

Final Report

Ammonium Niobium Oxalate - Testing of Effects to the Single Cell Green Alga *Pseudokirchneriella subcapitata*

Guidelines

OECD 201 (2011)

Study Director

Dr. Silke Falk

Date

21 May 2014

Testing Facility

Eurofins Agrosience Services
EcoChem GmbH
Eutinger Str. 24
D-75223 Niefern-Öschelbronn
Germany

Sponsor

CBMM Europe BV
WTC H-Tower, Zuidplein 96
NL-1077 XV Amsterdam
The Netherlands

Study Identification Code

Test item:	Ammonium Niobium Oxalate
Study Code:	S12-03660
Trial/Lab Phase Codes:	S12-03660 -L1_AAPs

Statement of Confidentiality

This report contains confidential and proprietary information of the sponsor which must not be disclosed to anyone except the employees of this company or to persons authorised by law or judicial judgement without the expressed and written approval of the sponsor.

Statement of Compliance with the Principles of Good Laboratory Practice

The study described in this report was conducted in compliance with the most recent edition of:

- The Principles of Good Laboratory Practice (GLP), (Chemical act, attachment 1, Germany).
- The OECD Principles of Good Laboratory Practice.

The German requirements are based on the OECD Principles of Good Laboratory Practice which are accepted by regulatory authorities throughout the European Community, the United States of America (FDA and EPA) and Japan (MHW, MAFF and METI) on the basis of intergovernmental agreements.

Head of testing facility
(Dr. Martin Feyerabend/Dr. Susanne Timmermann)

23 May 14 Susanne Timmermann
Date / Signature

Study director
(Dr. Silke Falk)

21 May '14 Silke Falk
Date / Signature

Ammonium Niobium
Oxalate

Final Report

S12-03660

Statement of Quality Assurance Unit

Study code: S12-03660

Study title: Ammonium Niobium Oxalate - Testing of Effects to the Single Cell Green Alga *Pseudokirchneriella subcapitata*

This study has been audited by the relevant Quality Assurance Unit(s) in accordance with the OECD principles of Good Laboratory Practice and respective national regulations. Dates of inspection and reporting are listed in this section, or in the phase reports supplied by the test site(s). Documents were audited as draft versions. Facilities and/or processes and systems are monitored as part of a regular program.

		Date of Audit	Date of Report to Principal Investigator	Date of Report to Study Director ¹⁾	Date of Report to Management ²⁾
Study Plan	-	30 Aug 2012	-	30 Aug 2012	30 Aug 2012
Study Plan 2	-	16 Oct 2013	-	16 Oct 2013	16 Oct 2013
Amendment 1	-	08 May 2014	-	08 May 2014	08 May 2014
Experimental Phase	Serial Dilution Weighing: Test Item	28 Oct 2013	-	28 Oct 2013	28 Oct 2013
Final Report	Biological Part	24 Mar 2014	-	25 Mar 2014	25 Mar 2014
Final Report	Analytical Part	07 Apr 2014	-	07 Apr 2014	07 Apr 2014

¹⁾ including Lead QA and test facility management if audit reported to Principal Investigator

²⁾ test site management if audit reported to Principal Investigator, otherwise test facility management

- not applicable

According to the inspections detailed above, and the QA Statements provided by the test sites it can be confirmed that the methods, procedures, and observations described in this final report are a full and accurate account of the raw data.

Quality assurance
(Dr. Ulrich Schwarz)

23 May 2014 U. Schwarz

Date / Signature

Contents

Statement of Confidentiality	2
Statement of Compliance with the Principles of Good Laboratory Practice.....	2
Statement of Quality Assurance Unit	3
1 Summary	7
2 Time Schedule	10
3 Study Objective.....	10
4 Materials and Methods.....	10
4.1 Test Item.....	10
4.2 Test Organism	11
4.3 Test Medium	11
4.4 Description of Test Method.....	12
4.4.1 Performance of Test and Test Design.....	12
4.4.2 Test Item Concentrations.....	12
4.4.3 Test Conditions.....	13
4.5 Data	13
4.5.1 Determination of Cell Numbers	13
4.5.2 Measurements.....	14
4.5.3 Analytical Determinations.....	14
4.6 Chemical Analysis.....	14
4.7 Evaluation	14
4.7.1 Calculation of Growth Rates	14
4.7.2 Calculation of Yield	15
4.7.3 Statistical Evaluation of Results.....	15
5 Deviations from the Study Plan	15
6 Results.....	16
6.1 Range-Finding Test	16
6.2 Main Test	16
6.3 Analytical Results	18
6.4 Statistical Evaluation of the Results.....	19
6.5 Results of pH and Temperature Measurements	19
7 Validity of the Test	20
8 Conclusion	21
9 Archiving	21
10 References.....	21
11 Distribution	22
11.1 Study Plan	22
11.2 Final Report.....	22
11.3 Raw Data.....	22
12 Appendix.....	23

Ammonium Niobium

Oxalate

Final Report

S12-03660

A 1	Main Test: Individual Cell Numbers	23
A 2	Main Test: Control, Growth Rates	24
A 3	Main Test: Control, Daily Growth Rates	24
A 4	Main Test: Coefficient of Variation (CV) of Control Daily Growth Rates	24
A 5	Calibration Data for Cell Numbers	25
A 6	Analytical Method for the Determination of Niobium in Ammonium Niobium Oxalate	29
A 7	Certificates	39

List of Tables

Table 1:	EC ₅₀ , NOEC and LOEC-values for Ammonium Niobium Oxalate after 72 h exposed to the test item.....	8
Table 2:	Preparation of the test solutions in the main test	13
Table 3:	Results of the range-finding test	16
Table 4:	Average cell number for each sampling time and concentration.....	16
Table 5:	Percentage inhibition of growth rate.....	17
Table 6:	Percentage inhibition of yield.....	18
Table 7:	Concentrations of niobium in the test medium	18
Table 8:	EC ₅₀ , NOEC and LOEC-values for Ammonium Niobium Oxalate after 72 h exposed to the test item.....	19
Table 9:	pH measurements during the test.....	19
Table 10:	Temperature during the test	20
Table 11:	Light intensity at test start.....	20
Table 12:	Calibration data for cell numbers.....	26
Table 13:	Recovery of niobium from test item / niobium reference item spiked into medium	32

List of Figures

Figure 1:	Main test, growth curves.....	17
Figure 2:	Calibration curve for cell numbers, main test (100 x amplification)	25
Figure 3:	Typical calibration data for analysis of niobium by ICP-MS	34
Figure 4:	Typical spectrum of a 0.1 µg/L niobium standard with the internal standards scandium, indium and lutetium.....	35
Figure 5:	Typical spectrum of a 40 µg/L niobium standard with the internal standards scandium, indium and lutetium.....	36
Figure 6:	Typical spectrum of a recovery sample (fortification level 2.33 mg/L test item in medium; dilution factor 50).....	36
Figure 7:	Typical spectrum of a recovery sample (fortification level 73.9 mg/L test item in medium; dilution factor 500)	37
Figure 8:	Typical spectrum of a sample (50 mg/L-0h fresh; dilution factor 525)	37
Figure 9:	Typical spectrum of a sample (50 mg/L-72h aged; dilution factor 525)	38
Figure 10:	Substance identity of ANO Ammonium Niobium Oxalate	39

Ammonium Niobium
 Oxalate

Final Report

S12-03660

Figure 11: Certificate of analysis of the test item	41
Figure 12: Certificate of analysis of niobium reference item	42
Figure 13: GLP Certificate of test facility	43

1 Summary

Report:	Falk, S. (2014): Ammonium Niobium Oxalate – Testing of Effects to the Single Cell Green Alga <i>Pseudokirchneriella subcapitata</i>
Source:	Eurofins Agrosience Services EcoChem GmbH. Eutinger Straße 24. 75223 Niefern-Öschelbronn. Germany; unpublished report no.: S12-03660, Issued: 21 May 2014
Guidelines:	OECD 201 (2011)
Deviations:	None
GLP:	Yes (certified laboratory)
Objective:	The aim of the study was the assessment of the effects of the test item on single cell green algae within the framework of laws for registration according to OECD Guideline 201.
Materials and methods:	Test item Ammonium Niobium Oxalate (ANO), Batch number: AD/4663, Content of ANO (analysed): 99.4 % (w/w). Test species <i>Pseudokirchneriella subcapitata</i>. Initial concentration of 0.5×10^4 cells/mL in each test vessel, were exposed in a static test system for 3 days. Following a static range-finding test with test item concentrations of 0.100, 1.00, 10.0 and 100 mg/L Ammonium Niobium Oxalate a static main test with test item concentrations of 0.149, 0.476, 1.53, 4.88, 15.6 and 50.0 mg/L Ammonium Niobium Oxalate was performed. Six replicates were performed for the control and three for each test item concentration. Additionally one replicate per test item concentration for sampling was run in parallel. The test was performed in 500 mL Erlenmeyer flasks under continuous illumination at $68 - 107 \mu\text{Em}^{-2}\text{s}^{-1}$ at cell culture level. The final volume in each main test vessel was 150 - 200 mL. After 1, 2 and 3 days, the cell growth was determined by fluorescence detection. The mean value of the cell concentration was plotted versus time to produce growth curves for each concentration. The EC_{50} value as well as LOEC and NOEC values were determined after 72 hours. The temperature was recorded after 0, 24, 48 and 72 hours. The pH-values of the test solutions were measured after 0 and 72 hours and the light intensity at test start (0 hours). Analytical samples were taken from all test item concentrations and from the control after 0, 24, 48 and 72 hours. The test item

Ammonium Niobium Oxalate

Final Report

S12-03660

concentrations 4.88, 15.6 and 50.0 mg/L were analysed at 0 and 72 hours to verify the actual test concentrations using a validated analytical method.

Dates of work:

28 Oct 2013 – 21 Feb 2014

Findings:

The measured concentrations of niobium in the test item solutions of 4.88, 15.6 and 50.0 mg/L were between 45 - 113 % of nominal concentration.

