FINAL REPORT

Study no.

X02a-130

2 hours Bio-elution Study on

Fe-Cr-Ni Powder Mixture

(68.6/16.4/10.7 mass fraction)

at a *0.2, 1 and 2 g/L loading* in a simulated gastric fluid

December 19, 2017

Statement

Title: 2 hours Bio-elution Study on **Fe-Cr-Ni Powder Mixture** (68.6/16.4/10.7 mass fraction) at a *0.2, 1 and 2 g/L loading* in a simulated gastric fluid.

Study no.: **X02a-130**

The study has been performed and conducted according to the OECD Principles of Good Laboratory Practices for experiments carried out by the *Test Facility* ECTX. The raw data and the reported results have been obtained by following the instructions of the study plan and the SOPs concerned. Any planned or unplanned changes to the study plan and SOPs applied have been recorded as an amendment or deviation.

The delegated analytical phase has not been performed in compliance with the OECD Principles of GLP but according to the criteria of the ISO 17025 standard by the accredited Dutch laboratory WLN Business B.V..

Noted and approved:

Tony Brouwers

Study Director

19/12/2017

Study completion date:

Quality Assurance Statement

Title: 2 hours Bio-elution Study on **Fe-Cr-Ni Powder Mixture** (68.6/16.4/10.7 mass fraction) at a *0.2, 1 and 2 g/L loading* in a simulated gastric fluid.

Study no.: **X02a-130**

The study has been audited by the Quality Assurance Unit to assure compliance with the study plan, standard operating procedures and according to the OECD Principles of GLP. The findings of each study phase were reported to management and the Study Director. The audits took place on the following dates:

Audited Period of Project Study phase	Date	Reporting of findings to Study Director and Test Facility Management
Study plan	18-09-2017	18-09-2017
Performance of the study: - weighing test item - experiment, gastric at pH 1.5	 19-09-2017 19-09-2017	30-11-2017
Raw data	30-11-2017	30-11-2017
Draft report	30-11-2017	30-11-2017
Final report	19-12-2017	19-12-2017

This statement indicates that the reported results accurately and completely reflect the raw data generated by the study.

To the best of our knowledge, there has been no significant deviation from the OECD Principles of GLP that could have adversely affected the integrity of the study.

Noted and approved:

19/12/2017.

Marleen Schreurs

Date:

Lead Quality Assurance Manager

Statement of Authentication

Title: 2 hours Bio-elution Study on **Fe-Cr-Ni Powder Mixture** (68.6/16.4/10.7 mass fraction) at a *0.2, 1 and 2 g/L loading* in a simulated gastric fluid.

Study no.: **X02a-130**

The experiments of the study have been performed in the *Test Facility* according to the OECD Principles of GLP. The delegated analytical phase has been carried out by the accredited Dutch laboratory WLN Business B.V.. The SOPs used by the *Test Site* have been accepted by the *Test Facility*. The raw data have been produced according to the instructions of the study plan and procedures. The results accurately reflect the raw data and show that the objectives of the study have been achieved.

Noted and approved:



19/12/2017

Tony Brouwers — Date:

Test Facility Manager

Contents

STATEMENT **2**

QUALITY ASSURANCE STATEMENT **3**

STATEMENT OF AUTHENTICATION **4**

CONTENTS **5**

1 SUMMARY **6**

2 IDENTIFICATION OF THE STUDY, TEST ITEM AND REFERENCE ITEM **8**

2.1 STUDY TITLE AND NUMBER 8

2.2 TEST ITEM IDENTIFICATION AND CHARACTERISATION 8

2.3 REFERENCE ITEM IDENTIFICATION AND CHARACTERISATION 9

3 INFORMATION CONCERNING SPONSOR, TEST FACILITY AND TEST SITE **11**

3.1 SPONSOR IDENTIFICATION 11

3.2 TEST FACILITY IDENTIFICATION 11

3.3 TEST SITE IDENTIFICATION 11

4 DATES **12**

5 TEST METHODS **13**

5.1 TEST GUIDANCE 13

5.2 STANDARD OPERATING PROCEDURES (SOPS) 13

6 TEST SYSTEM **14**

6.1 SIMULATED GASTRIC FLUID 14

7 EXPERIMENTAL DESIGN **15**

7.1 MATERIALS 15

7.2 PREPARATION OF THE TEST ITEM 15

7.3 TEST CONDITIONS 15

7.4 PERFORMANCE 15

7.5 DATA ANALYSIS 18

8 RESULTS **19**

9 CONCLUSION **22**

10 DEVIATIONS FROM THE ORIGINAL STUDY PLAN **23**

11 ARCHIVES **24**

12 REFERENCES **25**

ANNEXES **26**

1 Summary

Bio-elution refers to the *in vitro* extraction methods used to measure the degree to which a substance (e.g., metal or mineral ion) is dissolved in simulated biological fluids. Simulated biological fluids represent relevant exposure routes. The resulting value is the "bio-accessibility", and is defined as the "fraction of a substance that is soluble under physiological conditions and therefore potentially available for absorption into systemic circulation".

The objective of this study was to obtain knowledge about the bio-elution characteristics of **Fe-Cr-Ni Powder Mixture** (68.6/16.4/10.7 mass fraction) in simulated gastric fluid. This study has been conducted according to the recommended Standard Operating Procedure (SOP) for Bioelution Testing of Metals, Inorganic Metal Compounds, and Metal-Containing Complex Materials: Simulated Gastric Fluid (Eurometaux, July 2016) which is based on ASTM D5517-07: Standard Test Method for Determining the extractability of metals from art materials - ASTM, 2007 (American Society for Testing and Materials). The extent of dissolution of **Fe-Cr-Ni Powder Mixture** (68.6/16.4/10.7 mass fraction) with a particle size <100 µm was tested in a simulated gastric fluid at 37 °C and pH 1.5 for 2 hours (0.2 g/L, 1 g/L and 2 g/L loading) at an agitation speed of 100 revolutions per minute (rpm) for 1 hour followed by 1 hour of settling (without shaking). The bio-elution endpoint was based on the dissolved chromium, iron and nickel concentrations obtained after 2 hours of extraction.

The following deviation from the SOP was made:

- The samples for metal analyses were preserved by adding 0.12 mL concentrated HNO_3 per 12 mL instead of no preservation in the SOP.

The study was performed at ECTX. Analysis of the concentrations of dissolved element concentrations has been performed at WLN Business B.V. (The Netherlands), an ISO 17025 accredited laboratory, as delegated by ECTX.

The measured pH of the test medium at the start of the test was 1.50, i.e. within the specifications of pH 1.5 ± 0.1. The pH measured in the additional test item vessels (replica X) at the start of the test was 1.50 (0.2 g/L and 2 g/L loading) and 1.51 (1 g/L loading) i.e. within the specifications of pH 1.5 ± 0.1. At the 2 hours sampling point of the test, the pH in the blank control vessels, reference item vessels and test vessels was between 1.50 and 1.52.

