Final Report

Ammonium Niobium Oxalate - Assessment of Toxic Effects on *Daphnia magna* Using the 48 h Acute Immobilisation Test

Guidelines

OECD 202 (2004)

Study Director

Claudia Zawadsky, Dipl.-Ing. (FH)

Date

21 May 2014

Testing Facility

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Sponsor

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Study Identification Code

Test item:	Ammonium Niobium Oxalate
Study code:	S12-03659
Trial/Lab Phase code:	S12-03659-L1_AADm

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, Federal Republic of 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) 22 May 14 Date / Signature

Study director
(Dipl.-Ing. (FH) Claudia Zawadsky) 21 May 2014 Date / Signature

Statement of Quality Assurance Unit

Study code: S12-03659

Study title: Ammonium Niobium Oxalate - Assessment of Toxic Effects on *Daphnia magna* Using the 48 h Acute Immobilisation Test

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		09 Nov 2012	-	09 Nov 2012	09 Nov 2012
Amendment 1		15 Jan 2014	-	15 Jan 2014	15 Jan 2014
Amendment 2		20 Feb 2014	-	20 Feb 2014	20 Feb 2014
Experimental Phase	Preparation: Standard Solutions	22 Jan 2014	-	22 Jan 2014	22 Jan 2014
Final Report	Analytical Part	07 Apr 2014	-	07 Apr 2014	07 Apr 2014
Final Report	Biological Part	28 Apr 2014	-	28 Apr 2014	28 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
(Jürgen Schmidt)

22 May 2014 / [signature]
Date / Signature

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1 Summary

Report: ZAWADSKY, C. (2014): Ammonium Niobium Oxalate - Assessment of Toxic Effects on *Daphnia magna* Using the 48 h Acute Immobilisation Test.

Source: Eurofins Agroscience Services EcoChem GmbH, Eutinger Str. 24, D-75223 Niefern-Öschelbronn, Germany. Unpublished report No.: S12-03659. Issued: 21 May 2014.

Guidelines: OECD 202 (2004).

Deviations: None.

GLP: Yes (certified laboratory).

Study Objective: The toxicity of Ammonium Niobium Oxalate on *Daphnia magna* was tested in a 48 hour acute immobilisation test. The test was performed according to OECD Guideline 202.

Material and methods: Test item Ammonium Niobium Oxalate (ANO), Batch number: AD/4663, Content of ANO (analysed): 99.4 % (w/w). Test species: *Daphnia magna* Straus, Clone V, max. 24 hours old. Twenty organisms per test concentration (4 replicates of 5 animals each) were used. The duration of the test was 48 hours. Following a static range-finding test with concentrations of 0, 0.01, 0.1, 1.00, 10.0 and 100 mg/L a semi-static main test with concentrations of 0, 0.842, 2.19, 5.69, 14.8, 38.5 and 100 mg/L was performed. Test solutions were prepared by dilution of the test item in test medium and application of defined volumes of the test solutions to the test vessels. Endpoints reported are the EC_{50} and the NOEC after 24 and 48 hours. Temperature, pH-value and oxygen concentration of the test solutions measured after 0, 24 and 48 hours are reported. Hardness of the test water was measured on the day of application. Analytical samples taken at 0 hours (initial value) from fresh test solutions and after 24 hours from aged and fresh test solutions were analysed.

Dates of work: 04 Nov 2013 – 07 Mar 2014

Findings: The initial measured concentrations in the test item solutions ranged from 84 to 113 % of nominal with a mean initial concentration at 101 % of nominal. The measured concentrations in the aged test item solutions ranged from 11 to 45 % of nominal with a mean concentration at 25 % of nominal. Toxicological endpoints were therefore evaluated using nominal concentrations and actual concentrations based on the geometric means of the analysed concentration levels.

Table 1: EC_x and NOEC-values of daphnids exposed to the test item evaluated using nominal concentrations

Endpoint	Ammonium Niobium Oxalate [mg/L]	
	24 h	48h
NOEC	100	100
EC_{50}	> 100	> 100
95 % confidence limit of EC_{50}	-	-

- not applicable

Table 2: EC_x and NOEC-values of daphnids exposed to the test item evaluated using actual concentrations

Endpoint	Ammonium Niobium Oxalate [mg/L]	
	24 h	48h
NOEC	34.0	34.0
EC_{50}	> 34.0	> 34.0
95 % confidence limit of EC_{50}	-	-

- not applicable

The total hardness (as $CaCO_3$) of the untreated control was determined to be 10°dH (179 mg/L $CaCO_3$); the mean pH-value of the untreated control was determined to be 8.04 ± 0.23 (Std. Dev.), the mean temperature of the control and all test item concentrations was measured to be 19.6 ± 0.3 °C (Std. Dev.) and the mean oxygen concentration was determined to be 8.9 ± 0.2 mg/L (Std. Dev.).

Conclusions: According to the results of the test, the **EC_{50} (48 h)** for immobilisation was supposed to be **> 100 mg/L (nominal)** and **> 34.0 mg/L (actual).** The corresponding **NOEC (48 h)** was **100 mg/L (nominal)** and **34.0 mg/L (actual)** of test item.

2 Time Schedule

Study initiation date:	09 Jul 2013
Start of the experimental phase:	04 Nov 2013
End of the experimental phase (biological part):	07 Mar 2014
End of the experimental phase (analytical part):	07 Mar 2014
Draft report (biological part):	01 Apr 2014
Draft report (analytical part):	21 Mar 2014
Study completion date:	21 May 2014

3 Study Objective

The immobilisation effect of Ammonium Niobium Oxalate on *Daphnia magna* was tested in a 48 hour acute immobilisation test. The test was performed according to OECD Guideline 202 (2004).