Since some of the measured concentrations were below 80 % the biological endpoints were evaluated using the nominal and actual measured concentrations based on the arithmetic mean of the geometric mean of each test item concentration.

Table 1: EC₅₀, NOEC and LOEC-values for Ammonium Niobium Oxalate after 72 h exposed to the test item

Endpoint	Ammonium Niobium Oxalate [mg/L]	
	nominal	actual ⁴⁾
Growth rate ErC ₅₀ ¹⁾	28.5	21.1
Yield EyC ₅₀ ²⁾	1.10	0.813
LOEC ³⁾	1.53	1.13
NOEC ³⁾	0.476	0.352

¹⁾ Probit analysis following the Gompertz distribution

²⁾ Probit analysis following the logistic distribution

³⁾ following the most sensitive parameter growth rate

⁴⁾ Actual test item concentration was based on the geometric mean of each test item concentration.

Conclusions:

As indicated in Table 1 the test item the EC₅₀-value for growth rate (ErC₅₀) was 28.5 mg/L nominal (corresponding to 21.1 mg/L actual). The EC₅₀-value for yield (EyC₅₀) was 1.10 mg/L (corresponding to 0.813 mg/L actual). The LOEC was determined to be 1.53 mg/L (equivalent to 1.13 mg/L) and the NOEC was determined to be 0.476 mg/L (equivalent to 0.352 mg/L actual) for the most sensitive parameter growth rate.

All validity criteria of OECD Guideline for Testing of Chemicals No. 201 were fulfilled:

- Cell numbers, measured in the controls between 0 d and 3 d. were found to increase by a factor of 92.46 which exceeds the threshold of 16. It corresponds to a growth rate of 1.49651 d⁻¹ respectively (Appendix A 1 and Appendix A 2).
- The coefficient of variation of average growth in replicate control cultures was 3.0 % and did not exceed

6.5 % for the whole test period (Appendix A 2).

- The mean coefficient of variation for the section-by-section specific growth rates (days 0 – 1, 1 – 2 and 2 – 3) in the control cultures was 16 % and did not exceed 35 % (Appendix A 4).

2 Time Schedule

Study initiation date:	23 Oct 2013
Start of the experimental phase:	28 Oct 2013
End of the experimental phase (biological part):	30 Jan 2014
End of the experimental phase (analytical part):	21 Feb 2014
Draft report (biological phase):	11 Mar 2014
Draft report (analytical phase):	19 Mar 2014
Study completion date:	21 May 2014

3 Study Objective

The effect of the test item was tested in a growth inhibition test using the single cell green alga *Pseudokirchneriella subcapitata*. The test was based on the OECD Guideline 201: Freshwater Alga and Cyanobacteria, growth inhibition test (2006 corrected July 2011).

4 Materials and Methods

4.1 Test Item

Common Name:	Ammonium Niobium Oxalate (ANO)
Chemical Name	Reaction mass of ammonium diaqua[bis(oxalate)]oxoniobate(1-) hydrate and ammonium hydrogen oxalate oxalic acid (1:1:1) dehydrate
Test item code:	2012-003688
Batch number:	AD/4663
Content of ANO (analysed):	99.4 % (w/w)
Appearance/colour:	Powder/white
Certificate of analysis:	27 March 2014
Expiry date:	25 March 2015
Storage conditions:	Room temperature

Purity and Composition

All specifications of purity and composition of the test item were provided by the sponsor.

Stability and Homogeneity in the Test Solution

Homogeneity of test solutions was obtained by thorough stirring or mixing immediately before application. Stability in test solution was verified by analysis.

4.2 Test Organism

The test organism is *Pseudokirchneriella subcapitata* Hindák, strain SAG 61.81. The algae used in this study were purchased from MBM Sciencebridge GmbH, Hans-Adolf-Krebs-Weg 1, D-37075 Göttingen, Germany.

The algae were grown semi-continuously under sterile cultures in the laboratory. Old medium was periodically replaced by fresh mineral solution in order to keep the algae in an exponential growth state. Stock cultures were ordered regularly from the culture collection.

Culture conditions were as follows:

- Illumination: continuously from the top by light tubes, 60 - 120 $\mu\text{Em}^{-2}\text{s}^{-1}$ at cell culture level
- Temperature: 21 – 24 °C
- Culture flasks: 100 mL Erlenmeyer flasks
- Visually healthy cells were used for the test
- CO₂ supply by shaking on a rotating shaker, 105 rpm

Cells from this semi-continuous liquid stock culture were used for the test.

4.3 Test Medium

The medium used for the test was composed as follows (AAP-Medium, Annex 3 of OECD 201).

Stock solution No.	Substance	Conc. in test medium [mg/L]
I	NaHCO ₃	15.0
II	K ₂ HPO ₄	1.044
III	MgSO ₄ ·7H ₂ O	14.6
IV	NaNO ₃	25.5
	CaCl ₂ ·2H ₂ O	4.41
	MgCl ₂ ·6H ₂ O	12.16
V	H ₃ BO ₃	0.186
	MnCl ₂ ·4H ₂ O	0.415
	ZnCl ₂	0.00327
	CoCl ₂ ·6H ₂ O	0.00143
	CuCl ₂ ·2H ₂ O	1.2 · 10 ⁻⁵
	Na ₂ MoO ₄ ·2H ₂ O	0.00726
	FeCl ₃ ·6H ₂ O	0.160
	Na ₂ EDTA·2H ₂ O	0.300

The pH was adjusted to 7.5 ± 0.1

4.4 Description of Test Method

4.4.1 Performance of Test and Test Design

The algae were exposed to different concentrations of the test item under defined conditions in a synthetic growth medium during several generations. The initial cell density was adjusted to 0.5×10^4 algae per mL. By comparing the cell division under test conditions with and without the influence of the test item, inhibition of the cell multiplication in the test item treatments can be calculated compared to the untreated control. This inhibition is a value for toxicity. The cell numbers are determined by fluorescence detection.

At defined dates (1, 2 and 3 days after initial exposure), the number of cells in each replicate was evaluated. Thereby those concentrations were determined which resulted in 50 % inhibition of the cell growth rate (ErC_{50}) and yield (EyC_{50}), the concentration which did not cause any inhibition (NOEC) and the lowest observed effect concentration (LOEC) were determined.

4.4.2 Test Item Concentrations

Following a static range-finding test with test item concentrations of 0.100, 1.00, 10.0 and 100 mg/L a static main test was performed with the following range of test item concentrations (spaced by a factor of 3.2): 0.149, 0.476, 1.53, 4.88, 15.6 and 50.0 mg/L. There were six replicates for the control and three replicates for each of the test item concentrations. Additionally one replicate per test item concentration for sampling was run in parallel. The cell density was adjusted to an initial concentration of 0.5×10^4 cells/mL in each test vessel.

A stock solution (S1) was prepared by directly weighing 500 mg (S1) of the test item into 1000 mL test medium. The further concentrations V1 – V6 were made by diluting the appropriate solutions to give the required test concentrations. The preparation of the test solutions is presented in Table 2. All test item solutions were clear and transparent.

Table 2: Preparation of the test solutions in the main test

Target concentration	Test solution	Test item (required)	Dilution		Final volume	Volume per test vessel
[mg/L]	No.	[mg]	No.	[g]	[g]	[mL]
-	S1	500	-	-	1000 mL	-
50.0	V1	-	S1	100	1000	150 – 200
15.6	V2	-	S1	31.25	1000	150 – 200
4.88	V3	-	S1	9.77	1000	150 – 200
1.53	V4	-	V1	30.60	1000	150 – 200
0.476	V5	-	V2	30.51	1000	150 – 200
0.149	V6	-	V3	30.53	1000	150 – 200
0	Control	-	-	-	1200 ¹⁾	150 – 200

¹⁾ only AAP medium with algae

4.4.3 Test Conditions

Test procedure: static
Duration: 3 days (72 hours)
Initial cell density: 0.5×10^4 cells/mL
Temperature: 20.3 – 24.0 °C
pH of control: 7.09 – 7.68
Illumination: continuously from the top, $68 - 107 \mu\text{Em}^{-2}\text{s}^{-1}$
Culture flasks: 500 mL Erlenmeyer flasks with aluminium caps and two baffles
CO₂ supplied: by shaking on a rotating shaker, 105 rpm

4.5 Data

4.5.1 Determination of Cell Numbers

The cell numbers were determined by fluorescence measurement, using a calibration curve, where cell numbers (x-axis) were plotted versus fluorescence signals (y-axis). Calibration data acquisition was done within a sensitivity range of 100 x. Each replicate was measured twice. To establish a calibration curve, the cell numbers were counted with a Neubauer chamber after preparation of a dilution series of a logarithmic growing *Pseudokirchneriella subcapitata*, see Appendix A 5.

The daily fluorescence measurements were performed with a Fuji FLA-3000 laser emitter at 633 nm (Fuji Photo Film Co., LTD) and evaluated with BAS Reader (Control software for Fuji scanners) and AIDA software (Advanced Image Data Analyzer).

Additionally, the morphological appearance of the algae cells was observed microscopically at the end of the test.

4.5.2 Measurements

Measurements of pH-value were performed at $t = 0$ h and $t = 72$ h. The temperature was measured at day 0, 24, 48, and 72 hours and the light intensity at test start.

4.5.3 Analytical Determinations

Analytical data are required by the OECD guidelines to determine the concentration and stability of niobium over the entire test period.

In the main test samples were taken from all concentrations and from the control at test start ($t = 0$ hour) and after 24, 48 and 72 hours. The test item concentrations 4.88, 15.6 and 50.0 mg/L were analysed at 0 and 72 hours to verify test concentrations. The analytical sampling procedure is described in Appendix A 6.

4.6 Chemical Analysis

The analysis of samples from the test treatment solutions was performed in the analytical laboratories of the testing facility. The content of niobium in the treatment solution samples was determined by analysing with HPLC/MS-MS. The method was validated with regard to accuracy (recovery), linearity, precision and non-analyte interference of the analytical system. The analytical systems showed a sufficient specificity for niobium from the test medium as outlined in SANCO/3029/99 rev.4 11/07/00. The data for the method and the results of the validation and the analysis are part of the raw data and final report of this study (see Appendix A 6).