The temperature of the sampled blank control, reference item and test item vessels was between 36.8 °C and 37.0 °C, and corresponded to the required test conditions of 37 °C ± 1 °C.

The blank control vessels showed small concentrations of chromium above the accredited reporting limit of 0.5 µg/L Cr. The blank measurements of iron and nickel were below their corresponding accredited reporting limits of 10 µg/L Fe and 0.5 µg/L Ni.

In the blank corrected test item vessels with a nominal loading of **0.2 g/L Fe-Cr-Ni Powder Mixture**, the following average dissolved element concentrations were measured after 2 hours of exposure to the simulated gastric fluid (pH 1.5):

- 0.0147 ± 0.0013 mg/L Cr ($CV_{between\text{-}vessel}$ = 9 %; N = 3),
- 63.8 ± 1.3 mg/L Fe ($CV_{between\text{-}vessel}$ = 2 %; N = 3),
- 5.05 ± 0.27 mg/L Ni ($CV_{between\text{-}vessel}$ = 5 %; N = 3).

Based on the chromium content (16.4 % Cr), iron content (68.6 % Fe) and nickel content (10.7 % Ni) in the test item mixture and the average dissolved element concentrations in the test solutions:

- a release of chromium of 0.045 % of content,
- a release of iron of 46 % of content,
- a release of nickel of 24 % of content,

could be calculated at the 2 hours endpoint.

In the blank corrected test item vessels with a nominal loading of **1 g/L Fe-Cr-Ni Powder Mixture**, the following average dissolved element concentrations were measured after 2 hours of exposure to the simulated gastric fluid (pH 1.5):

- 0.0300 ± 0.0010 mg/L Cr ($CV_{between\text{-}vessel}$ = 3 %; N = 3),
- 253 ± 7 mg/L Fe ($CV_{between\text{-}vessel}$ = 3 %; N = 3),
- 29.2 ± 1.1 mg/L µg/L Ni ($CV_{between\text{-}vessel}$ = 4 %; N = 3).

Based on the chromium content (16.4 % Cr), iron content (68.6 % Fe) and nickel content (10.7 % Ni) in the test item mixture and the average dissolved element concentrations in the test solutions:

- a release of chromium of 0.018 % of content,
- a release of iron of 37 % of content,
- a release of nickel of 27 % of content,

could be calculated at the 2 hours endpoint.

In the blank corrected test item vessels with a nominal loading of **2 g/L Fe-Cr-Ni Powder Mixture**, the following average dissolved element concentrations were measured after 2 hours of exposure to the simulated gastric fluid (pH 1.5):

- 0.0432 ± 0.0004 mg/L Cr ($CV_{between\text{-}vessel}$ = 1 %; N = 3),
- 437 ± 3 mg/L Fe ($CV_{between\text{-}vessel}$ = 1 %; N = 3),
- 40.1 ± 1.1 mg/L Ni ($CV_{between\text{-}vessel}$ = 3 %; N = 3).

Based on the chromium content (16.4 % Cr), iron content (68.6 % Fe) and nickel content (10.7 % Ni) in the test item mixture and the average dissolved element concentrations in the test solutions:

- a release of chromium of 0.013 % of content,
- a release of iron of 32 % of content,
- a release of nickel of 19 % of content,

could be calculated at the 2 hours endpoint.

2 Identification of the Study, Test Item and Reference Item

2.1 Study title and number

Title: 2 hours Bio-elution Study on **Fe-Cr-Ni Powder Mixture** (68.6/16.4/10.7 mass fraction) at a *0.2, 1 and 2 g/L loading* in a simulated gastric fluid.

Study no.: **X02a-130**

2.2 Test Item identification and characterisation

The test item, an **Fe-Cr-Ni Powder Mixture**, was prepared for each test vessel separately to ensure a correct element ratio (see *7.2 Preparation of the test Item*).

The information about the identification and characterisation of the test item has been delivered by the Sponsor at the start of the study.

Test Item identification

Test item name:	**Fe-Cr-Ni Powder Mixture**
Manufacturer:	The mixtures were prepared at ECTX
Supplier:	See *2.3 Reference Item identification and characterisation* for the information about the constituents.
ECTX no.:	-

Test Item characterisation

Element(s) of interest:	Chromium, iron and nickel
Composition regarding the elements of interest:	Mass based mixture: 1. 68.6 parts iron powder 2. 16.4 parts chromium powder 3. 10.7 parts nickel powder
Physical form:	Solid, (in the powder form)
Colour:	Grey
Particle size distribution:	<44 µm, See constituent elements under *2.3 Reference Item identification and characterisation*

Storage conditions and Expiry date

n.a.

Safe Handling Precautions

n.a. Safety data sheets were available for each of the constituents (see *2.3 Reference Item identification and characterisation*.

2.3 Reference Item identification and characterisation

As the reference items (three), the individual powders were used, i.e. iron powder, chromium powder and nickel powder:

Reference Item identification (Iron powder)

Supplier:	Sigma Aldrich BVBA Brusselsesteenweg 288, B-3090 Overijse, Belgium
Supplier contact:	+32 (0)3/899.13.01

ECTX no.:	Description	Acros Art. no.	Lot no.
R0039/01	Iron powder >97 % ; -325 mesh (<44 µm)	209309	STBG7186

CAS no.: 7439-89-6
Molecular weight: 55.85 g/mol
Stored under argon. Moisture sensitive. Safety data sheet is available.
A certificate of chemical analysis of the tested batch is added to this study report (Annex 9).
Particle Size Distribution (PSD):
D(v, 0.1) = 7.12 µm
D(v, 0.5) = 22.34 µm
D(v, 0.9) = 40.28 µm
A certificate of analysis of the PSD is added under Annex 10
Specific Surface Area (N-BET)
0.30 m²/g
A certificate of analysis of the Specific Surface Area (N-BET) is added under Annex 10

Reference Item identification (Chromium powder)

Supplier:	Sigma Aldrich BVBA Brusselsesteenweg 288, B-3090 Overijse, Belgium
Supplier contact:	+32 (0)3/899.13.01

ECTX no.:	Description	Acros Art. no.	Lot no.
R0040/01	Chromium powder 99.999 % ; -325 mesh (44 µm)	266299	MKBP5427V

CAS no.: 7440-47-3
Molecular weight: 52.00 g/mol
Air sensitive. Safety data sheet is available.
A certificate of chemical analysis of the tested batch is added to this study report (Annex 7).
Particle Size Distribution (PSD):
D(v, 0.1) = 17.72 µm
D(v, 0.5) = 45.91 µm
D(v, 0.9) = 82.96 µm
A certificate of analysis of the PSD is added under Annex 8
Specific Surface Area (N-BET)
0.08 m²/g
A certificate of analysis of the Specific Surface Area (N-BET) is added under Annex 8

