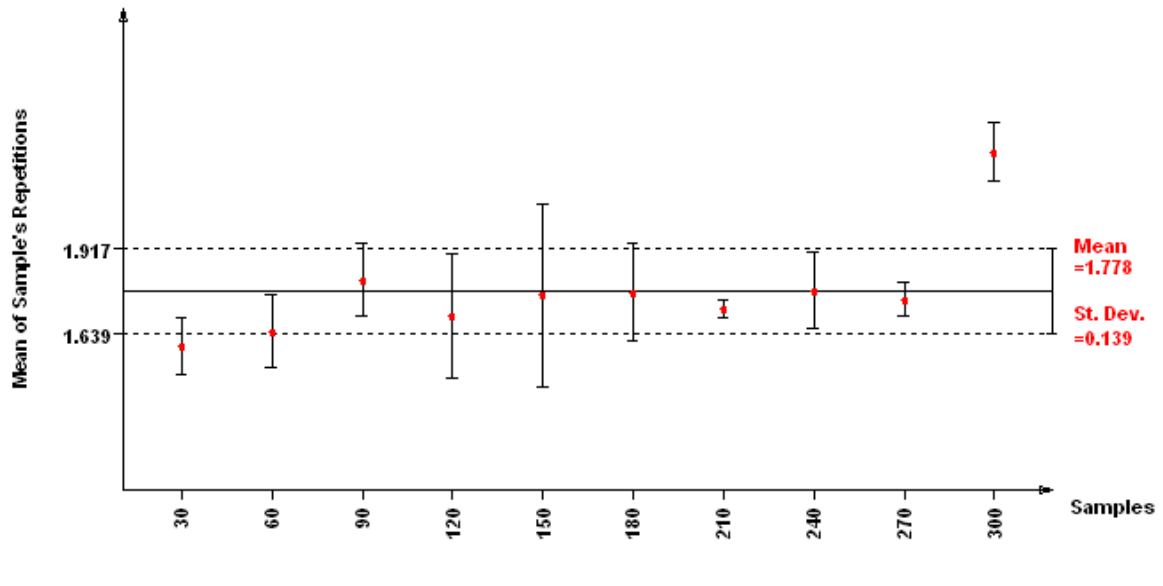
**ANOVA Table**

Source of Variation	SS	d.f.	MS	StDev	F	F-crit 95%	F-crit 99%
Between Units	0.554	9	0.062	0.139	2.698	3.020	4.942
Within Units	0.228	10	0.023	0.151			
Total	0.783	19					

**Snedecor F-Test**

Differences between units statistically significant? (a=95%) :No  
Differences between units statistically significant? (a=99%) :No