4.7 Evaluation

The measured cell numbers (N_n) in the test cultures and controls were tabulated together with the concentrations of the test item and the times of measurement (t_n). The mean value of the cell numbers for each test item concentration and the control was plotted versus time to produce growth curves.

4.7.1 Calculation of Growth Rates

The average specific growth rate for a specific period is calculated as the logarithmic increase of the cell numbers for each single vessel in the controls and test item treatments:

$$\mu_{i-j}(\text{day}^{-1}) = \frac{\ln N_j - \ln N_i}{t_j - t_i}$$

where

μ_{i-j} : average specific growth rate from time i to j

N_j : average of cell numbers at t_j

N_i : average of cell numbers at t_i

The percentage inhibition of growth rates (% I_μ) is calculated as the difference between the growth rates of the control (μ_c) and the growth rates in the treatment (μ_t):

$$\% I_\mu = \frac{\mu_c - \mu_t}{\mu_c} \cdot 100$$

4.7.2 Calculation of Yield

Yield was calculated as the cell numbers at the end of the test minus the starting cell numbers, for each single vessel of controls and treatments. For each test concentration and control, a mean value for yield along with variance estimates was calculated. The percent inhibition in yield (% I_y) was calculated for each treatment replicate as follows:

$$\% I_y = \frac{Y_c - Y_t}{Y_c} \cdot 100$$

where

% I_y : percent inhibition of yield

Y_c : mean value for yield in the control group

Y_t : mean value for yield for the treatment group

4.7.3 Statistical Evaluation of Results

The statistical evaluation for 72 hours was performed for growth rate and yield using SAS[®] (2002–2010). A test for normality of the data was performed by calculating the Shapiro-Wilk's statistic and the homogeneity of variance of the data was performed by calculating the Levene Test. The NOEC and LOEC were determined by using the Dunnett's t-test for growth rate and Jonckheere-Terpstra test for yield, left sided. The ErC_{50} and EyC_{50} values were evaluated using the probit analysis following Gompertz distribution (growth rate) and logistic distribution (yield).

5 Deviations from the Study Plan

The study was performed according to the study plan dated 23 Oct 2013 and amendment No 1 dated 12 May 2014 without any deviation.

This report reflects the conduct of the study.

6 Results

6.1 Range-Finding Test

The range-finding test was carried out at a test item concentration range between 0.100 and 100 mg/L, spaced by a factor of 10. The average cell numbers for each concentration and time of sampling are shown in Table 3.

Table 3: Results of the range-finding test

Test item conc. [mg/L]	Average cell numbers/mL ¹⁾			
	0d	1d	2d	3d
0 (Control)	0.5	2.11	8.85	34.03
0.100	0.5	2.19	8.46	29.82
1.00	0.5	1.71	5.11	14.87
10.0	0.5	1.61	3.26	6.68
100	0.5	0.86	0.90	0.94

¹⁾ Algae counts are divided by 10000. At the start, the cell density was adjusted to 0.5×10^4 cells/mL

6.2 Main Test

The main test was performed with the following test item concentrations: 0.149, 0.476, 1.53, 4.88, 15.6 and 50.0 mg/L. The average cell numbers for each concentration and sampling point are shown in Table 4 and the resulting growth curves are demonstrated in Figure 1. Table 5 shows the percentage inhibition of growth rate and Table 6 the inhibition of yield.

Table 4: Average cell number for each sampling time and concentration

Test item conc. nominal [mg/L]	Test item conc. actual [mg/L]	Average cell numbers/mL ¹⁾			
		0d	1d	2d	3d
0 (Control)	0	0.5	2.86	12.51	46.23
0.149	0.110	0.5	2.47	12.12	43.84
0.476	0.352	0.5	2.56	13.65	49.79
1.53	1.13	0.5	1.92	5.56	13.56
4.88	3.61	0.5	1.88	4.34	9.17
15.6	11.5	0.5	1.77	2.72	4.30
50.0	37.0	0.5	1.88	3.16	5.56

¹⁾ Algae counts are divided by 10000. At the start, the cell density was adjusted to 0.5×10^4 cells/mL

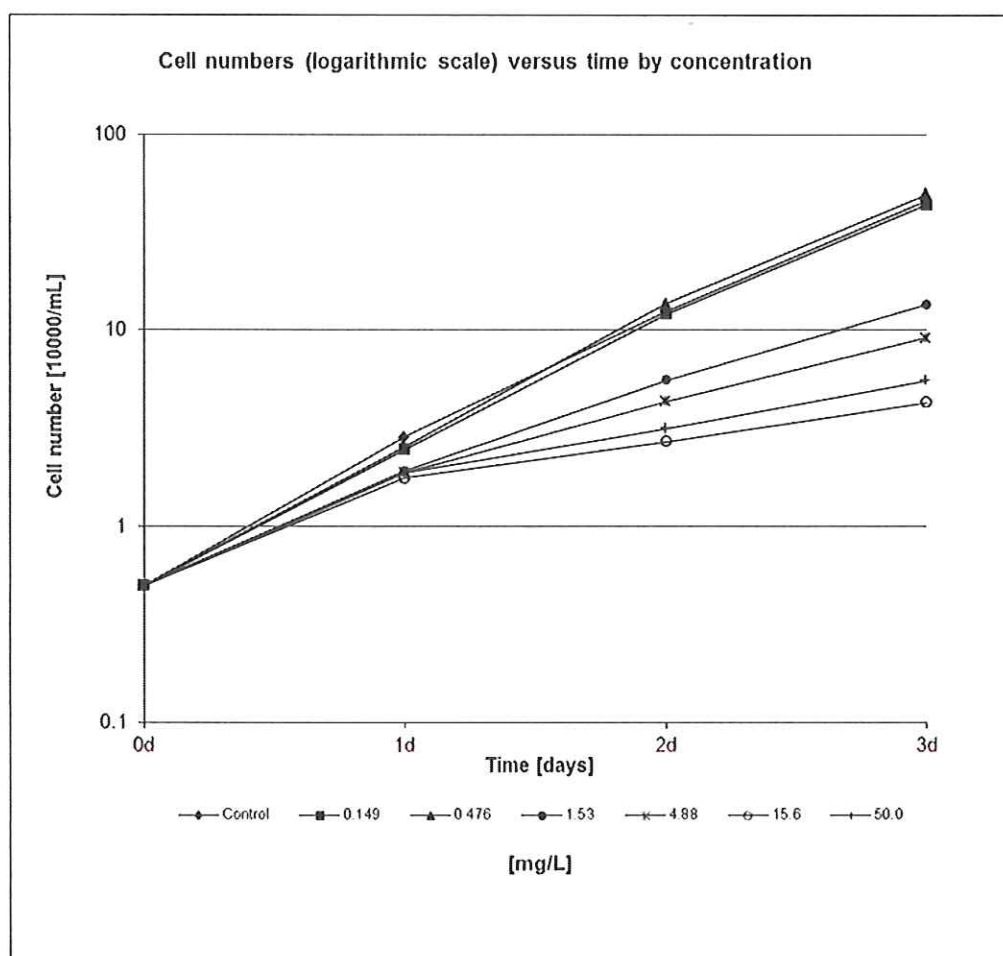


Figure 1: Main test, growth curves

Table 5: Percentage inhibition of growth rate

Test item conc. nominal [mg/L]	Test item conc. actual [mg/L]	Inhibition of growth rate [%]		
		0d - 1d	0d - 2d	0d - 3d
0 (Control)	0	0.0	0.0	0.0
0.149	0.110	8.4	0.7	1.2
0.476	0.352	6.3	-3.1	-2.2
1.53	1.13	22.9	24.7	26.6*
4.88	3.61	24.2	32.8	35.8*
15.6	11.5	27.6	47.1	52.1*
50.0	37.0	24.1	42.3	46.4*

*statistically significant compared to the control

Table 6: Percentage inhibition of yield

Test item conc. nominal [mg/L]	Test item conc. actual [mg/L]	Inhibition of yield [%]		
		0d - 1d	0d - 2d	0d - 3d
0 (Control)	0	0.0	0.0	0.0
0.149	0.110	16.5	3.2	5.2
0.476	0.352	12.7	-9.5	-7.8
1.53	1.13	39.8	57.9	71.4
4.88	3.61	41.5	68.0	81.0*
15.6	11.5	46.2	81.5	91.7*
50.0	37.0	41.5	77.9	88.9*

*statistically significant compared to the control

The individual cell numbers of each test item concentration and each control replicate are presented in Appendix A1. The individual values of the controls for growth rate, daily growth rate and coefficient of variation are presented in Appendix A2, A3 and A4.

The morphology of the algae cells was observed microscopically. The cells were considered normal for the control and all test item concentrations.

6.3 Analytical Results

The test was performed under static conditions. The concentration of the active substance was verified in test medium by analysing the content of niobium in the test concentrations 4.88, 15.6 and 50.0 mg/L at 0 and 72 hours. The results are presented in Table 7.

Table 7: Concentrations of niobium in the test medium

Test item	Niobium	Sampling	Niobium			
nominal conc.	nominal conc.			% of nominal	Geometric mean	Test item
[mg/L]	[mg/L]	[h]	[mg/L]		[%]	conc. actual [mg/L] ¹⁾
0 (Control)	0	0	n.d.	-	-	-
		72	n.d.	-		
4.88	0.839	0	0.549	65	68	3.61
		72	0.607	72		
15.6	2.68	0	3.04	113	71	11.5
		72	1.20	45		
50.0	8.60	0	8.16	95	82	37.0
		72	6.06	70		
Arithmetic mean:					74	

Limit of quantification (LOQ) = 2.33 mg/L test item, corresponds to 0.400 mg Nb/L

¹⁾ Based on the arithmetic mean of the geometric mean of each test item concentration

²⁾ Based on the analysed content of Nb 17.2 %

The measured concentrations of niobium in the test item solutions of 4.88, 15.6 and 50.0 mg/L were between 45 - 113 % of nominal concentration.