Reference Item identification (Nickel powder)

Supplier:	Particle Technology Labs 555 Rogers Street, Downers Grove, IL 60515, USA
Supplier contact:	+1-(630)-969-2703 x118

ECTX no.:	Description	PTL no.	Lot no.
T0220/01	Nickel metal powder >99 % ; d_{90}: 1.84 µm	N36F.1.4-PTL	-

Nickel metal powder (ECTX no.: T0220/01) was subject in ECTX study X02a-085.
A certificate of chemical analysis of the tested batch is added to this study report (Annex 11).
Particle Size Distribution (PSD):
D(v, 0.1) = 0.77 µm
D(v, 0.5) = 1.20 µm
D(v, 0.9) = 1.84 µm
A Laboratory report of the PSD is added under Annex 12
Specific Surface Area (N-BET)
2.58 m^2/g
A Laboratory report of the Specific Surface Area (N-BET) is added under Annex 13

3 Information concerning Sponsor, Test Facility and Test Site

3.1 Sponsor identification

Sponsor:	**Eurometaux** Avenue de Broqueville, 12 B-1150 Brussels Belgium
Study Monitor on behalf of the Sponsor:	**Violaine Verougstraete**

3.2 Test Facility identification

ECTX bvba, Havenstraat 46/0.01, B-3500 Hasselt, Belgium.	
Study Director:	**Tony Brouwers**
Lead Quality Assurance Manager:	Marleen Schreurs
Test Facility Manager:	Tony Brouwers

3.3 Test Site identification

WLN Business B.V., Rijksstraatweg 85, NL-9756 AD Glimmen, The Netherlands. (ISO 17025 Accredited) RVA (Raad Voor Accreditatie) Accreditation certificate no. L075 valid from 12-11-1993 to 01-12-2017.	
Contributing Scientist:	Jan Bonnema
Quality Control Manager:	Jeroen Boekenoogen
Test Site Management:	Jeroen van Nuil / Harm de Kleine

4 Dates

Study Initiation Date:	**18-09-2017**
Experimental Starting Date:	**19-09-2017**
Experimental Completion Date:	**22-09-2017** (with specific analyses)
Study Completion Date:	Date of approval of this Final Report by the Study Director
Remarks:	The test was carried out on **19-09-2017**
	The samples were delivered to the analytical lab (WLN) on **20-09-2017**
	Specific analyses were carried out on **21-09-2017**

5 Test methods

5.1 Test guidance

The conduct of the study is based on the Standard Operating Procedure (SOP) for Bioelution Testing of Metals, Inorganic Metal Compounds, and Metal-Containing Complex Materials: Simulated Gastric Fluid (Eurometaux, July 2016).

5.2 Standard Operating Procedures (SOPs)

5.2.1 SOPs of ECTX

The SOPs versions used in the study at the Test Facility were valid at the start of the experiment and were listed below:

- SOP IN-01: Instrumentation – µpipettes,
- SOP IN-02: Instrumentation – pH, temperature, dissolved oxygen and conductivity measurements,
- SOP AB-09: Recipe book,
- SOP IA-01: Instrumentation – balances,
- SOP TX-02: bio-elution tests,
- SOP AB-10: Cleaning and maintenance,
- SOP AB-11: Waste management,
- SOP AB-14: Analytical measurements by an external laboratory.

5.2.2 SOPs of WLN Business B.V.

The SOPs versions used in the study at the Test Site were valid at the start of the experiment and were listed below:

- SOP WLN-MHB.07: Receipt, handling and storage of samples,
- SOP WLN-CM.W.11.1: Elemental determination by ICP-MS technique, SFS-EN ISO 17294-2,
- SOP WLN-OK.KHB.05 5.10: Reporting of results,
- SOP WLN-OK.P.39.1: Discarding of waste/samples.

6 Test System

6.1 Simulated gastric fluid

Simulated gastric fluid (0.07 N HCl) was prepared according to recipe 63 of SOP AB-09 (recipe book). The pH of the medium was brought to pH 1.50, using a NaOH solution. The chemical composition of the test medium is shown in the following table:

HCl (30 %)	7.41 mL
Ultrapure water	Up to 1 L
NaOH solution	Few drops to adjust pH to pH 1.5 ± 0.1 (as reported in ASTM 5517)

7 Experimental Design

7.1 Materials

- Usual laboratory equipment,
- Microbalance Mettler Toledo XP6 (SNR24043156),
- Analytical balance Mettler Toledo XP204S (SNR1129023848),
- Balance Mettler Toledo XP8002S (SNR1129020806),
- Thermometer Testo 875-1i (SNR2738028), pH-meter (HACH HQ40d multimeter SNR080100016916),
- Thermostatic shaker Thermo Scientific SHKE8000-8CE (SNR109245-80) (stroke length: 2.54 cm),
- New, pre-cleaned and acid rinsed closed borosilicate 3.3 Erlenmeyer flasks (250 mL),
- New, pre-cleaned and acid rinsed, closed borosilicate 3.3 Erlenmeyer flasks (2 L),
- Demineralised water was prepared by a MilliQ Integral 5 System: conductivity 18 MΩ; Total Organic Carbon = 3 ppb C,
- Simulated gastric fluid (SOP AB-09: Recipe book: recipe 63),
- 10N NaOH solution,
- Dispensette, Brand Dispensette III digital 5-50 mL (SNR08K21110),
- 0.2 µm Pall Acrodisc® 25 mm syringe filters (PN 4612),
- 12 mL Latex-and oil-free syringes of for sampling (HSW Norm-Ject Ref 4100.000Spec),
- 15 ml 120x17 mm conical PP sample tubes, (Deltalab Ref 429910),
- Automatic pipettes (Brand Transferpette®, Eppendorf Multipette®),
- Concentrated HNO_3 (67-69%), PlasmaPur, SCP SCIENCE 250-037-175PL,
- ICP-MS: Thermo Scientific iCAP™ Q ICP-MS (Waterlaboratorium Noord).

7.2 Preparation of the Test Item

The test item was prepared for each test vessel separately according to the overview of the test setup (*7.4.1 Test set-up*). The actual 'powder' loading in the test vessels deviates slightly from the nominal loading (95.7 % instead of 100 %). In this manner, however, the results are directly comparable to the dissolution results of stainless steel 316L (ECTX Study: X02a-084) which contained in addition to iron, chromium and nickel other traces of elements.