### Graph Nested Designs Cu



## Homogeneity Study - Nested Design

### Output for : Nested Designs Zn

#### Data for the Unit: 30

##### **Sub-Units**

Repetitions	30/1	30/2
1	22.9	23.6

*measurement unit : mg/kg*

#### Data for the Unit: 60

##### **Sub-Units**

Repetitions	60/1	60/2
1	24.6	25.1

*measurement unit : mg/kg*

#### Data for the Unit: 90

##### **Sub-Units**

Repetitions	90/1	90/2
1	24.8	27.1

*measurement unit : mg/kg*

#### Data for the Unit: 120

##### **Sub-Units**

Repetitions	120/1	120/2
1	21.8	26.7

*measurement unit : mg/kg*

#### Data for the Unit: 150

##### **Sub-Units**

Repetitions	150/1	150/2
1	28	22.8

*measurement unit : mg/kg*

#### Data for the Unit: 180

##### **Sub-Units**

Repetitions	180/1	180/2
1	24.8	27.2

*measurement unit : mg/kg*

**Data for the Unit: 210**

**Sub-Units**

Repetitions	210/1	210/2
1	24.9	23.4

*measurement unit : mg/kg*

**Data for the Unit: 240**

**Sub-Units**

Repetitions	240/1	240/2
1	24.4	26.1

*measurement unit : mg/kg*

**Data for the Unit: 270**

**Sub-Units**

Repetitions	270/1	270/2
1	23.9	25.9

*measurement unit : mg/kg*

**Data for the Unit: 300**

**Sub-Units**

Repetitions	300/1	300/2
1	30.2	32.7

*measurement unit : mg/kg*

**ANOVA Table**

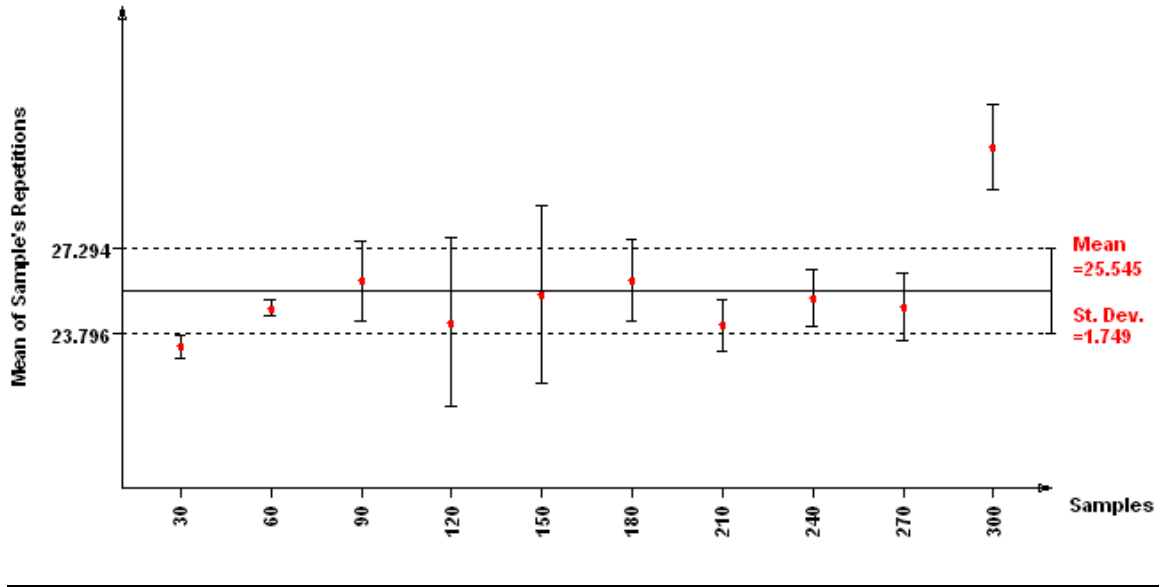
Source of Variation	SS	d.f.	MS	StDev	F	F-crit 95%	F-crit 99%
Between Units	90.275	9	10.031	1.749	2.564	3.020	4.942
Within Units	39.115	10	3.912	1.978			
Total	129.390	19					

**Snedecor F-Test**

Differences between units statistically significant? (a=95%) :No  
Differences between units statistically significant? (a=99%) :No



### Graph Nested Designs Zn



## Homogeneity Study - Repeatability

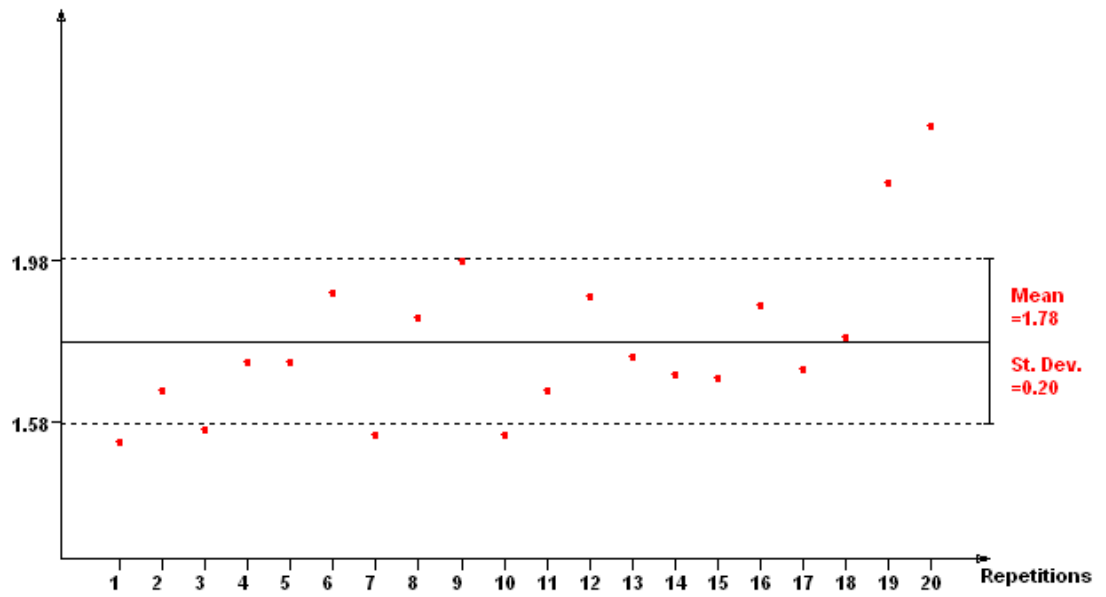
### Output for : Repeatability Cu

S/N	Sample Value
1	1.53
2	1.66
3	1.56
4	1.73
5	1.73
6	1.9
7	1.55
8	1.84
9	1.98
10	1.55
11	1.66
12	1.89
13	1.74
14	1.7
15	1.69
16	1.87
17	1.71
18	1.79
19	2.17
20	2.31

*measurement unit : mg/kg*

Mean	1.78
STDev	0.20
CV(%)	11.42

### Graph Repeatability Cu



## **Homogeneity Study - Repeatability**

**Output for : Repeatability Zn**

S/N	Sample Value
1	22.9
2	23.6
3	24.6
4	25.1
5	24.8
6	27.1
7	21.8
8	26.7
9	28
10	22.8
11	24.8
12	27.2
13	24.9
14	23.4
15	24.4
16	26.1
17	23.9
18	25.9
19	30.2
20	32.7

*measurement unit : mg/kg*

Mean	25.5
STDev	2.6
CV(%)	10.2

### Graph Repeatability Zn