Since some of the measured concentrations were below 80 % the biological endpoints were evaluated using the nominal and actual measured concentrations based on the arithmetic mean of the geometric mean of each test item concentration.

6.4 Statistical Evaluation of the Results

In Table 8 the endpoint values are summarised based on the test item concentration.

Table 8: EC₅₀, NOEC and LOEC-values for Ammonium Niobium Oxalate after 72 h exposed to the test item

Endpoint	Ammonium Niobium Oxalate [mg/L]			
	Nominal	95% confidence limit	Actual ⁴⁾	95% confidence limit
ErC ₅₀ ¹⁾	28.5	18.3 – 51.2	21.1	13.5 – 37.9
EyC ₅₀ ²⁾	1.10	0.796 – 1.46	0.813	0.588 – 1.08
LOEC ³⁾	1.53		1.13	
NOEC ³⁾	0.476		0.352	

¹⁾ Probit analysis following the Gompertz distribution

²⁾ Probit analysis following the logistic distribution

³⁾ following the most sensitive parameter growth rate

⁴⁾ Actual test item concentration was based on the geometric mean of each test item concentration.

Statistically significant inhibitory effects were observed after 72 hours from the nominal concentration 1.53 mg/L (nominal) (equivalent to 1.13 mg/L actual) and above.

6.5 Results of pH and Temperature Measurements

The results from the measurement of the growth conditions during the test are shown in the following tables (Table 9, Table 10 and Table 11).

Table 9: pH measurements during the test

Test item conc. [mg/L]	t = 0 h	t = 72 h
0 (Control)	7.09	7.68
0.149	7.11	8.52
0.476	7.12	8.65
1.53	7.15	8.16
4.88	6.99	8.02
15.6	6.89	7.95
50.0	5.90	7.87

Table 10: Temperature during the test

Time [h]	Temperature [°C]	
	min	max
0	21.0	22.0
24	20.3	21.6
48	20.3	24.0
72	20.4	23.6

Table 11: Light intensity at test start

L	Light intensity [$\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$]						Mean
	A	B	C	D	E	F	
1	85	100	100	97	84	73	90
2	88	103	107	98	84	74	92
3	80	101	107	101	85	77	92
4	82	99	103	97	82	73	89
5	78	93	94	90	78	68	84
Mean	83	99	102	97	83	73	89
RSD	4.8	3.8	5.3	4.2	3.4	4.4	12.7

Light intensity was measured six times at five different locations (L) above the shaker

7 Validity of the Test

All validity criteria of OECD Guideline for Testing of Chemicals No. 201 were fulfilled:

- Cell numbers, measured in the controls between 0 d and 3 d. were found to increase by a factor of 92.46 which exceeds the threshold of 16. It corresponds to a growth rate of 1.49651 d^{-1} respectively (Appendix A 1 and Appendix A 2).
- The coefficient of variation of average growth in replicate control cultures was 6.5 % and did not exceed 7 % for the whole test period (Appendix A 2).
- The mean coefficient of variation for the section-by-section specific growth rates (days 0 – 1, 1 – 2 and 2 – 3) in the control cultures was 16 % and did not exceed 35 % (Appendix A 4).

8 Conclusion

Inhibitory effects for the test item (Ammonium Niobium Oxalate) were observed at nominal concentrations of 1.53 mg/L (corresponding to 1.13 mg/L actual) and above after 72 hours for the most sensitive parameter growth rate. Therefor the LOEC was determined to be 1.53 mg/L (equivalent to 1.13 mg/L) and the NOEC was determined to be 0.476 mg/L (equivalent to

0.352 mg/L actual). For the test item the EC₅₀-value for growth rate (ErC₅₀) was 28.5 mg/L nominal (corresponding to 21.1 mg/L actual). The EC₅₀-value for yield (EyC₅₀) was 1.10 mg/L (corresponding to 0.813 mg/L actual).

9 Archiving

For the periods demanded by the principles of GLP the following documents and materials will be archived:

- Study plan, raw data, comments of the sponsor on the draft report and the final report.
- All documentation generated by the Quality Assurance Unit.
- A sample of the test item.

All documents and materials will be stored in the archives of Eurofins Agrosience Services EcoChem GmbH. The premises for storing the documents and materials are settled according to the principles of Good Laboratory Practice in the organisation of the testing facility.

10 References

- EUROPEAN COMMISSION, DIRECTORATE GENERAL HEALTH AND CONSUMER PROTECTION (2000): Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A. Section 4) and Annex III (part A. Section 5) of Directive 91/414. SANCO/3029/99 rev. 4. 11/07/2000.
- OECD (1998): OECD Principles on Good Laboratory Practice (as revised in 1997). OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. ENV/MC/CHEM(98)17.
- OECD 201 (2006): OECD Guidelines for Testing of Chemicals No. 201. Freshwater Alga and Cyanobacteria; Growth inhibition Test. Adopted: 23 March 2006, Annex 5 corrected: 28 July 2011).
- SAS INSTITUTE INC. 2002-2010. SAS[®] Proprietary Software 9.3; Cary. NC. USA.

11 Distribution

11.1 Study Plan

Original: Testing facility (1 x)

Copy*: Sponsor (1x)

* paper copy or pdf-file possible

11.2 Final Report

Original: Testing facility (1 x)

Sponsor (1 x)

11.3 Raw Data

Original: Testing facility

12 Appendix

A 1 Main Test: Individual Cell Numbers

Test item conc. [mg/L]	Cells/mL ¹⁾				Yield
	0d	1d	2d	3d	0d-3d
Control	0.5	2.78	9.77	33.01	32.51
	0.5	2.81	10.00	36.76	36.26
	0.5	2.78	11.43	39.63	39.13
	0.5	2.86	15.38	62.59	62.09
	0.5	2.82	11.32	39.21	38.71
	0.5	3.10	17.17	66.15	65.65
Mean	0.5	2.86	12.51	46.23	45.73
0.149	0.5	2.24	9.35	30.36	29.86
	0.5	2.61	13.38	42.27	41.77
	0.5	2.57	13.63	58.89	58.39
Mean	0.5	2.47	12.12	43.84	43.34
0.476	0.5	2.70	15.88	55.46	54.96
	0.5	2.50	11.26	39.70	39.20
	0.5	2.48	13.81	54.22	53.72
Mean	0.5	2.56	13.65	49.79	49.29
1.53	0.5	2.00	6.00	14.92	14.42
	0.5	1.70	5.72	13.40	12.90
	0.5	2.07	4.96	12.37	11.87
Mean	0.5	1.92	5.56	13.56	13.06
4.88	0.5	1.95	4.78	10.86	10.36
	0.5	1.73	3.37	6.47	5.97
	0.5	1.95	4.86	10.19	9.69
Mean	0.5	1.88	4.34	9.17	8.67
15.6	0.5	1.97	3.10	4.69	4.19
	0.5	1.59	2.57	3.93	3.43
	0.5	1.76	2.50	4.28	3.78
Mean	0.5	1.77	2.72	4.30	3.80
50.0	0.5	1.93	3.22	5.42	4.92
	0.5	1.83	3.22	5.73	5.23
	0.5	1.87	3.04	5.53	5.03
Mean	0.5	1.88	3.16	5.56	5.06

¹⁾ cell numbers are divided by 10⁴

A 2 Main Test: Control, Growth Rates

	Growth rates. μ [d ⁻¹]		
	0d - 1d	0d - 2d	0d - 3d
Control	1.71560	1.48623	1.39665
	1.72633	1.49787	1.43252
	1.71560	1.56469	1.45758
	1.74397	1.71311	1.60992
	1.72988	1.55986	1.45403
	1.82455	1.76816	1.62836
Mean	1.74266	1.59832	1.49651
Std. Dev.	0.04148	0.11601	0.09760
CV (%)	2.4	7.3	6.5

A 3 Main Test: Control, Daily Growth Rates

	Growth rates. μ [d ⁻¹]		
	0d - 1d	1d - 2d	2d - 3d
Control	1.71560	1.25687	1.21749
	1.72633	1.26940	1.30183
	1.71560	1.41379	1.24334
	1.74397	1.68225	1.40354
	1.72988	1.38983	1.24236
	1.82455	1.71176	1.34876
Mean	1.74266	1.45398	1.29289
Std. Dev.	0.04148	0.19860	0.07230
CV (%)	2.4	13.7	5.6

A 4 Main Test: Coefficient of Variation (CV) of Control Daily Growth Rates

Mean daily growth rates μ [d ⁻¹]	Standard deviation	CV [%]
1.39665	0.27692	20
1.43252	0.25496	18
1.45758	0.23916	16
1.60992	0.18137	11
1.45402	0.25002	17
1.62836	0.24862	15
	Mean CV	16