7.3 Test conditions

- Temperature controlled at 37 °C ± 1 °C, in the dark,
- pH initially set at pH 1.5 ± 0.1,
- Agitation speed 100 rpm in a temperature controlled orbital laboratory shaker (orbital size: 1 inch, 2.54 cm).

7.4 Performance

7.4.1 Test set-up

250 mL borosilicate glass Erlenmeyer's were used for the blank control vessel, the reference item vessels (loading of 0.2 g/L) and the test item vessels (loading of 1 g/L and 2 g/L).

2 L borosilicate glass Erlenmeyer's were used for the test item vessels (loading of 0.2 g/L). The simple mixture was trying to reproduce the percent amount of Fe, Ni and Cr in SS316L. However, SS316L also contains about 4% of other elements. Thus the mass of the simple mixture added up to 0.192 g/L instead of 0.2 g/L, 0.96 g/L instead of 1 g/L, and 1.92 g/L instead of 2 g/L.

- In the blank control vessel, 50 mL gastric test medium (37 °C ± 1 °C) was added, only.
- In each reference item vessel (iron, chromium, nickel), 50 mL gastric test medium (37 °C ± 1 °C) was added to obtain a loading of 0.2 g/L iron, chromium and nickel, respectively.
- The four test item vessels of 1 g/L and 2 g/L loading were prepared by adding 50 mL gastric test medium (37 °C ± 1 °C) to the weighed test item mixture. The four test item vessels of 0.2 g/L loading were prepared by adding 500 mL gastric test medium (37 °C ± 1 °C) to the weighed test item mixture.
  One of the four replicates of each loading, further noted as replica X, was prepared, only for the measurement of the initial pH (at the start of the test) and did not have any further utility in the test.

The pH of the solution of replicas X were measured to ensure that the initial pH is at pH 1.5 ± 0.1. This procedure was used to avoid cross contamination.

After covering the vessels with a screwcap and swirling the flasks to mix the test item and the medium, the flasks were placed in the dark into a thermostatic orbital shaker (37 °C ± 1 °C) at an agitation rate of 100 rpm for one hour. After that, the flasks were settled at 37 °C ± 1 °C for another hour.

An overview of the test set-up is shown in the following table:

Time	Target loading (g/L)	Replica number	Fe weighed	Cr weighed	Ni weighed	Test medium added
0 hours	0.2	*replica X	68.614 mg	16.411 mg	10.718 mg	500 mL
	1	*replica X	34.313 mg	8.221 mg	5.350 mg	50 mL
	2	*replica X	68.605 mg	16.404 mg	10.720 mg	50 mL
2 hours	0	blank	-	-	-	50 mL
	0.2	iron	10.030 mg	-	-	50 mL
	0.2	chromium	-	10.008 mg	-	50 mL
	0.2	nickel	-	-	10.040 mg	50 mL
	0.2	replica1	68.601 mg	16.429 mg	10.723 mg	500 mL
	0.2	replica2	68.588 mg	16.412 mg	10.710 mg	500 mL
	0.2	replica3	68.629 mg	16.418 mg	10.742 mg	500 mL
	1	replica1	34.302 mg	8.204 mg	5.359 mg	50 mL
	1	replica2	34.321 mg	8.208 mg	5.348 mg	50 mL
	1	replica3	34.326mg	8.227 mg	5.355 mg	50 mL
	2	replica1	68.614 mg	16.390 mg	10.711 mg	50 mL
	2	replica2	68.587 mg	16.417 mg	10.727 mg	50 mL
	2	replica3	68.596 mg	16.408 mg	10.704 mg	50 mL

* replica X was added to check the initial impact of the test item to the pH of the test system and was discarded after the start of the test.

7.4.2 Sampling during the test

The following sampling procedure was used at the sampling time to collect and preserve the samples for ICP-MS analyses:

At the end of the incubation period (2 hours = 1-hour shaking at 100 rpm and 1-hour setting), each vessel was swirled to homogenise the solution before sampling, to avoid a concentration gradient after setting.

Sampling was performed for each test vessel as indicated in the sampling scheme below. In each test vessel, a 12-mL sample was taken twice with a 12 mL syringe at a depth of two-thirds of the supernatant. The samples were filtrated through a 0.2 µm syringe filter and transferred to uniquely labelled 15-mL PP sample tubes. The samples were preserved by adding 0.12 mL concentrated HNO_3 per 12 mL. The samples were covered (to avoid evaporation and concentration) and stored at room temperature in the dark until shipment to the Test Site (see *3.3 Test Site identification*).

Each sample was labelled with a unique number and with an expiry date that is one month after sampling:

e.g. X02a-130-**1** where:

X02a-130 is the study number,

1 is the sample number.

7.4.3 Sampling scheme

For the test vessels with simulated gastric fluid the following sampling scheme was applied:

Sampling time (h)	**Number of samples**							**Total number of samples**
	Medium	Reference item + medium			Test item + medium			
	(blank) 0 g/L	Fe Loading 0.2 g/L	Cr Loading 0.2 g/L	Ni Loading 0.2 g/L	Loading 0.2 g/L	Loading 1 g/L	Loading 2 g/L	
2	2*1	2*1	2*1	2*1	2*3	2*3	2*3	26
Total number of samples:								26

A detailed list is given in Annex 5.

7.4.4 Observations and measurement of temperature and pH

At the start of the test (0h), pH was measured in the additional replicas with test item at a 0.2 g/L, 1 g/L and 2 g/L loading (named replica X). The addition of the test item to the test system had no significant influence on the initial pH of the test system.

After sampling, temperature and pH were measured in the remaining blank control and test solutions after sampling.

The details are presented in Annex 1, 2a, 2b and 2c.

7.4.5 Shipment of the samples to the Test Site

The samples and datasheet DSH026 Sample Transfer (external), which contained a chain of custody and a detailed sample list with storage conditions were transferred to the Test Site (see *3.3 Test Site identification*) in an appropriate manner according to SOP AB-14 (see *5.2.1 SOPs of ECTX*).

7.4.6 Analyses of the samples by the Test Site

The determination of dissolved chromium, iron and nickel concentrations in the blank control, reference item and test item vessels were carried out at the Test Site (see *3.3 Test Site identification*), using an ICP-MS (SOP WLN-CM.W.11.1 Elemental determination by ICP-MS technique, SFS-EN ISO 17294-2). To describe the dissolution behaviours, even below the accredited reporting limits of WLN and to allow more accurate toxicity calculations, raw data were used to perform calculations. Rounded values were used to at least one more decimal place than the corresponding accredited reporting limit of WLN, if relevant. The accredited reporting limit with an accuracy of 90 % and 110 % is 0.5 µg/L for chromium and nickel and 10 µg/L for iron. For values between the detection limit and the validated reporting limits, an accuracy of 90 % - 110 % cannot be guaranteed.