4 Material and Methods

4.1 Test / Reference Item(s)

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

Reference Item			
Name / Code	potassium dichromate	Batch number	112403J
Test item code	2012-004778	Appearance / colour	solid / orange
CAS number	7778-50-9	Purity analysed	99.95 % w/w
Density	2.69 g/cm^3	Risk symbol(s)	T+, N
Certificate of analysis	28 June 2011	Expiry date	30 June 2016
Stability in application solution	sufficient for the test purpose (at least 1h)	Storage conditions	room temperature (15 to 25 °C)

All specifications given on the certificate of analysis, provided by the sponsor/supplier, are essential for correct identification of the test item for use under GLP. They have not been verified by the test facility and no claim of GLP compliance will be made for these data except where this is explicitly claimed on the certificate of analysis. Additional specifications for test item characterisation may originate from (non-GLP) sources other than the sponsor/supplier.

4.2 Test Organisms

Daphnia magna STRAUS, Clone V, was used as the test organism. The animals are continuously bred in the laboratory and were originally purchased in a healthy condition from the Umweltbundesamt (Federal Environment Agency) in Berlin/Germany.

Daphnia magna was reared as single culture where one daphnid is kept per 100 mL. The pH-value of the aerated water was within a range of 6.0 – 9.0. The dissolved oxygen was above 60 % saturation and the total hardness 140 - 250 mg/L (as CaCO3), corresponding to 7.8 - 14°dH. The animals were fed with single cell green algae (*Desmodesmus subspicatus*, formerly *Scenedesmus subspicatus*) at least three times a week.

The daphnids were reared at a temperature of 20 ± 2 °C in a climatic chamber with 16 hours of illumination and 8 hours of darkness. The medium was changed three times per week. A pipette was used to separate the young daphnids from the adults.

Freshly hatched daphnids less than 24 hours old were used for the test.

4.3 Test Medium

The test medium was water composed of dechlorinated drinking water and deionised water. At test initiation the pH-value of the control (untreated test medium) was 7.89, the dissolved oxygen was 8.6 mg/L and the total hardness was 10°dH (179 mg/L as $CaCO_3$).

4.4 Description of Test Method

4.4.1 Performance of the Test and Test Design

The daphnids were exposed to increasing concentrations of the test item for 48 hours. Two concentrations of the reference item potassium dichromate (1.0 mg/L, 2.0 mg/L) and a control were tested as well.

4.4.2 Preparation of Test Solutions

The concentrations in the main test were spaced by a factor of 2.6. The large spacing factor of 2.6 was selected since a flat dose response relationship was observed in previous tests. The necessary amount of Ammonium Niobium Oxalate for preparing the stock solution was weighed on weighing scoops and transferred to a volumetric flask. Test medium (see 0) was added up to the bench mark and the solution was homogenised by shaking. The lower test solutions were prepared by dilution of the stock solution. Defined volumes of the prepared solution were transferred to each test vessels. The test solution volume was 50 mL per test vessel (see Table 3). All test vessels were saturated with test item prior to test start.

Table 3: Stock solution for application in the main test

Target concentration	Test item (required)	Dilution solution		Final volume /amount	Volume per test vessel	Solution
[mg/L]	[mg]	No.	[g]		[mL]	No.
100	100	-	-	1000 mL	50	S1
38.5	-	S1	385.0	1000 g	50	V1
14.8	-	V1	384.4	1000 g	50	V2
5.69	-	V2	384.5	1000 g	50	V3
2.19	-	V3	384.9	1000 g	50	V4
0.842	-	V4	384.5	1000 g	50	V5
0	-	-	-	-	50	Control

4.4.3 Test Conditions

Test procedure:	semi-static
Duration:	48 hours
Temperature:	18.8 – 20.1 °C
Oxygen concentration:	≥ 8.6 mg/L
pH-value:	7.14 – 8.39
Exposure to light:	16 hours photoperiod daily
Feeding:	none
Test vessels:	four 100 mL glass beakers per concentration, each filled with 50 mL
Loading:	10 mL of test solution for each animal
Aeration:	none
Number of animals:	20 per concentration in 4 replicates of 5

4.5 Data

4.5.1 Observations

After 24 h and 48 h the immobilised daphnids were counted. All daphnids not able to swim within 15 seconds after gentle agitation of the test vessel were considered to be immobilised. If present, behavioural changes of daphnids were recorded at 24 and 48 hours after starting the test.

4.5.2 Measurements

The test temperature and the pH-value as well as the oxygen concentration of the test media were measured at all concentrations at t = 0 h (fresh), 24 h (fresh and aged) and 48 h (aged) in one replicate per test item concentration (see Appendix A 1).

4.5.3 Analytical Determinations

Analytical data are required by the guidelines for verification of test item concentrations as well as the stability of the test item over the entire test period.

Analytical samples were taken from all test concentrations and control(s) at test start, after 24 and 48 hours.

The control and all test item concentrations were analysed at t = 0 hours from fresh test solutions and at t = 24 hours from aged and fresh test solutions.

The analytical sampling for the verification of the test concentrations from test solutions was performed as described in Appendix A 2.

4.6 Chemical Analysis

The analysis of samples from the test solutions was performed at the testing facility. 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 the analyte 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 given in Appendix A 2.

4.7 Statistical Evaluation of Results

The 24 h and 48 h EC_{50} are the estimated concentrations where 50 % of the daphnids were immobilised after 24 and 48 hours, respectively.

Since no immobilisation above the allowed control immobilisation of 10 % was observed at the highest test item concentration of 100 mg test item/L, no statistical determination was indicated.

The NOEC was established based on the highest concentration at which the immobilisation is not higher than the allowed control immobilisation (≤ 10 % immobilisation).

5 Deviations from the Study Plan

The study was performed according to the study plan dated 09 July 2013, the study plan amendment No. 1 dated 20 January 2014 and the study plan amendment No. 2 dated 25 February 2014 without any deviation.