A 5 Calibration Data for Cell Numbers

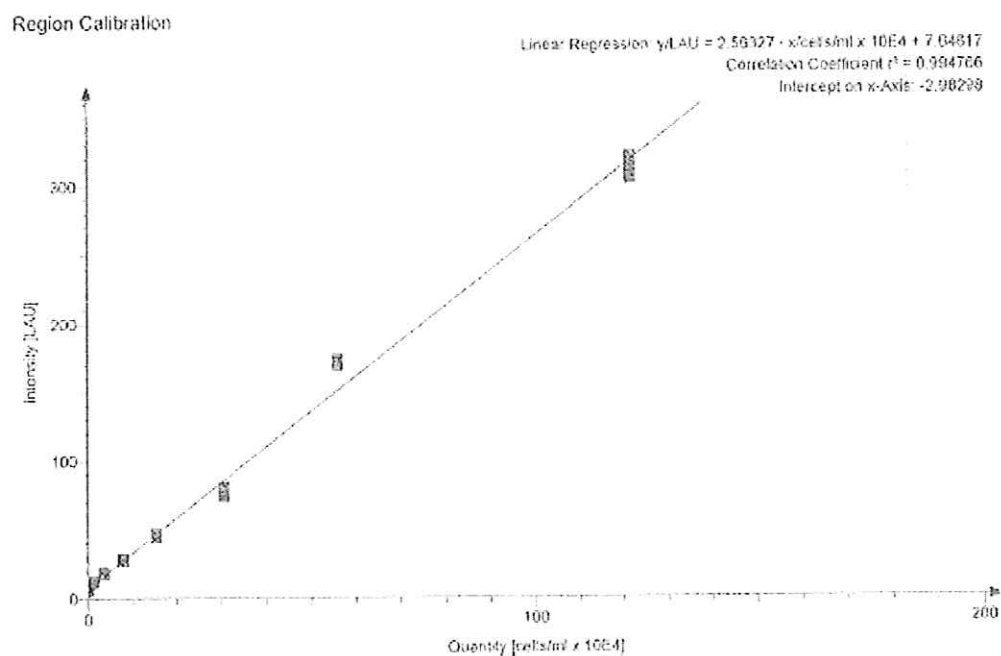


Figure 2: Calibration curve for cell numbers, main test (100 x amplification)

Table 12: Calibration data for cell numbers

Well-No	Intensity-Bkg [LAU] ¹⁾	Intensity-Bkg [%] ²⁾	Std. Quantity [cells/ml x 10 ⁴] ³⁾	Recalc. Quantity [cells/ml x 10 ⁴] ⁴⁾
5	8.2	0.26	0	0.23
6	8.3	0.26	0	0.24
7	8.2	0.26	0	0.23
8	8.5	0.27	0	0.32
9	10.1	0.32	0.25	0.94
10	10.6	0.34	0.25	1.16
11	9.7	0.31	0.25	0.79
12	9.8	0.31	0.25	0.83
13	12.2	0.39	1.25	1.76
14	12.3	0.39	1.25	1.81
15	11.9	0.38	1.25	1.65
16	11.7	0.37	1.25	1.59
17	18.0	0.57	3.50	4.03
18	17.9	0.57	3.50	3.99
19	18.3	0.58	3.50	4.17
20	19.2	0.61	3.50	4.49
21	28.2	0.90	7.75	8.04
22	28.9	0.92	7.75	8.29
23	27.8	0.88	7.75	7.87
24	28.4	0.90	7.75	8.10
25	46.8	1.49	15.25	15.29
26	46.3	1.47	15.25	15.08
27	44.5	1.41	15.25	14.39
28	44.5	1.41	15.25	14.39
29	78.6	2.49	30.50	27.67
30	80.8	2.56	30.50	28.52
31	74.3	2.36	30.50	26.02
32	76.2	2.42	30.50	26.76
33	168.9	5.36	56.00	62.91
34	171.2	5.43	56.00	63.81
35	173.5	5.51	56.00	64.71
36	170.2	5.40	56.00	63.40
37	320.8	10.18	121.25	122.18
38	313.9	9.96	121.25	119.47
39	304.7	9.67	121.25	115.90
40	306.4	9.72	121.25	116.53
41	7.6	0.24	0	-0.02
42	8.5	0.27	0	0.33
43	7.4	0.23	0	-0.11

¹⁾ Intensity (Linear Arbitrary Units)

²⁾ Intensity background

³⁾ Cell counts by microscope

⁴⁾ Calculated cell counts by AIDA

Ammonium Niobium
Oxalate

Final Report

S12-03660

Table 12 (continued): Calibration data for cell numbers

Well-No	Intensity-Bkg [LAU] ¹⁾	Intensity-Bkg [%] ²⁾	Std. Quantity [cells/ml x 10 ⁴] ³⁾	Recalc. Quantity [cells/ml x 10 ⁴] ⁴⁾
44	7.5	0.24	0	-0.07
45	8.0	0.25	0	0.15
46	7.1	0.23	0	-0.22
47	7.8	0.25	0	0.06
48	6.5	0.21	0	-0.43
49	6.8	0.22	0	-0.34
50	6.9	0.22	0	-0.31
51	6.8	0.21	0	-0.34
52	7.3	0.23	0	-0.14
53	6.8	0.22	0	-0.33
54	6.6	0.21	0	-0.40
55	6.6	0.21	0	-0.40
56	7.1	0.23	0	-0.21
57	6.7	0.21	0	-0.35
58	6.7	0.21	0	-0.39
59	7.5	0.24	0	-0.05
60	7.3	0.23	0	-0.15
61	6.8	0.22	0	-0.31
62	6.5	0.21	0	-0.43
63	6.8	0.21	0	-0.35
64	6.5	0.21	0	-0.45
65	6.8	0.21	0	-0.34
66	7.2	0.23	0	-0.17
67	7.0	0.22	0	-0.25
68	7.2	0.23	0	-0.17
69	7.0	0.22	0	-0.25
70	6.6	0.21	0	-0.40
71	6.8	0.22	0	-0.33
72	7.3	0.23	0	-0.12
73	6.8	0.22	0	-0.33
74	7.6	0.24	0	-0.02
75	7.2	0.23	0	-0.18
76	7.5	0.24	0	-0.05
77	6.7	0.21	0	-0.36
78	6.4	0.20	0	-0.49
79	6.7	0.21	0	-0.38
80	6.7	0.21	0	-0.37
81	7.1	0.22	0	-0.22
82	7.2	0.23	0	-0.18

¹⁾ Intensity (Linear Arbitrary Units)

²⁾ Intensity background

³⁾ Cell counts by microscope

⁴⁾ Calculated cell counts by AIDA

Table 12 (continued): Calibration data for cell numbers

Well-No	Intensity-Bkg [LAU] ¹⁾	Intensity-Bkg [%] ²⁾	Std. Quantity [cells/ml x 10 ⁴] ³⁾	Recalc. Quantity [cells/ml x 10 ⁴] ⁴⁾
83	7.3	0.23	0	-0.15
84	6.8	0.21	0	-0.34
85	7.2	0.23	0	-0.17
86	6.6	0.21	0	-0.40
87	6.8	0.22	0	-0.32
88	7.0	0.22	0	-0.26
89	7.7	0.25	0	0.03
90	6.7	0.21	0	-0.38
91	7.8	0.25	0	0.07
92	7.0	0.22	0	-0.24
93	6.7	0.21	0	-0.38
94	7.9	0.25	0	0.10
95	6.5	0.21	0	-0.44
96	6.7	0.21	0	-0.36

¹⁾ Intensity (Linear Arbitrary Units)

²⁾ Intensity background

³⁾ Cell counts by microscope

⁴⁾ Calculated cell counts by AIDA

A 6 Analytical Method for the Determination of Niobium in Ammonium Niobium Oxalate

Summary

An analytical method for the determination of niobium in Ammonium Niobium Oxalate was validated with regard to recovery, linearity of detector response, repeatability and specificity. The analytical method fulfills the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000 and is characterized as follows:

- Method principle:** Samples were digested in a microwave oven with nitric acid, if necessary diluted with water, bidistilled grade, and measured by ICP-MS.
- Specificity:** Niobium was identified by the mass to charge ratio (m/z) of a specific isotope in comparison with a certified reference item.
- Linearity:** The calibration function was linear within the range from 0.1 µg/L to 40 µg/L of niobium with $r = 1.0000$.
- Recovery:** The recovery was determined by fortification of medium with the test item and niobium reference item, respectively, at the concentration levels given below:

Fortification level test item (mg/L)	Fortification level niobium (mg/L)	n	Mean recovery ± RSD (%)
2.33	0.400	5	104 ± 2
73.9	12.7	5	80 ± 3

- Repeatability:** The relative standard deviation per fortification level is within the guideline requirements ($\leq 20\%$).
- Blanks:** Residues of niobium in the medium used for recovery samples were below 30 % of LOQ.
- LOQ:** The limit of quantification was 0.400 mg/L niobium.
- LOD:** The limit of detection was defined as 30 % of LOQ (= 0.120 mg/L niobium).

Test Item

The characterization of the test item is given in Figure 11.

Reference Item

The characterization of the reference item is given in Figure 12. Dilutions for calibration of ICP-MS analysis were prepared in 1 % nitric acid from this stock solution.

Material and Methods

Equipment

Volumetric pipettes (Eppendorf): 0.5-5 mL, 10-100 μ L, 100-1000 μ L

ICP-MS 7700x (Agilent)

Microwave digestion system speedwave, Berghof

Reagents

Nitric acid, 69 % for trace analysis (Fluka No. 84385)

Water, bidistilled grade (prepared at laboratory)

Indium ICP Standard, 1001 mg/L In, (SCP Science 140-051-49x)

Scandium ICP Standard, 999 mg/L Sc, (SCP Science 140-051-21x)

Lutetium ICP Standard, 998 mg/L Lu, (SCP Science 140-051-71x)

Outline of the Method

The samples were stored refrigerated (nominally 1-10 °C) until analysis. At the analytical laboratory, the samples were tempered to room temperature and shaken well.

1 mL of the sample was given into a microwave vessel. Then 10 mL of 69 % nitric acid were added and the sample was digested for about 45 min in a microwave oven with a temperature profile (120 – 210 °C). The whole sample was quantitatively transferred into a 50 mL tube with water. The 50 mg/L-0h fresh/72h aged samples were diluted by a factor of 10 and the 15.6 mg/L-0h fresh/72h aged samples were diluted by a factor of 2 with water, bidistilled grade, to be in the linear range of the calibration curve. The other samples were not diluted. All samples were measured by ICP-MS.

Analysis by ICP-MS

ICP system:	Agilent 7700x with autosampler
Carrier Gas:	Argon
Flow of Carrier Gas:	0.99 L/min
Tune Step:	hehe
Oxide rate:	< 2 %
Nebulizer Pump:	0.1 rps

Detection parameters for ICP-MS experiments:

Compound	Isotope (m/z)
Niobium	93 (quantifier)

Within the sequence, the detector linearity was confirmed over the calibration range of interest by constructing a calibration function within the range from 0.1 mg/L to 40 mg/L niobium.