The analyses were not performed in compliance with the GLP principles, but according to the ISO 17025 standard.

Each sample, possibly after proper dilution, was measured once.

7.4.7 Reports from the Test Site

An analytical report of the delegated phase including the raw data has been written and approved by the Test Site Scientist. The analytical report does not contain a QA Statement because the tests have been carried out according to the criteria of the ISO 17025 standard which does not require a QA Statement.

The analytical report of the delegated phase was sent to the Study Director on 22-09-2017 and is added as an annex to this final report (Annex 6).

7.5 Data analysis

The data from the analytical laboratory are expressed in µg element per L solution.

These data are recalculated (blank corrected) and expressed in mg element per g test item and as percent metal release of the total available element.

The results of the individual metals are also expressed as released element per surface (mg/m²), based on the results of the N-BET analyses.

The dataset of *n* vessels was analysed. The between-vessel mean, standard deviation and coefficient of variation of the measured dissolved element concentrations were calculated using the equations in the table below:

	Mean	Standard deviation	Coefficient of variation
Between	$x_{avg} = \frac{1}{n}\sum_{i=1}^{n} x_i$	$s_{between} = \left(\frac{1}{n-1}\sum_{i=1}^{n}\left(x_i - x_{avg}\right)^2\right)^{1/2}$	$CV_{between} = \frac{s_{between}}{x_{avg}} \cdot 100\%$

A between-vessel coefficient of variation of <20% is a quality criterion for the 2 hours endpoint.

With very low concentrations close to the detection limit of the analytical method, such as may be with blanks or with a substance that releases element concentrations close to the detection limit, the between-vessel coefficient of variation can be expected to exceed this target coefficient of variation. Moreover, should the substance be characterised by a wide particle size range, the possibility exists that this coefficient of variation target may not be readily met.

8 Results

The temperature of the test solutions at the sampling point was between 36.8 °C and 37.0 °C, which was in line with the test conditions of 37 °C ± 1 °C (Annex 1, 2a, 2b and 2c).

The measured pH of the test medium at the start of the test was 1.50, i.e. within the specifications of pH 1.5 ± 0.1. The pH measured in the additional test item vessels (replica X) at the start of the test was 1.50 for both loadings, i.e. within the specifications of pH 1.5 ± 0.1. At the 2 hours sampling point of the test, the pH in the blank control vessels, reference item vessels and test vessels was between 1.50 and 1.52. (Annex 1, 2a, 2b and 2c)

The raw data of the dissolved element concentrations in the test samples, measured by the Test Site WLN Business B.V., are presented in Annex 6.

Measurements of the blank control vessels showed small concentrations of chromium above the accredited reporting limit of 0.5 µg/L Cr. The blank measurements of iron and nickel were below their corresponding accredited reporting limits of 10 µg/L Fe and 0.5 µg/L Ni.

The following observations could be made in the reference item vessels containing the individual metal powders of the elements of interest (Cr, Fe and Ni):

- Remaining, undissolved, metal powder could be observed in the Erlenmeyer flasks at the end of the experiment indicating that the dissolution was not complete. Determinations of the dissolved chromium content of 20.1 µg/L (i.e. 0.010 %), iron content of 84.2 mg/L (i.e. 42 %) and nickel content of 32.5 mg/L (i.e. 16 %) could confirm these observations.
- Based on the measured specific surface area of the chromium powder (i.e. 0.08 m^2/g), a release per surface of 1.3 mg/m^2 was calculated.
- Based on the measured specific surface area of the iron powder (i.e. 0.30 m^2/g), a release per surface of 1.4×10^3 mg/m^2 was calculated.
- Based on the measured specific surface area of the nickel powder (i.e. 2.58 m^2/g), a release per surface of 63 mg/m^2 was calculated.

The following observations could be made in the test vessels with a nominal loading of **0.2 g/L Fe-Cr-Ni Powder Mixture**:

- Remaining test item (grey particles) could be observed on the filter and in the Erlenmeyer flasks at the end of the experiment.
- An average dissolved chromium concentration of 0.0147 ± 0.0013 mg/L Cr (or 0.0734 ± 0.0065 mg/g nominal mixture) was measured after 2 hours of exposure to the simulated gastric fluid (pH 1.5) with a between-vessel variation of 9 % which met the 20 % quality criterion at the 2 hours endpoint.

  Based on the chromium content in the **Fe-Cr-Ni Powder Mixture** (i.e. 16.4 %) and the average dissolved chromium concentration, a chromium release of 0.045 % could be calculated at the 2 hours endpoint.
- An average dissolved iron concentration of 63.8 ± 1.3 mg/L Fe (or 319 ± 6 mg/g nominal mixture) was measured after 2 hours of exposure to the simulated gastric fluid (pH 1.5) with a between-vessel variation of 2 % which met the 20 % quality criterion at the 2 hours endpoint.

  Based on the iron content in the **Fe-Cr-Ni Powder Mixture** (i.e. 68.6 %) and the average dissolved iron concentration, an iron release of 46 % could be calculated at the 2 hours endpoint.

- An average dissolved nickel concentration of 5.05 ± 0.27 mg/L Ni (or 25.2 ± 1.3 mg/g nominal mixture) was measured after 2 hours of exposure to the simulated gastric fluid (pH 1.5) with a between-vessel variation of 5 % which met the 20 % quality criterion at the 2 hours endpoint.

  Based on the nickel content in the **Fe-Cr-Ni Powder Mixture** (i.e. 10.7 %) and the average dissolved nickel concentration, a nickel release of 24 % could be calculated at the 2 hours endpoint.

The following observations could be made in the test vessels with a nominal loading of **1 g/L Fe-Cr-Ni Powder Mixture**:

- Remaining test item (grey particles) could be observed on the filter and in the Erlenmeyer flasks at the end of the experiment.
- An average dissolved chromium concentration of 0.0300 ± 0.0010 mg/L Cr (or 0.0300 ± 0.0010 mg/g nominal mixture) was measured after 2 hours of exposure to the simulated gastric fluid (pH 1.5) with a between-vessel variation of 3 % which met the 20 % quality criterion at the 2 hours endpoint.

  Based on the chromium content in the **Fe-Cr-Ni Powder Mixture** (i.e. 16.4 %) and the average dissolved chromium concentration, a chromium release of 0.018 % could be calculated at the 2 hours endpoint.
- An average dissolved iron concentration of 253 ± 7 mg/L Fe (or 253 ± 7 mg/g nominal mixture) was measured after 2 hours of exposure to the simulated gastric fluid (pH 1.5) with a between-vessel variation of 3 % which met the 20 % quality criterion at the 2 hours endpoint.