This report reflects the conduct of the study.

6 Results

6.1 Main Test

After 24 and 48 hours of exposure no immobilisation above the allowed control immobilisation was observed in the control and up to the highest test item concentration of 100 mg/L.

The results are presented in Table 4 and Table 5.

Table 4: Results of the main test, 24 h values

Concentration Test Item	Control	0.842	2.19	5.69	14.8	38.5	100
		[mg/L]					
	immobilised daphnids after 24 h						
Group 1	0	0	1	0	0	0	0
Group 2	0	0	0	0	0	0	1
Group 3	0	0	0	1	0	0	0
Group 4	0	0	0	0	0	0	0
Σ	0	0	1	1	0	0	1
%	0	0	5	5	0	0	5

Table 5: Results of the main test, 48 h values

Concentration Test Item	Control	0.842	2.19	5.69	14.8	38.5	100
		[mg/L]					
	immobilised daphnids after 48 h						
Group 1	0	0	1	0	0	0	0
Group 2	0	0	0	0	0	0	1
Group 3	0	0	0	1	0	0	0
Group 4	0	0	0	0	0	0	0
Σ	0	0	1	1	0	0	1
%	0	0	5	5	0	0	5

After 24 and 48 hours a fine, white precipitate was observed at the test item concentration of 14.8 mg/L. More precipitate was observed at the test item concentration of 38.5 mg/L and at the highest test item concentration of 100 mg/L the bottom of the test vessel was completely covered with precipitate.

6.2 Toxic Reference

In order to check the validity of the results, the toxicity of the reference item potassium dichromate was tested at 1.0 and 2.0 mg/L with 20 test organisms per test concentration. The results are presented in Table 6.

Table 6: Results of the toxic reference test, started on 21 Jan 2014

$K_2Cr_2O_7$ [mg/L]	24 h		48 h	
	1.0	2.0	1.0	2.0
	immobilised daphnids			
Group 1	0	3	1	5
Group 2	0	3	1	5
Group 3	1	3	1	5
Group 4	0	5	1	5
Σ	1	14	4	20
%	5	70	20	100

The results indicate an EC_{50} (24 h) of the reference item potassium dichromate between 1.0 and 2.0 mg/L. Since the results fall within the historical data generated with the reference item at the testing facility and are within the range of 0.6 – 2.1 mg/L recommended by the test guideline OECD 202, the daphnids were suitable for the determination of the toxicological effects of the test item.

7 Analytical Results

The analytical verification of test item concentrations in daphnid test medium was done by analysing the content of Ammonium Niobium Oxalate in the samples during the test. Samples from control and all test item concentrations were analysed at test start t = 0 h from fresh test solutions and after 24 hours from aged and fresh test solutions. The analysed concentrations are presented in Table 7.

Table 7: Determined concentration of Ammonium Niobium Oxalate

Test item nominal [mg/L]	Nb[2] nominal [mg/L]	Sampling	Nb [mg/L]	Nb % of nominal	Nb Geometric mean[1]	Test item actual [mg/L]
0	0	0h fresh 24h fresh 24h aged	n.d. n.d. n.d.	- - -	-	-
0.842	0.145	0h fresh 24h fresh 24h aged	0.147 0.139 0.066	102 96 45	67	0.564
2.19	0.377	0h fresh 24h fresh 24h aged	0.349 0.399 0.136	93 106 36	60	1.31
5.69	0.979	0h fresh 24h fresh 24h aged	0.922 0.987 0.258	94 101 26	50	2.85
14.8	2.55	0h fresh 24h fresh 24h aged	2.70 2.66 0.52	106 104 20	46	6.81
38.5	6.62	0h fresh 24h fresh 24h aged	5.59 7.22 0.71	84 109 11	33	12.7
100	17.2	0h fresh 24h fresh 24h aged	17.3 19.5 1.94	101 113 11	34	34.0
Mean value fresh test solutions				101		
Mean value aged test solutions				25		

- = not calculated; n.d. = not detectable; LOQ = 0.251 mg/L Ammonium Niobium Oxalate corresponding to 0.0432 mg/L Nb

[1] mean values of fresh test solutions and single values of aged test solutions per concentration level were used for calculation of geometric means

[2] based on the analysed content of Nb 17.2 %

The initial measured concentrations in the test item solutions ranged from 84 to 113 % of nominal with a mean initial concentration at 101 % of nominal. The measured concentrations in the aged test item solutions ranged from 11 to 45 % of nominal with a mean concentration at 25 % of nominal.

7.1 Statistical Evaluation

All toxicological endpoints were evaluated using nominal concentrations and actual concentrations based on the geometric means of the analysed concentration levels of the test item (see Table 8 and Table 9).

Table 8: EC_x and NOEC-values of daphnids exposed to the test item evaluated using nominal concentrations

Endpoint	Ammonium Niobium Oxalate [mg/L]	
	24 h	48h
NOEC	100	100
EC_{50}	> 100	> 100
95 % confidence limit of EC_{50}	-	-

- not applicable

Table 9: EC_x and NOEC-values of daphnids exposed to the test item evaluated using actual concentrations

Endpoint	Ammonium Niobium Oxalate [mg/L]	
	24 h	48h
NOEC	34.0	34.0
EC_{50}	> 34.0	> 34.0
95 % confidence limit of EC_{50}	-	-

- not applicable

According to the results of the test, the EC_{50} (48 h) for immobilisation was supposed to be **> 100 mg/L (nominal)** and **> 34.0 mg/L (actual)**. The corresponding NOEC (48 h) was **100 mg/L (nominal)** and **34.0 mg/L (actual)** of test item.

7.2 Validity of the Results

According to the OECD 202 guideline this study can be regarded as valid, since

- in the control not more than 10 % of animals were immobilised
- the dissolved oxygen concentration was ≥ 3 mg/L at the end of the test in control and test vessels

8 Archiving

All data and study documents will be archived in accordance with the SOP's of the Test Facility.