Calculation of Concentrations

The concentrations of niobium were calculated according to the following equation by reference to the mean response as follows:

$$C = \frac{c_{\text{sample}} \cdot f}{1000}$$

C concentration in the sample (mg/L)

c_{sample} analyzed concentration of the sample, as calculated from the calibration function ($\mu\text{g/L}$)

f dilution factor

1000 conversion factor from $\mu\text{g/L}$ to mg/L

Method Validation

The method was fully validated according to guideline SANCO/3029/99 rev. 4.

Recovery and Repeatability

Recovery samples were prepared by fortification of medium with the test item and niobium reference item, respectively, as follows.

For low recovery 2 mL of a 2.002 mg/L niobium stock solution in 1 % nitric acid were given into a tube. The tube was filled up to 10 mL with medium and shaken well.

For high recovery about 500 mg test item were weighed into a 50 mL tube. The tube was filled up to the mark with water, bidistilled grade, and shaken well. 0.37 mL of this stock solution were given into a 50 mL tube. The tube was filled up to the mark with medium and shaken well.

1 mL of the recovery samples was given into a microwave vessel. Then 10 mL of 69 % nitric acid were added and the sample was digested for about 45 min in a microwave oven with a temperature profile (120 – 210 °C). The whole sample was quantitatively transferred into a 50 mL tube with water. Low recovery samples were not diluted but high recovery samples were diluted by a factor of 10 with water, bidistilled grade, to be in the linear range of the calibration curve. All samples were measured by ICP-MS.

Table 13: Recovery of niobium from test item / niobium reference item spiked into medium

Nominal test item (mg/L)	Nominal niobium (mg/L)	Found niobium (mg/L)	Recovery (%)	Mean Recovery ± RSD (%)
2.33	0.400	0.412	103	104 ± 2
		0.410	103	
		0.414	104	
		0.419	105	
		0.429	107	
73.9	12.7	10.1	80	80 ± 3
		9.92	78	
		9.95	78	
		10.3	81	
		10.5	83	

Mean recoveries and relative standard deviations per fortification fulfil the criteria of guideline SANCO/3029/99 (70 – 110 % mean recovery, ≤ 20 % RSD).

Limit of Quantification, Limit of Detection and Blanks

The limit of quantification (LOQ) was defined as the lowest fortification level with mean recoveries ranging from 70 % to 110 % at a relative standard deviation (RSD) of ≤ 20 %. These criteria were fulfilled for the 0.400 mg/L niobium fortification level.

The limit of detection (LOD) was defined as 30 % of the limit of quantification (= 0.120 mg/L of niobium).

Residues of niobium in the medium used for recovery samples were below 30 % of LOQ.

Linearity

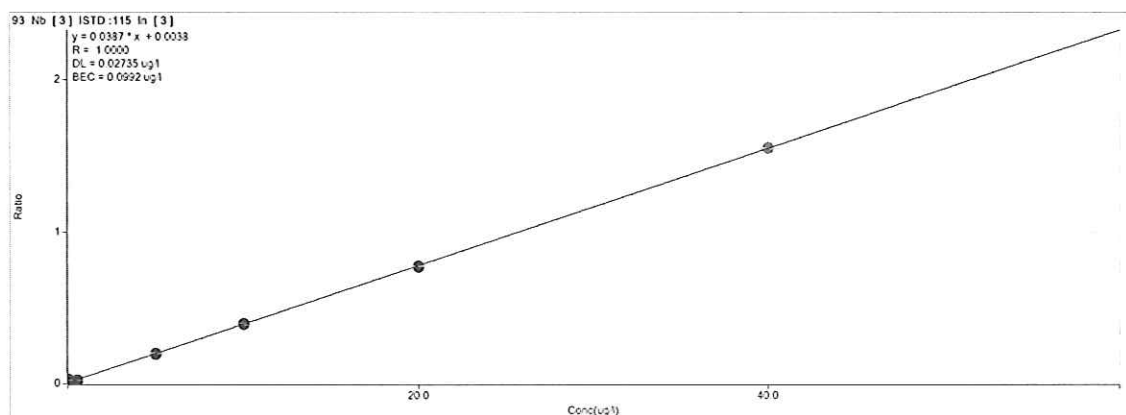
The detector response for ICP-MS analysis was linear within the range from 0.1 $\mu\text{g/L}$ to 40 $\mu\text{g/L}$ of niobium with $r = 1.0000$ (see Figure 3).

Indium was used as internal standard in every measured sample (115 m/z).

Specificity

A highly specific detection system was used (MS). Niobium was identified by mass to charge ratio (m/z) in comparison with a certified reference item. No relevant interferences occurred at the response of niobium. The analytical method can therefore be regarded as highly specific and selective for niobium.

Calibration Data



Nominal concentration (µg/L)	Ratio	Calculated concentration (µg/L)
0*	0.003837	0
0.100	0.007209	0.0872
0.501	0.021076	0.446
1.00	0.023824	1.13
5.01	0.197142	5.00
10.0	0.392620	10.1
20.0	0.775232	19.9
40.0	1.551754	40.0

*water with 1 % nitric acid.

Figure 3: Typical calibration data for analysis of niobium by ICP-MS

Spectra

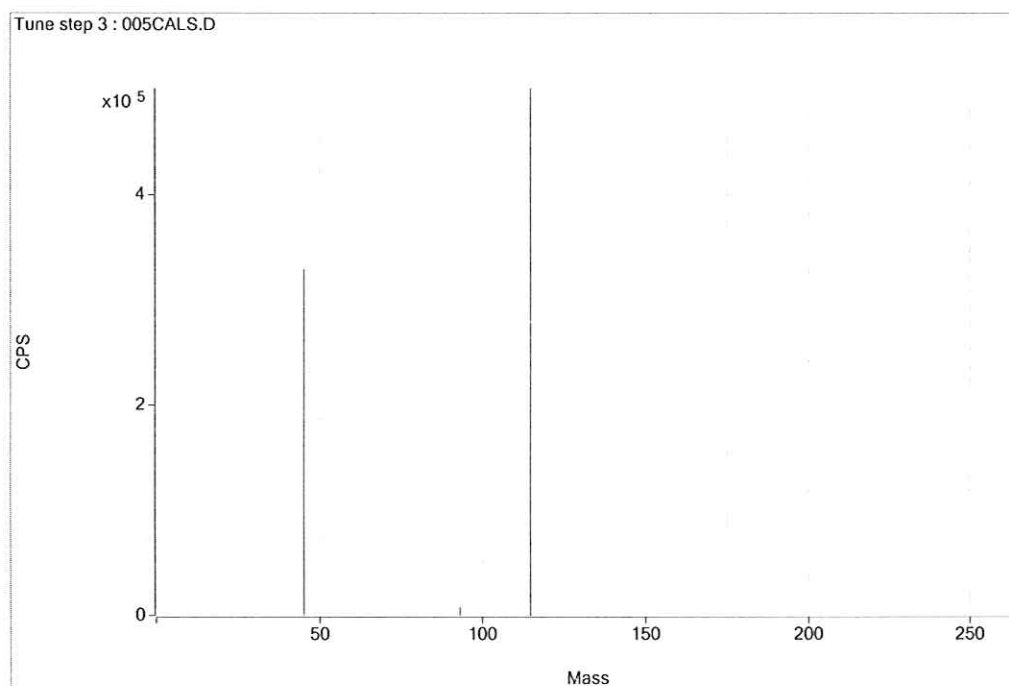
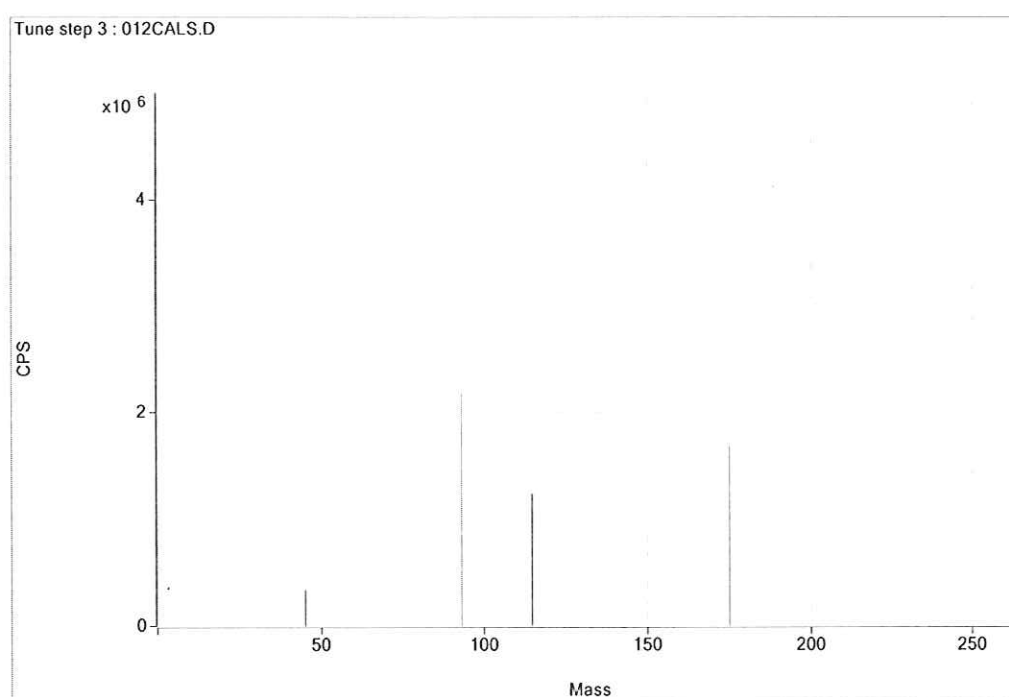


Figure 4: Typical spectrum of a 0.1 µg/L niobium standard with the internal standards scandium, indium and lutetium



Ammonium Niobium
Oxalate

Final Report

S12-03660

Figure 5: Typical spectrum of a 40 µg/L niobium standard with the internal standards scandium, indium and lutetium