  Based on the iron content in the **Fe-Cr-Ni Powder Mixture** (i.e. 68.6 %) and the average dissolved iron concentration, an iron release of 37 % could be calculated at the 2 hours endpoint.
- An average dissolved nickel concentration of 29.2 ± 1.1 mg/L Ni (or 29.2 ± 1.1 mg/g nominal mixture) was measured after 2 hours of exposure to the simulated gastric fluid (pH 1.5) with a between-vessel variation of 4 % which met the 20 % quality criterion at the 2 hours endpoint.

  Based on the nickel content in the **Fe-Cr-Ni Powder Mixture** (i.e. 10.7 %) and the average dissolved nickel concentration, a nickel release of 27 % could be calculated at the 2 hours endpoint.

The following observations could be made in the test vessels with a nominal loading of **2 g/L Fe-Cr-Ni Powder Mixture**:

- Remaining test item (grey particles) could be observed on the filter and in the Erlenmeyer flasks at the end of the experiment.
- An average dissolved chromium concentration of 0.0432 ± 0.0004 mg/L Cr (or 0.0216 ± 0.0002 mg/g nominal mixture) was measured after 2 hours of exposure to the simulated gastric fluid (pH 1.5) with a between-vessel variation of 1 % which met the 20 % quality criterion at the 2 hours endpoint.

  Based on the chromium content in the **Fe-Cr-Ni Powder Mixture** (i.e. 16.4 %) and the average dissolved chromium concentration, a chromium release of 0.013 % could be calculated at the 2 hours endpoint.
- An average dissolved iron concentration of 437 ± 3 mg/L Fe (or 219 ± 2 mg/g nominal mixture) was measured after 2 hours of exposure to the simulated gastric fluid (pH 1.5) with a between-vessel variation of 1 % which met the 20 % quality criterion at the 2 hours endpoint.

  Based on the iron content in the **Fe-Cr-Ni Powder Mixture** (i.e. 68.6 %) and the average dissolved iron concentration, an iron release of 32 % could be calculated at the 2 hours endpoint.
- An average dissolved nickel concentration of 40.1 ± 1.1 mg/L Ni (or 20.1 ± 0.6 mg/g nominal mixture) was measured after 2 hours of exposure to the simulated gastric fluid (pH 1.5) with a between-vessel variation of 3 % which met the 20 % quality criterion at the 2 hours endpoint.

Based on the nickel content in the **Fe-Cr-Ni Powder Mixture** (i.e. 10.7 %) and the average dissolved nickel concentration, a nickel release of 19 % could be calculated at the 2 hours endpoint.

All raw data can be found in Annex 1-13 of this study report.

9 Conclusion

During this study on the bio-elution of **Fe-Cr-Ni Powder Mixture**, the following could be observed after 2 hours of exposure to simulated gastric fluid (pH 1.5):

- At a **nominal loading of 0.2 g/L**, following amounts were measured:
  - For chromium an average value of 0.0147 mg/L Cr ($CV_{between\text{-}vessel}$ = 9 %; N = 3) was found after 2 hours of extraction, corresponding with a chromium release related to the total chromium content of 0.045 %.
  - For iron an average value of 63.8 mg/L Fe ($CV_{between\text{-}vessel}$ = 2 %; N = 3) was found after 2 hours of extraction, corresponding with an iron release related to the total iron content of 46 %.
  - For nickel an average value of 5.05 mg/L Ni ($CV_{between\text{-}vessel}$ = 5 %; N = 3) was found after 2 hours of extraction, corresponding with a nickel release related to the total nickel content of 24 %.
- At a **nominal loading of 1 g/L**, following amounts were measured:
  - For chromium an average value of 0.0300 mg/L Cr ($CV_{between\text{-}vessel}$ = 3 %; N = 3) was found after 2 hours of extraction, corresponding with a chromium release related to the total chromium content of 0.018 %.
  - For iron an average value of 253 mg/L Fe ($CV_{between\text{-}vessel}$ = 3 %; N = 3) was found after 2 hours of extraction, corresponding with an iron release related to the total iron content of 37 %.
  - For nickel an average value of 29.2 mg/L Ni ($CV_{between\text{-}vessel}$ = 4 %; N = 3) was found after 2 hours of extraction, corresponding with a nickel release related to the total nickel content of 27 %.
- At a **nominal loading of 2 g/L**, following amounts were measured:
  - For chromium an average value of 0.0432 mg/L Cr ($CV_{between\text{-}vessel}$ = 1 %; N = 3) was found after 2 hours of extraction, corresponding with a chromium release related to the total chromium content of 0.013 %.
  - For iron an average value of 437 mg/L Fe ($CV_{between\text{-}vessel}$ = 1 %; N = 3) was found after 2 hours of extraction, corresponding with an iron release related to the total iron content of 32 %.
  - For nickel an average value of 40.1 mg/L Ni ($CV_{between\text{-}vessel}$ = 3 %; N = 3) was found after 2 hours of extraction, corresponding with a nickel release related to the total nickel content of 19 %.

The results can be assumed reliable since the test conditions stayed constant during the experiment.

10 Deviations from the original Study Plan

None.

11 Archives

All the materials and records necessary to reconstruct the study have been maintained in the archives of ECTX in a file coded **X02a-130**. The Study Plan, the lab notebook, the raw data, the correspondence and final report has been stored in a locked filing cabinet. A representative sample of the iron powder (R0039/01: 10.1695 g) and chromium powder (R0040/01: 10.6035 g) was archived (19-09-2017) in suitably sealed vials and stored under the storage conditions mentioned. At the sponsor's request, the remaining test item is stored at ECTX for possible further studies. A representative sample of the nickel metal powder (10.0353 g) was already archived earlier (study X02a-085; 11-11-2016) in a suitably sealed vial and stored under the storage conditions mentioned.

The stock solutions, specimens and test and calibration solutions used during the study have not been archived and have been disposed of by ECTX and WLN Business B.V. according to the instructions of their corresponding SOPs when their expiry date has been exceeded or when their original characteristics could not be guaranteed anymore.

The duration of archiving is five (5) years after the date of approval of the Final Report by the Study Director. After this period the Sponsor decides if:

- The archiving of the records and materials should be continued;
- The archived material should be sent to the Sponsor;
- The archived material should be destroyed.