- Archived data and documents will be retained for a period from the issue of the final report, in accordance with the local national regulatory requirements (determined by the country of origin of the Study Director).
- Study specific documents will be stored in the GLP Archives listed below.
- Facility-based records and documentation of QA of the involved test sites will be stored in the respective GLP Archives according to the applicable national regulations.
- A sample of the test item will be stored in the dedicated archive at the test facility at which it is under test.

At least the following documents will be archived:

Document or material	Location of GLP Archive	Original/Copy
Study plan and amendments	Test Facility	Original
Raw data	Test Facility	Original
Final report (and report amendments)	Test Facility	Original

At the end of the archiving period study-specific data or material will NOT be disposed of without the prior written consent of the Sponsor.

9 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 202 (2004): OECD Guidelines for Testing of Chemicals No. 202. *Daphnia* sp., Acute Immobilisation Test. Adopted: 13 April 2004.

10 Appendix

A 1 Temperature, pH-Value and O_2 Concentration

Main Test

The temperature, pH-value and the O_2 concentration of the test solutions of the main test were measured at t = 0, 24 and 48 hours. The results are presented in Table 10 - Table 12.

Table 10: Temperature of the test solutions

	nominal test item concentration [mg/L]						
	Control	0.842	2.19	5.69	14.8	38.5	100
Time [h]	Temperature [°C]						
0 fresh	19.5	19.8	19.8	19.9	20.0	20.0	20.0
24 fresh	19.4	19.6	19.7	19.7	19.4	19.4	19.5
24 aged	19.7	19.6	19.6	19.4	19.3	18.8	18.9
48 aged	20.1	19.8	19.9	19.8	19.7	19.8	19.3
Mean	19.7	19.7	19.8	19.7	19.6	19.5	19.4
Std. dev.	0.3	0.1	0.1	0.2	0.3	0.5	0.5
Mean	19.6						
Std. dev.	0.3						

Table 11: pH-values of the test solutions

	nominal test item concentration [mg/L]						
	Control	0.842	2.19	5.69	14.8	38.5	100
Time [h]	pH						
0 fresh	7.89	7.95	7.96	7.96	7.85	7.79	7.14
24 fresh	7.80	7.84	7.87	7.87	7.84	7.55	7.33
24 aged	8.22	8.37	8.38	8.39	8.39	8.37	8.26
48 aged	8.25	8.34	8.36	8.36	8.37	8.31	8.27
Mean	8.04	8.13	8.14	8.15	8.11	8.01	7.75
Std. dev.	0.23	0.27	0.27	0.27	0.31	0.40	0.60
Mean	8.05						
Std. dev.	0.34						

Table 12: O_2 concentration of the test solutions

	nominal test item concentration [mg/L]						
	Control	0.842	2.19	5.69	14.8	38.5	100
Time [h]	**Oxygen [mg/L]**						
0 fresh	8.6	8.7	8.6	8.6	8.7	8.6	8.6
24 fresh	8.7	8.7	8.7	8.7	8.7	8.7	8.7
24 aged	8.8	8.9	9.0	9.0	9.0	9.0	9.1
48 aged	9.0	9.0	9.1	9.3	9.3	9.2	9.2
Mean	8.8	8.8	8.9	8.9	8.9	8.9	8.9
Std. dev.	0.2	0.2	0.2	0.3	0.3	0.3	0.3
Mean	8.9						
Std. dev.	0.2						

A 2 Analytical Method for the Determination of 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 fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000 and is characterized as follows:

Method principle: Samples were diluted with hydrochloric acid and nitric acid 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 niobium with r = 0.9996 .

Recovery: The recovery was determined by fortification of medium with the test item at the concentration levels given below:

Fortification level test item (mg/L)	Fortification level niobium (mg/L)	n	Mean recovery ± RSD (%)
0.251	0.0432	5	98 ± 1
121	20.8	5	102 ± 1

Repeatability: The relative standard deviation per fortification level is within the guideline requirements (≤ 20 %).

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

LOQ: The limit of quantification was 0.0432 mg/L niobium.

LOD: The limit of detection was defined as 30 % of LOQ (= 0.0130 mg/L niobium).

Test Item

The characterization of the test item is given in Figure 10.

Reference Item

The characterization of the reference item is given in Figure 11. Dilutions for calibration of ICP-MS analysis were prepared in 5 % hydrochloric acid from this stock solution.

Material and Methods

Equipment

Volumetric pipettes (Eppendorf): 0.5-5 mL, 10-100 µL, 100-1000 µL
ICP-MS 7700x (Agilent)

Reagents

Nitric acid, 69 % for trace analysis (Fluka No. 84385)
Hydrochloric acid, 30 % suprapur (Merck 1.00318)
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 at room temperature until analysis. At the analytical laboratory, the samples were shaken well. If necessary the samples were diluted with 2 % hydrochloric acid and 5 % nitric acid prior to analysis 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 µg/L to 40 µg/L niobium.

Calculation of Concentrations

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

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

C concentration in the sample (mg/L)

c_{sample} analyzed concentration of the sample, as calculated from the calibration function (µg/L)

f dilution factor

1000 conversion factor from µg/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 as follows.

For low 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. Afterwards the solution was diluted by a factor of 10 with water, bidistilled grade. 0.125 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. The recovery samples were diluted by a factor of 10 with 2 % hydrochloric acid and 5 % nitric acid and measured by ICP-MS.

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. 0.6 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. The recovery samples were diluted by a factor of 1000 with 2 % hydrochloric acid and 5 % nitric acid and measured by ICP-MS.