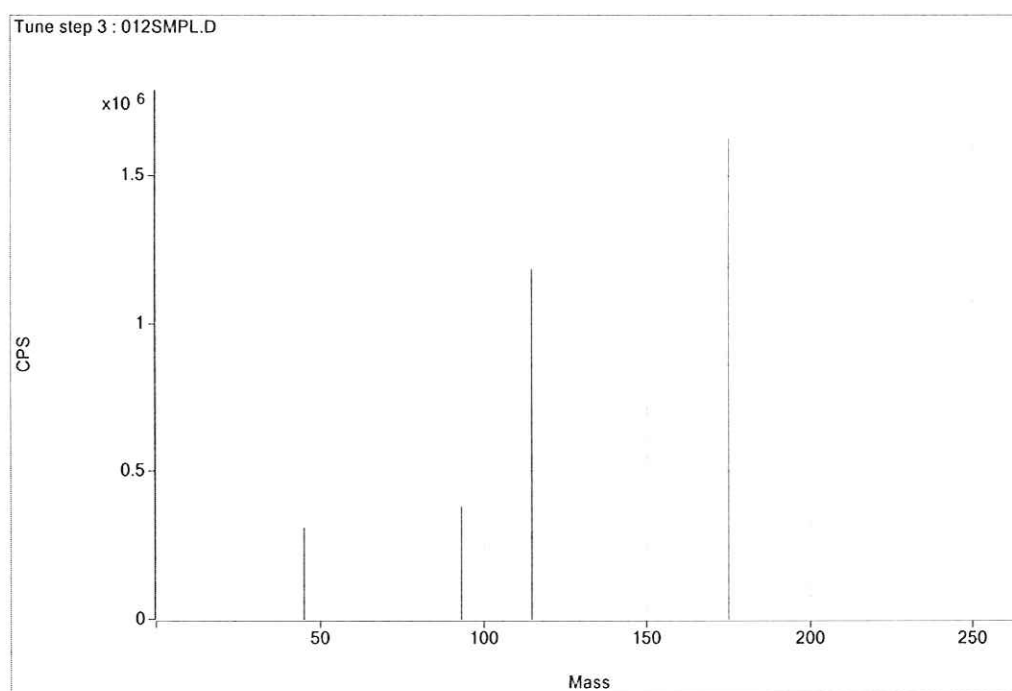


Figure 6: Typical spectrum of a recovery sample (fortification level 2.33 mg/L test item in medium; dilution factor 50)

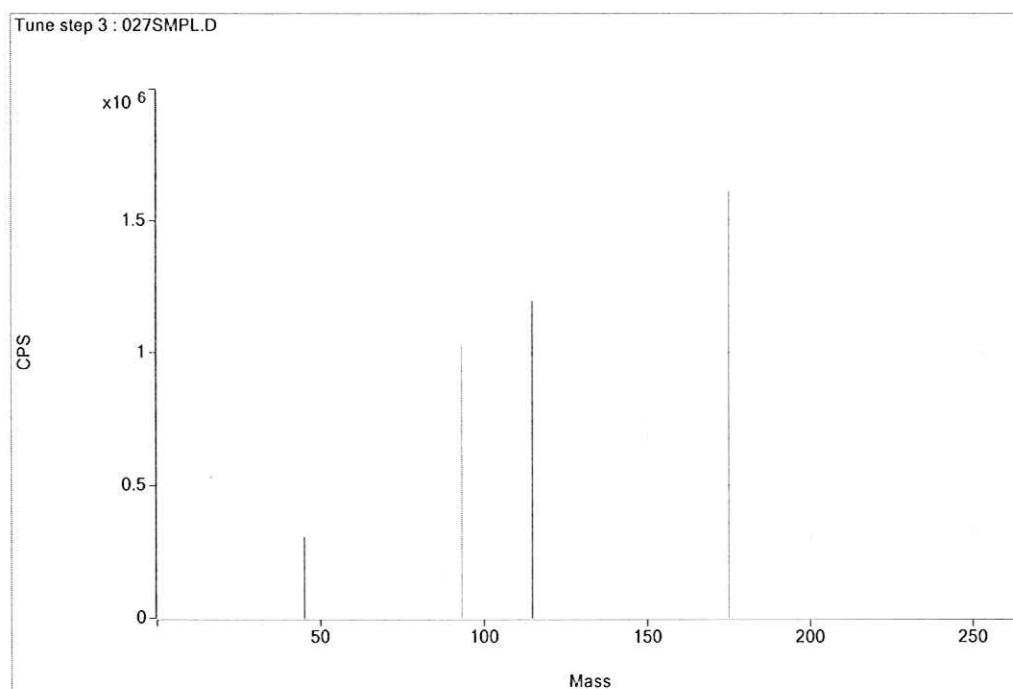


Figure 7: Typical spectrum of a recovery sample (fortification level 73.9 mg/L test item in medium; dilution factor 500)

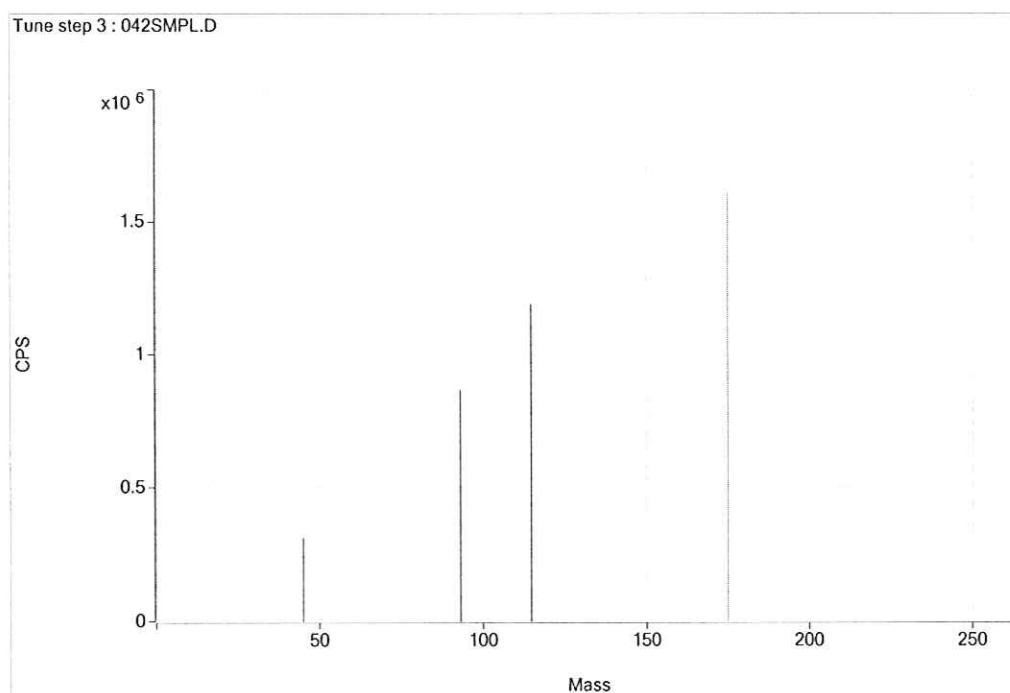


Figure 8: Typical spectrum of a sample (50 mg/L-0h fresh; dilution factor 525)

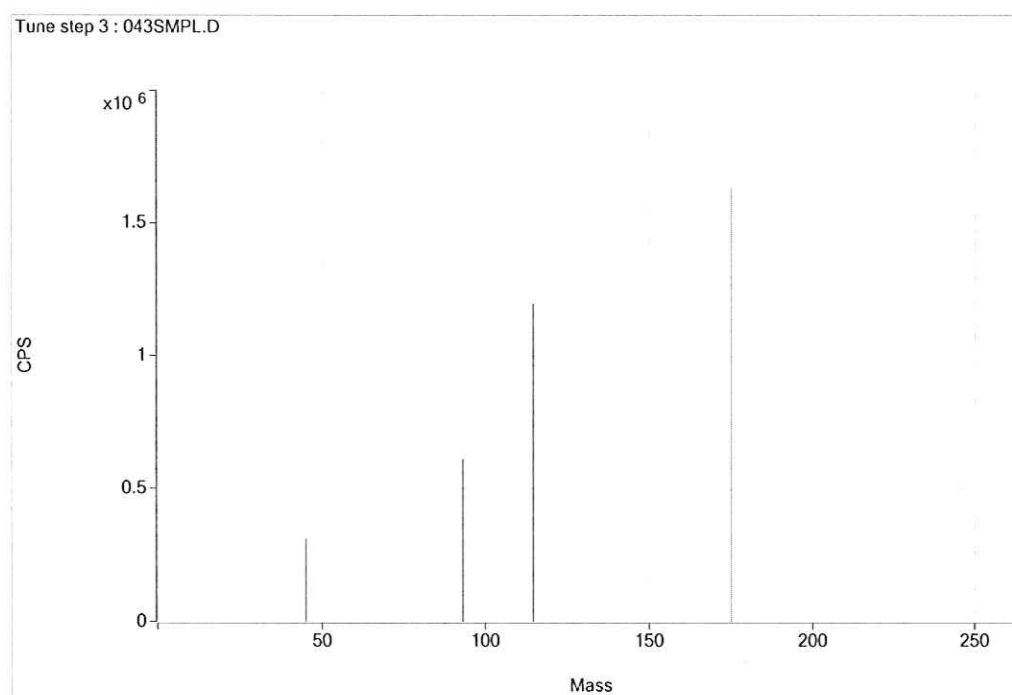


Figure 9: Typical spectrum of a sample (50 mg/L-72h aged; dilution factor 525)

A 7 Certificates



1. Substance identity of ANO (CBMM)

The substance ANO (Ammonium Niobium Oxalate, Sponsor CBMM) was examined. The following data according substance identity have to be indicated on "test item" in the study reports.