12 References

a) Eurometaux (2016): **Standard Operating Procedure (SOP) for Bioelution Testing of Metals, Inorganic Metal Compounds, and Metal-Containing Complex Materials: Simulated Gastric Fluid** (Eurometaux Brussels, July 2016).

b) ASTM D5517-14 (2014): **Standard Test Method for Determining Extractability of Metals from Art Materials**, Philadelphia, PA: American Society for Testing and Materials, 2014.

c) Henderson R.G., V. Verougstraete, K. Anderson, J.J. Arbildua, T.O. Brock, T. Brouwers, D. Cappellini, K.Delbeke, G. Herting, G. Hixon, I. Odnevall Wallinder, P.H. Rodriguez, F. Van Assche, P. Wilrich and A.R. Oller (2014). **Interlaboratory Validation of Bioaccessibility Testing for Metals.** Regul Toxicol and Pharmacol. 70(1): 170-181.

d) OECD (1981-2016) **OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring.**

Annexes

Annex 1:

Data of measured temperature, pH and dissolved element concentrations during the 2 hours exposure period of the reference items (0.2 g/L loading) exposed to the simulated gastric fluid at pH 1.5).
(BLANK CORRECTED if the blank value is higher than reporting limit. Otherwise blank assumed 0.)

The results are also expressed as element release per g reference powder, the percentage of available element eluted and the corresponding element release per surface.

reference item		2h				
pH 1.5 (1 h at 100 rpm)		µg/L Cr	µg/L Fe	µg/L Ni	T (°C)	pH
Accred. Reporting Limit		0.5	10	0.5		
test medium at 0 h					37.1	1.50
blank	spl1	0.7	<10	<0.5	37.0	1.50
	spl2	0.7	<10	<0.5		
	x_{avg}	0.7	<10	<0.5		
Cr powder	Total loading[a]	200160			36.9	1.50
(R0040/01)	Meas. spl1	20.0	<10	<0.5		
SSA: 0.08 m²/g	Meas. spl2	20.2	<10	<0.5		
	dissolution x_{avg}	20.1	<10	<0.5		
	absolute **Cr** release (mg/g ref item)	0.101 mg/g **Cr** powder				
	% of total available **Cr** eluted	0.010 %				
	Cr release/surface	1.3 mg/m²				
Fe powder	Total loading[a]		200600		37.0	1.52
(R0039/01)	Meas. spl1	<0.5	85854	<0.5		
SSA: 0.30 m²/g	Meas. spl2	<0.5	82525	<0.5		
	x_{avg}	<0.5	84190	<0.5		
	absolute **Fe** release (mg/g ref item)	421 mg/g **Fe** powder				
	% of total available **Fe** eluted	42 %				
	Fe release/surface	1.4 × 10³ mg/m²				
Ni powder	Total loading[a]			200800	37.0	1.50
(T0220/01)	Meas. spl1	<0.5	<10	32340		
SSA: 2.58 m²/g	Meas. spl2	<0.5	<10	32753		
	x_{avg}	<0.5	<10	32547		
	absolute **Ni** release (mg/g ref item)	163 mg/g **Ni** powder				
	% of total available **Ni** eluted	16 %				
	Ni release/surface	63 mg/m²				

a loading based on the actual weighed amount of reference metal powder

x_{avg} within vessel average of 2 samples (N=2)

s_{within} within vessel Standard Deviation (N=2)

CV_{within} within vessel Coefficient of Variation where v is the replica test vessel number (N=2)

Annex 2a:

Data of measured temperature, pH and dissolved element concentrations during the 2 hours extraction period of **0.2 g/L Fe-Cr-Ni Powder Mixture** in the simulated gastric fluid at pH 1.5.
(BLANK CORRECTED if the blank value is higher than reporting limit. Otherwise blank assumed 0.).

0.2 g/L test item		2h				
pH 1.5 (1 h at 100 rpm)		µg/L Cr	µg/L Fe	µg/L Ni	T	
Accred. Reporting Limit		0.5	10	0.5	(°C)	pH
test medium at 0 h					37.1	1.50
replicaX at 0 h					36.8	1.50
blank	spl1	0.7	<10	<0.5	37.0	1.50
	spl2	0.7	<10	<0.5		
	$x_{avg,1}$	0.7	<10	<0.5		
	$s_{within,1}$	0.0	-	-		
	$CV_{within,1}$ (%)	2	-	-		
replica1	spl1	16.1	62755	4800	36.9	1.50
	spl2	16.3	61903	4672		
	$x_{avg,1}$	16.2	62329	4736		
	$s_{within,1}$	0.2	602	90		
	$CV_{within,1}$ (%)	1	1	2		
replica2	spl1	13.9	64258	5186	36.9	1.51
	spl2	13.9	64735	5204		
	$x_{avg,2}$	13.9	64496	5195		
	$s_{within,2}$	0.0	337	13		
	$CV_{within,2}$ (%)	0	1	0		
replica3	spl1	14.1	64547	5228	36.8	1.50
	spl2	13.9	64585	5182		
	$x_{avg,3}$	14.0	64566	5205		
	$s_{within,3}$	0.1	27	33		
	$CV_{within,3}$ (%)	1	0	1		
	x_{avg}	14.7	63797	5045		
	$s_{between}$	1.3	1272	268		
	$CV_{between}$ (%)	9	2	5		
	s	1.2	1179	244		
	CV (%)	8	2	5		

$x_{avg,v}$	within vessel average of 2 samples where v is the replica test vessel number (N=2)
$s_{within,v}$	within vessel Standard Deviation where v is the replica test vessel number (N=2)
$CV_{within,v}$	within vessel Coefficient of Variation where v is the replica test vessel number (N=2)
x_{avg}	average of 3 test vessels (2 samples per test vessel; blank corrected, if the blank values are higher than the reporting limit. Otherwise blank assumed as 0) (N=6)
$s_{between}$	between vessel Standard Deviation (N=3)
$CV_{between}$	between vessel Coefficient of Variation (N=3)
s	overall Standard Deviation (N=6)
CV	overall Coefficient of Variation (N=6)

Annex 2b:

Data of measured temperature, pH and dissolved element concentrations during the 2 hours extraction period of **1 g/L Fe-Cr-Ni Powder Mixture** in the simulated gastric fluid at pH 1.5.
(BLANK CORRECTED if the blank value is higher than reporting limit. Otherwise blank assumed 0.).