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

Nominal test item (mg/L)	Nominal niobium (mg/L)	Found niobium (mg/L)	Recovery (%)	Mean Recovery ± RSD (%)
0.251	0.0432	0.0428	99	98 ± 1
		0.0429	99	
		0.0425	98	
		0.0421	97	
		0.0421	97	
121	20.8	21.4	103	102 ± 1
		21.2	102	
		21.4	103	
		21.5	103	
		21.0	101	

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 0.0432 mg/L niobium fortification level.

The limit of detection (LOD) was defined as 30 % of the limit of quantification (= 0.0130 mg/L of niobium).

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

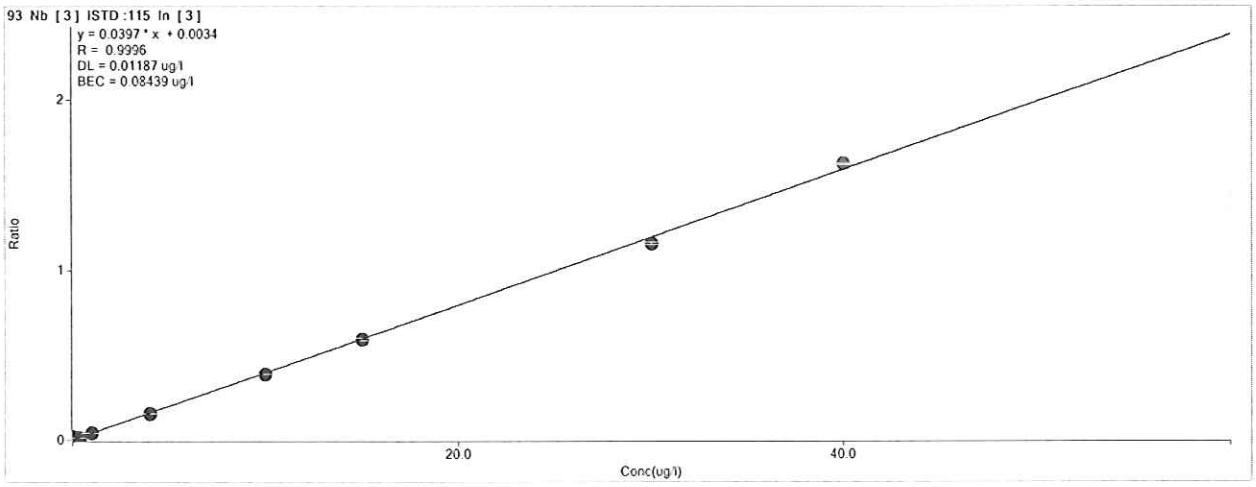
Linearity

The detector response for ICP-MS analysis was linear within the range from 0.1 µg/L to 40 µg/L niobium with r = 0.9996 (see Figure 1).
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*	0.003350	0
0.100	0.006709	0.0846
0.500	0.017288	0.351
1.00	0.040296	0.931
4.00	0.155522	3.83
10.0	0.390775	9.76
15.0	0.595828	14.9
30.0	1.156810	29.1
40.0	1.623875	40.8

* 5 % hydrochloric acid

Figure 1: Typical calibration data for analysis of niobium by ICP-MS

Spectra

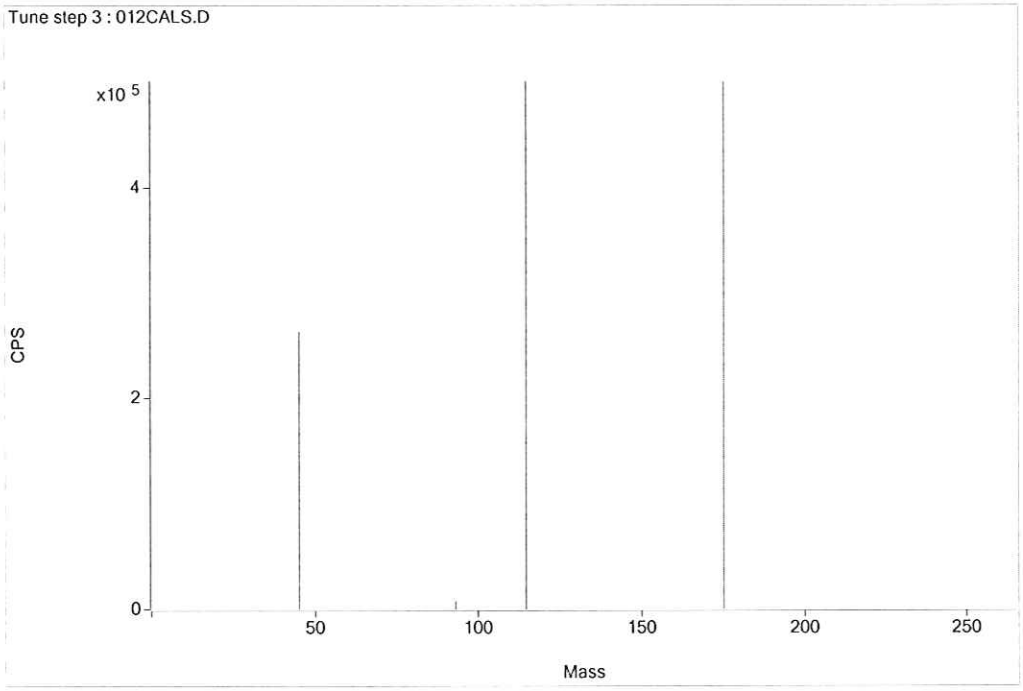


Figure 2: Typical spectrum of a 0.1 µg/L niobium standard with the internal standards scandium, indium and lutetium