Test item: ANO (common name)

Batch / Lot number: AD/4663

Chemical name: Reaction mass of ammonium diaqua[bis(oxalate)]oxoniobate(1-) hydrate and ammonium hydrogen oxalate oxalic acid (1:1:1) dehydrate

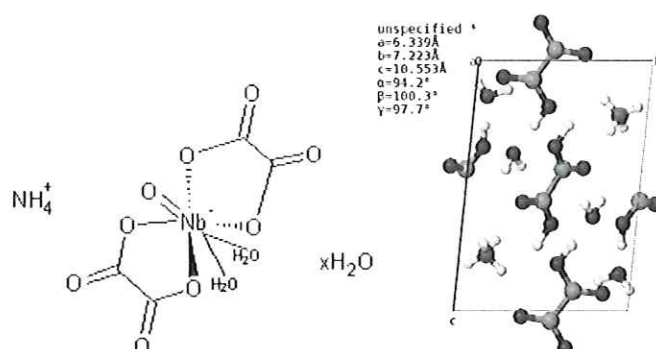
Type of substance: Multi-constituent substance

Purity: $\geq 96\%$

Molecular weight range: 339.012 - 446.261

The reported molecular weight (MW) is indicated for the reaction mass, of which the constituent 1 contains crystal water x ranged from 0 to 8 ($\text{NH}_4[\text{NbO}(\text{C}_2\text{O}_4)_2 \cdot 2\text{H}_2\text{O}] \cdot x\text{H}_2\text{O}$). Also the MW of 339.012 refers to the constituent 1 ($x=0$) and 446.261 to the constituent 2.

Structural formula:



Main constituents:

Ca. 70% (68-74% (w/w)) constituent 1: $(\text{NH}_4[\text{NbO}(\text{C}_2\text{O}_4)_2 \cdot 2\text{H}_2\text{O}] \cdot x\text{H}_2\text{O})$; $x=0-8$

16.09.2013

or 3-noc-16-09-2013

1

Figure 10: Substance identity of ANO Ammonium Niobium Oxalate



Ca. 27% (24-28% (w/w)) constituent 2: $(\text{NH}_4(\text{C}_2\text{HO}_4) \cdot (\text{C}_2\text{H}_2\text{O}_4) \cdot 2(\text{H}_2\text{O}))$

Impurities:

Ca. 2.5% (1-3% (w/w)) free water

Ca. 0.5% (0.1-1% (w/w)) organic and inorganic impurities (Na, K, Cl and SO_4 , as well as possible small quantity of reaction residue of oxalate and ammonium)

Constituent 1 $(\text{NH}_4[\text{NbO}(\text{C}_2\text{O}_4)_2 \cdot 2\text{H}_2\text{O}] \cdot x\text{H}_2\text{O})$; $x=0-8$

IUPAC name: Ammonium oxobis(ethanedioato) bisniobate(V) hydrates

Molecular formula: $\text{C}_4\text{H}_8\text{NNbO}_{11} \cdot x\text{H}_2\text{O}$, $x=0-8$

Molecular weight range: 339.012 - 483.134 (MW range is calculated for crystal water range $x=0-8$)

Constituent 2 $(\text{NH}_4(\text{C}_2\text{HO}_4) \cdot (\text{C}_2\text{H}_2\text{O}_4) \cdot 2\text{H}_2\text{O})$

IUPAC name: Ammonium hydrogen ethanedioate ethanedioic acid dehydrate

Molecular formula: $\text{C}_8\text{H}_9\text{N}_2\text{O}_{16} \cdot 4\text{H}_2\text{O}$

Molecular weight: 466.261

Appearance: white powder

CERTIFICATE OF ANALYSIS

Sample: **ANO**

Active ingredient: Ammonium Niobium Oxalate

Batch No.: AD/4663

Sponsor: CBMM Europe BV

Analysis date: 04 November 2013

Expiration date: 25 March 2015*

Assay: The content of ANO was determined as Niobium by ICP-MS. Niobium was quantified using a certified reference item as external standard and as internal standard Indium was used. The mass of 93 (Niobium) was used for quantification.

This study has been performed in compliance with the principles of Good Laboratory Practice.

The determination of the active ingredient is given in study S13-04815.

Result: **ANO: 99.4 % (w/w)**
(Mean of 6 determinations, RSD: 1.30 %)

*: according to sponsor's information

Niefern, March 27, 2014



Christina Wild, M.Sc.

Managing Director:
Dr. Martin Feyerabend
Nikolaus Kögler

Registered office: Niefern
District court Mannheim
HRB 500704

Bank Details:

Nord-LB
Bank Code: 250 500 00
Account No.: 150 778 512
Swift-Code: NOLADE2HXXX
IBAN Code: DE61 2505 0000 0150 77 65 12
VAT No.: DE 144 196 150

Eurofins Agrosience Services EcoChem GmbH

Eutinger Strasse 24
D-75223 Niefern-Öschelbronn
Fon: +49 (0) 7233 / 9627-0
Fax: +49 (0) 7233 / 9627-680
Email: info_niefern@eurofins.com

<http://www.eurofins.com/agrosienceservices>

Figure 11: Certificate of analysis of the test item



Certificate of Analysis

Certipur® Reference Material

Niobium ICP Standard 1000 mg/l Nb

1.70337.0100

Lot No.: HC242206

This Certificate of Analysis is based on the data from the accredited Merck Calibration Laboratory for ICP-OES, according to DIN EN ISO / IEC 17025.

Composition: Ammonium hexafluoro niobate in water

Assay: 1001 mg/kg
1001 mg/l (calculated)

Analysis: ICP-OES

Measurement $\pm 5 \text{ mg/kg } (\pm 0.5\%)$

Uncertainty: This value represents the expanded uncertainty (*U*) for a coverage probability of 95%. Refer to page 2 for further details.

Traceability: This ICP Standard has been measured applying high precision ICP-OES
And is directly traceable to the NIST SRM® 3137, lot 080502

Trace impurities µg/ml:

Ag <0.02	Cr <0.02	In <0.02	Ni <0.02	Sb <0.02	Ti <0.02
Al <0.50	Cu <0.02	Ir <0.02	Os <0.20	Sc <0.02	Tm <0.02
As <0.20	Dy <0.02	K <0.20	P <0.20	Se <0.20	U <0.02
Au <0.02	Er <0.02	La <0.02	Pb <0.05	Si <0.30	V <0.02
B <0.05	Eu <0.02	Li <0.02	Pd <0.02	Sm <0.02	W <0.20
Ba <0.02	Fe <0.05	Lu <0.02	Pr <0.02	Sn <0.02	Y <0.02
Be <0.02	Ga <0.02	Mg <0.02	Pl <0.02	Sr <0.02	Yb <0.02
Bi <0.20	Gd <0.02	Mn <0.02	Rb <0.02	Ta <0.10	Zn <0.02
Ca <0.10	Ge <0.02	Mo <0.02	Re <0.02	Tb <0.02	Zr <0.02
Cd <0.02	Hf <0.05	Na <0.10	Rh <0.02	Te <0.20	
Ce <0.02	Hg <0.02	Nb *	Ru <0.02	Th <0.02	
Co <0.02	Ho <0.02	Nd <0.02	S <0.20	Ti <0.05	

Date of release: 2012-01-17

Minimum shelf life: 2015-01-31

A. Yildirim

Dipl.-Ing. Ayfer Yildirim
(responsible laboratory manager quality control)

Merck KGaA · 64271 Darmstadt, Germany · Tel.: +49 (0) 6151 72 0
EMD Chemicals Inc., One Int. Plaza, Suite 300 · Philadelphia, PA 19113, USA, Tel. 1-888-367-3275

Figure 12: Certificate of analysis of niobium reference item

Ammonium Niobium
Oxalate

Final Report

S12-03660



Baden-Württemberg

LANDESANSTALT FÜR UMWELT, MESSUNGEN UND NATURSCHUTZ BADEN-WÜRTTEMBERG

Gute Laborpraxis / Good Laboratory Practice

GLP-Bescheinigung / Statement of GLP Compliance

(gemäß / according to § 19 b Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in: Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EC at:

☒ Prüfeinrichtung / Test facility

☐ Prüfstandort / Test site

Eurofins Agrosience Services EcoChem GmbH

Eutinger Straße 24

75223 Niefern-Öschelbronn

(Unverwechselbare Bezeichnung und Adresse / Unequivocal name and address)

Prüfungen nach Kategorien / Areas of Expertise

(gemäß / according to ChemVwV-GLP Nr. 5.3 / OECD guidance)

1 Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften	Physical-chemical testing
4 Ökotoxikologische Prüfungen zur Bestimmung der Auswirkungen auf aquatische und terrestrische Organismen	Environmental toxicity studies on aquatic and terrestrial organisms
5 Prüfungen zum Verhalten im Boden, im Wasser und in der Luft; Prüfungen zur Bioakkumulation und zur Metabolisierung	Studies on behavior in water, soil and air; bioaccumulation
6 Prüfungen zur Bestimmung von Rückständen	Residue studies
7 Prüfungen zur Bestimmung der Auswirkungen auf Mesokosmen und natürliche Ökosysteme	Studies on effects on mesocosms and natural ecosystems
8 Analytische Prüfungen an biologischen Materialien	Analytical and clinical chemistry testing

Datum der Inspektion / Date of Inspection

(Tag Monat Jahr / day month year)

10.10.2013

Die/Der genannte Prüfeinrichtung/Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

The above mentioned test facility/test site is included in the national GLP Compliance Programme and is inspected on a regular basis.

Based on the inspection report it can be confirmed, that this test facility/test site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Unterschrift / Datum / Signature / Date

Volker Giraud

Dr. Volker Giraud

Leiter der Abteilung Technischer Umweltschutz



Karlsruhe, 08.01.2014

(Name und Funktion der verantwortlichen Person / Name and function of responsible person)

LUBW Landesanstalt für Umwelt, Messungen und Naturschutz Baden-Württemberg

Postfach 10 01 63, 76231 Karlsruhe

(Name und Adresse der GLP-Überwachungsbehörde / Name and address of GLP Monitoring Authority)

Figure 13: GLP Certificate of test facility