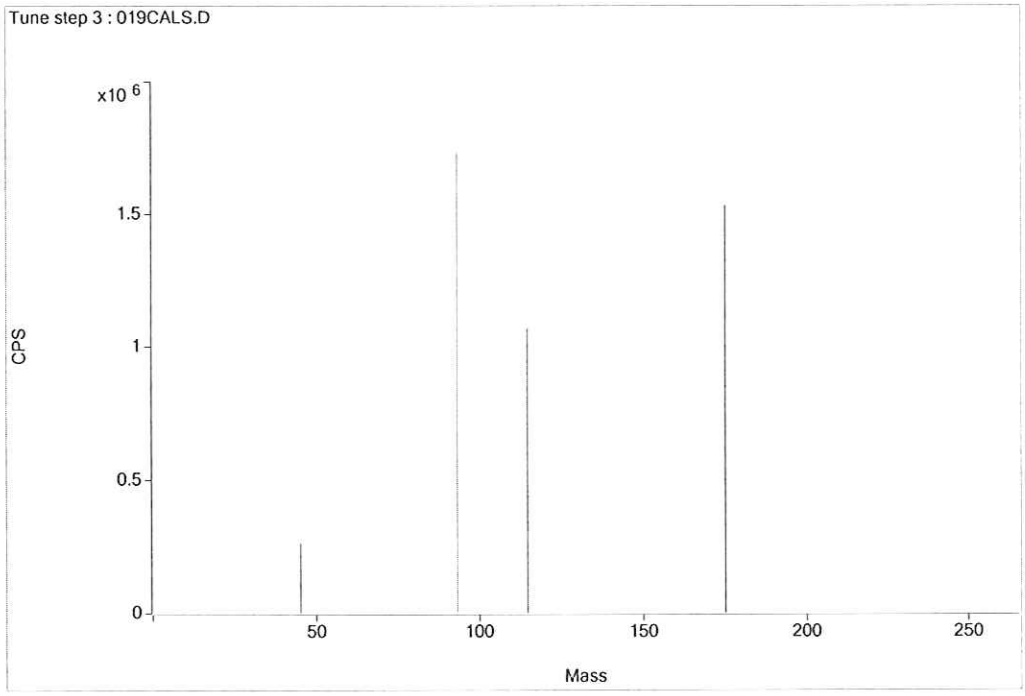


Figure 3: Typical spectrum of a 40 µg/L niobium standard with the internal standards scandium, indium and lutetium

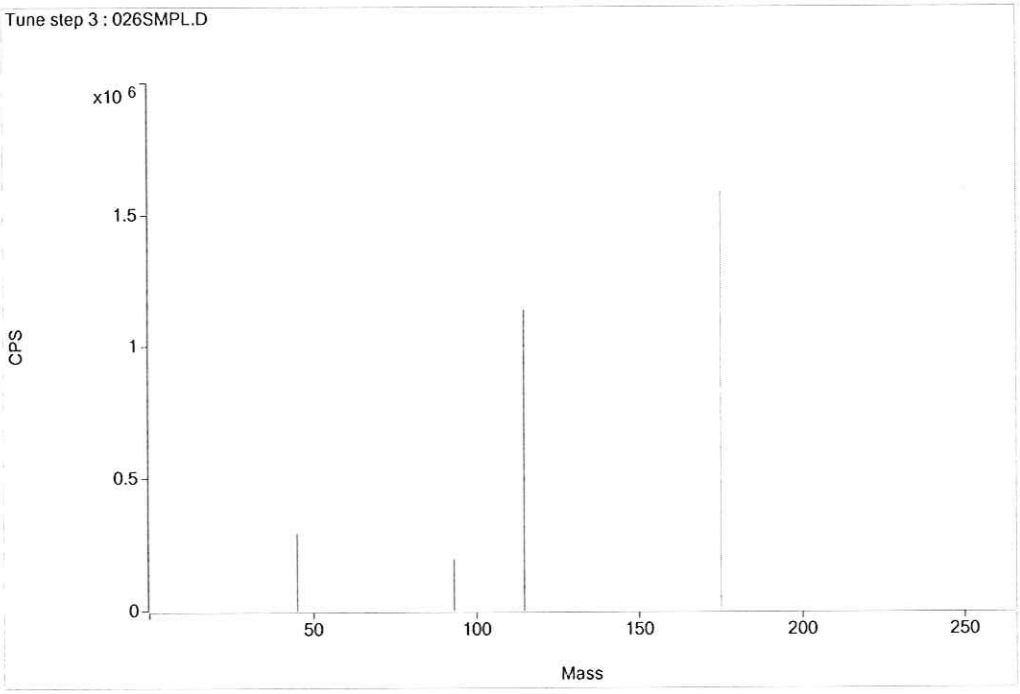


Figure 4: Typical spectrum of a recovery sample (fortification level 0.251 mg/L test item in medium; dilution factor 10)

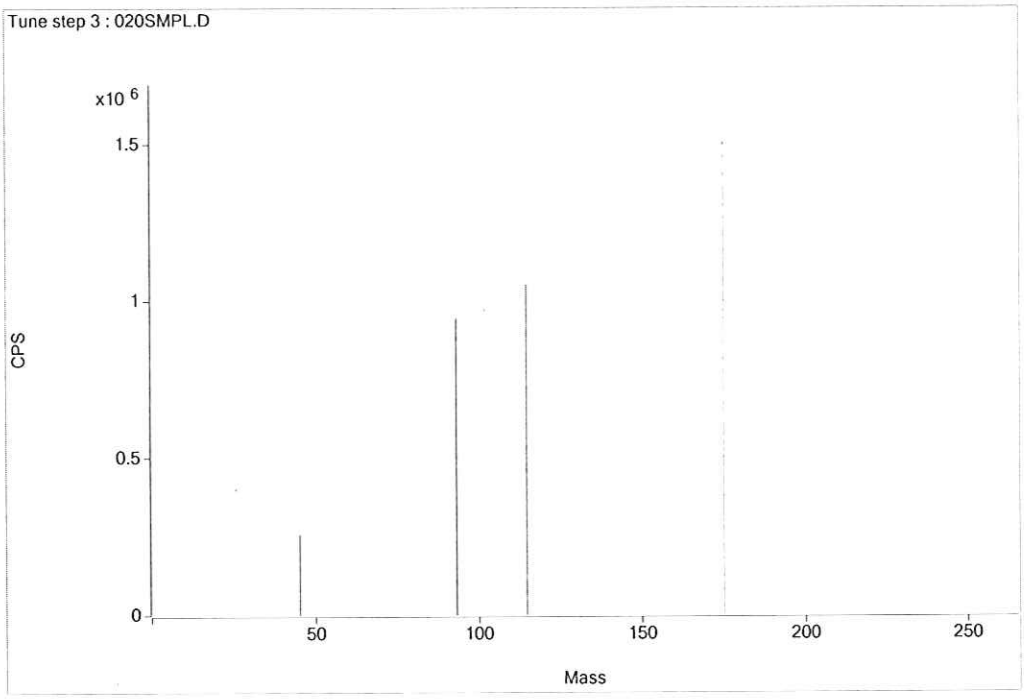


Figure 5: Typical spectrum of a recovery sample (fortification level 121 mg/L test item in medium; dilution factor 1000)

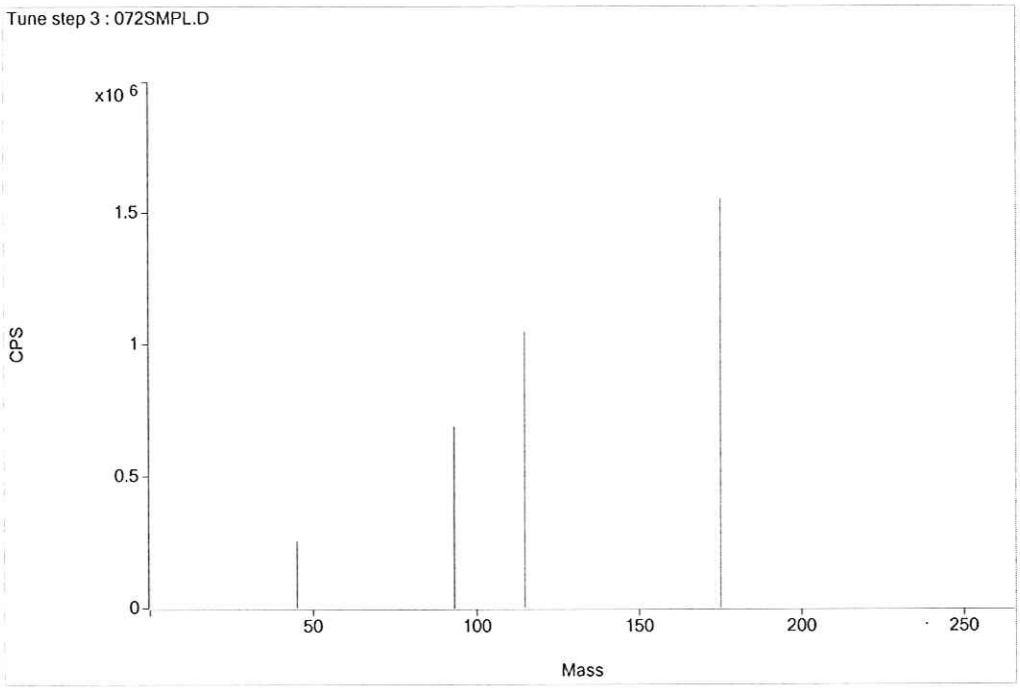


Figure 6: Typical spectrum of a sample (100 mg/L-0h fresh; dilution factor 1000)

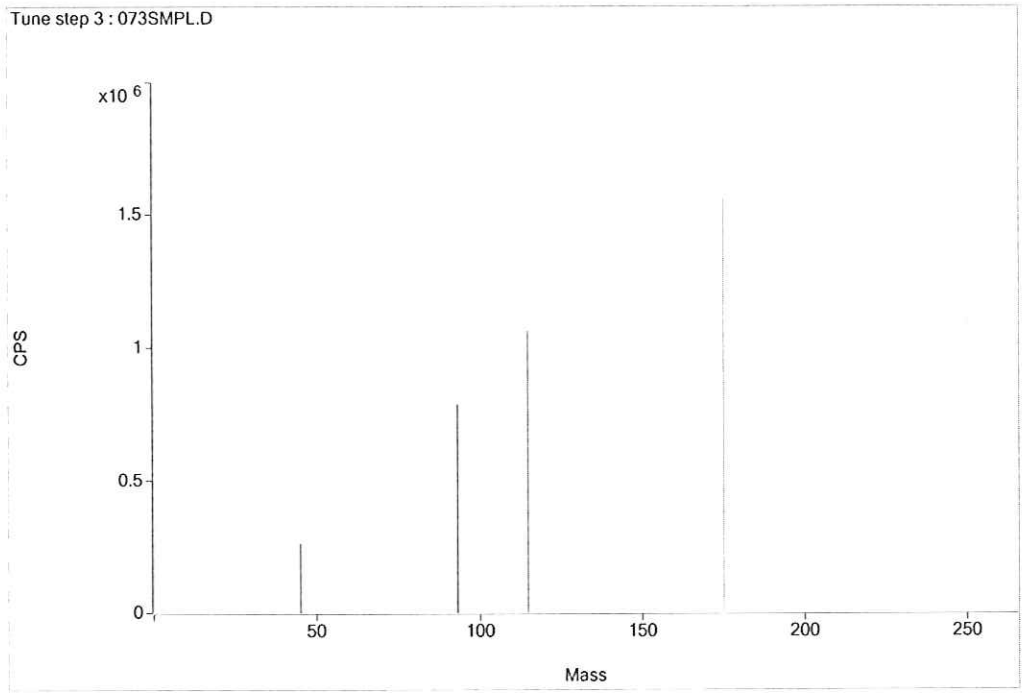


Figure 7: Typical spectrum of a sample (100 mg/L-24h fresh; dilution factor 1000)

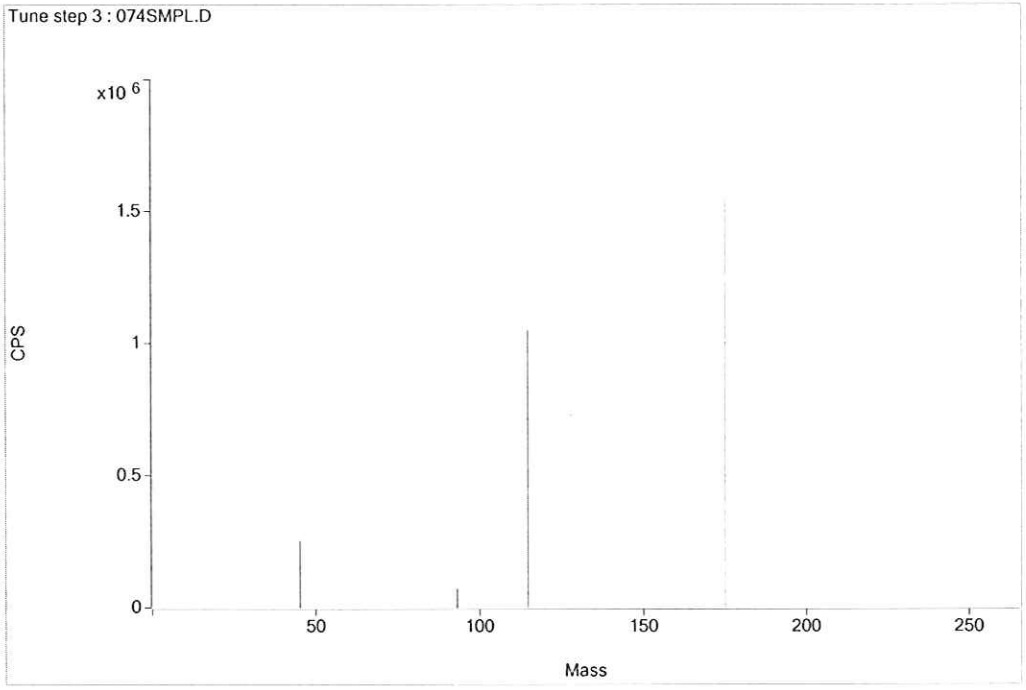


Figure 8: Typical spectrum of a sample (100 mg/L-24h aged; dilution factor 1000)

A 3 Certificates

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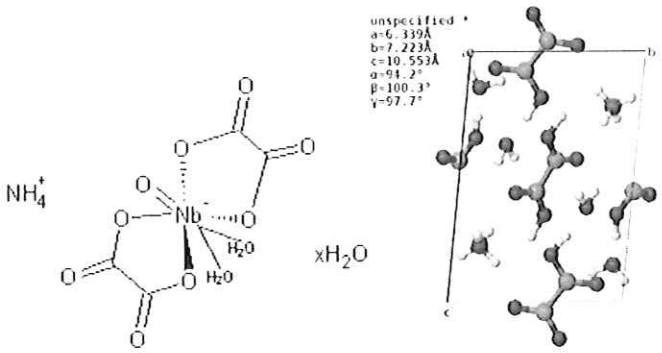
1. **Substance identity of ANO (CBMM)**

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:	≥ 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 ($NH_4[NbO(C_2O_4)_2 \cdot 2H_2O] \cdot xH_2O$). Also the MW of 339.012 refers to the constituent 2.

Structural formula:



Main constituents:

Ca. 70% (68-74% (w/w)) constituent 1: ($NH_4[NbO(C_2O_4)_2 \cdot 2H_2O] \cdot xH_2O$); x=0-8

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Figure 9: Substance identity of ANO Ammonium Niobium Oxalate

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Ca. 27% (24-28% (w/w)) constituent 2: ($NH_4(C_2HO_4) \cdot (C_2H_2O_4) \cdot 2(H_2O)$)

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 SO4, as well as possible small quantity of reaction residue of oxalate and ammonium)

Constituent 1 ($NH_4[NbO(C_2O_4)_2 \cdot 2H_2O] \cdot xH_2O$); x=0-8
IUPAC name: Ammonium oxobis(ethanedioato) bisniobate(V) hydrates
Molecular formula: C4H8NNbO11.xH2O, x= 0 – 8
Molecular weight range: 339.012 - 483.134 (MW range is calculated for crystal water range x= 0-8)

Constituent 2 ($NH_4(C_2HO_4) \cdot (C_2H_2O_4) \cdot 2H_2O$)
IUPAC name: Ammonium hydrogen ethanedioate ethanedioic acid dehydrate
Molecular formula: C8H9N2O16.4H2O
Molecular weight: 466.261

Appearance: white powder

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Figure 9 (continued): Substance identity of ANO Ammonium Niobium Oxalate

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Eurofins Agroscience Services EcoChem GmbH · Eutinger Str. 24 · D-75223 Niefern-Öschelbronn

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 Kügler

Registered office: Niefern
District court Mannheim
HRB 500704

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http://www.eurofins.com/agroscienceservices

Figure 10: 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** **Analysis: ICP-OES**
1001 mg/l (calculated)

Measurement Uncertainty: **± 5 mg/kg (± 0.5%)**
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	Tl	<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	Pt	<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 11: Certificate of analysis of niobium reference item

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 Agroscience Services EcoChem GmbH

Eutinger Straße 24

75223 Niefern-Öschelbronn

(Unverwechselbare Bezeichnung und Adresse / Unequivocal name and adress)

Prüfungen nach Kategorien / Areas of Expertise

(gemäß / according 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 12: GLP Certificate of test facility