1 g/L test item		2h				
pH 1.5 (1 h at 100 rpm)		µg/L Cr	µg/L Fe	µg/L Ni	T	
Accred. Reporting Limit		0.5	10	0.5	(°C)	pH
test medium at 0 h					37.1	1.50
replicaX at 0 h					36.6	1.51
blank	spl1	0.7	<10	<0.5	37.0	1.50
	spl2	0.7	<10	<0.5		
	$x_{avg,1}$	0.7	<10	<0.5		
	$s_{within,1}$	0.0	-	-		
	$CV_{within,1}$ (%)	2	-	-		
replica1	spl1	29.0	238277	27908	36.9	1.51
	spl2	29.0	250430	28256		
	$x_{avg,1}$	29.0	244354	28082		
	$s_{within,1}$	0.0	8594	246		
	$CV_{within,1}$ (%)	0.1	4	1		
replica2	spl1	30.2	252895	29938	37.0	1.51
	spl2	30.0	259516	30574		
	$x_{avg,2}$	30.1	256206	30256		
	$s_{within,2}$	0.2	4682	449		
	$CV_{within,2}$ (%)	0.6	2	1		
replica3	spl1	30.9	256962	29424	36.9	1.50
	spl2	30.8	257708	29203		
	$x_{avg,3}$	30.9	257335	29314		
	$s_{within,3}$	0.1	527	156		
	$CV_{within,3}$ (%)	0.3	0	1		
	x_{avg}	30.0	252631	29217		
	$s_{between}$	1.0	7191	1090		
	$CV_{between}$ (%)	3	3	4		
	s	0.9	7783	1004		
	CV (%)	3	3	3		

$x_{avg,v}$	within vessel average of 2 samples where v is the replica test vessel number (N=2)
$s_{within,v}$	within vessel Standard Deviation where v is the replica test vessel number (N=2)
$CV_{within,v}$	within vessel Coefficient of Variation where v is the replica test vessel number (N=2)
x_{avg}	average of 3 test vessels (2 samples per test vessel; blank corrected, if the blank values are higher than the reporting limit. Otherwise blank assumed as 0) (N=6)
$s_{between}$	between vessel Standard Deviation (N=3)
$CV_{between}$	between vessel Coefficient of Variation (N=3)
s	overall Standard Deviation (N=6)
CV	overall Coefficient of Variation (N=6)

Annex 2c:

Data of measured temperature, pH and dissolved element concentrations during the 2 hours extraction period of **2 g/L Fe-Cr-Ni Powder Mixture** in the simulated gastric fluid at pH 1.5.
(BLANK CORRECTED if the blank value is higher than reporting limit. Otherwise blank assumed 0.).

2 g/L test item		2h				
pH 1.5 (1 h at 100 rpm)		µg/L Cr	µg/L Fe	µg/L Ni	T	
Accred. Reporting Limit		0.5	10	0.5	(°C)	pH
test medium at 0 h					37.1	1.50
replicaX at 0 h					36.8	1.50
blank	spl1	0.7	<10	<0.5	37.0	1.50
	spl2	0.7	<10	<0.5		
	$x_{avg,1}$	0.7	<10	<0.5		
	$s_{within,1}$	0.0	-	-		
	$CV_{within,1}$ (%)	2	-	-		
replica1	spl1	42.7	441711	40643	37.0	1.51
	spl2	42.7	428390	39602		
	$x_{avg,1}$	42.7	435050	40122		
	$s_{within,1}$	0.0	9420	736		
	$CV_{within,1}$ (%)	0.1	2	2		
replica2	spl1	43.6	441333	41611	37.0	1.52
	spl2	43.4	431630	40958		
	$x_{avg,2}$	43.5	436481	41285		
	$s_{within,2}$	0.1	6861	462		
	$CV_{within,2}$ (%)	0.2	2	1		
replica3	spl1	43.0	444260	39695	36.9	1.51
	spl2	43.7	437379	38366		
	$x_{avg,3}$	43.3	440819	39030		
	$s_{within,3}$	0.5	4866	940		
	$CV_{within,3}$ (%)	1.2	1	2		
	x_{avg}	43.2	437450	40146		
	$s_{between}$	0.4	3004	1127		
	$CV_{between}$ (%)	1	1	3		
	s	0.4	6254	1159		
	CV (%)	1	1	3		

$x_{avg,v}$	within vessel average of 2 samples where v is the replica test vessel number (N=2)
$s_{within,v}$	within vessel Standard Deviation where v is the replica test vessel number (N=2)
$CV_{within,v}$	within vessel Coefficient of Variation where v is the replica test vessel number (N=2)
x_{avg}	average of 3 test vessels (2 samples per test vessel; blank corrected, if the blank values are higher than the reporting limit. Otherwise blank assumed as 0) (N=6)
$s_{between}$	between vessel Standard Deviation (N=3)
$CV_{between}$	between vessel Coefficient of Variation (N=3)
s	overall Standard Deviation (N=6)
CV	overall Coefficient of Variation (N=6)

Annex 3a:

Bioaccessibility at 2 hours released from **0.2 g/L Fe-Cr-Ni Powder Mixture** in simulated gastric fluid (BLANK CORRECTED if the blank value is higher than reporting limit. Otherwise blank assumed 0.).

	Cr release (mg/g Test Item)	Fe release (mg/g Test Item)	Ni release (mg/g Test Item)
replica1	0.0808	312	23.7
replica2	0.0694	322	26.0
replica3	0.0700	323	26.0
x_{avg}	0.0734	319	25.2
$s_{between}$	0.0065	6	1.3
CV (%)	9	2	5

x_{avg} = average of 3 test vessels (N=3).
$s_{between}$ = standard deviation (N=3).
CV = Coefficient of Variation (%).

Annex 3b:

Bioaccessibility at 2 hours released from **1 g/L Fe-Cr-Ni Powder Mixture** in simulated gastric fluid (BLANK CORRECTED if the blank value is higher than reporting limit. Otherwise blank assumed 0.).

	Cr release (mg/g Test Item)	Fe release (mg/g Test Item)	Ni release (mg/g Test Item)
replica1	0.0290	244	28.1
replica2	0.0301	256	30.3
replica3	0.0309	257	29.3
x_{avg}	0.0300	253	29.2
$s_{between}$	0.0010	7	1.1
CV (%)	3	3	4

x_{avg} = average of 3 test vessels (N=3).
$s_{between}$ = standard deviation (N=3).
CV = Coefficient of Variation (%).

Annex 3c:

Bioaccessibility at 2 hours released from **2 g/L Fe-Cr-Ni Powder Mixture** in simulated gastric fluid (BLANK CORRECTED if the blank value is higher than reporting limits. Otherwise blank assumed 0.).

	Cr release (mg/g Test Item)	Fe release (mg/g Test Item)	Ni release (mg/g Test Item)
replica1	0.0214	218	20.1
replica2	0.0217	218	20.6
replica3	0.0217	220	19.5
x_{avg}	0.0216	219	20.1
$s_{between}$	0.0002	1.5	0.6
CV (%)	1	1	3

x_{avg} = average of 3 test vessels (N=3).
$s_{between}$ = standard deviation (N=3).
CV = Coefficient of Variation (%).

Annex 4a:

Summarised results of the average dissolved element concentrations, element release per g test item mixture, element release as a percentage of the nominal mixture loading and the percentage of available element eluted, after 2 hours of extraction of **0.2 g/L Fe-Cr-Ni Powder Mixture** in the simulated gastric fluid at pH 1.5:

Fe-Cr-Ni Powder Mixture: 2-h gastric at pH 1.5 at **0.2 g/L** loading			
Analyte		$x_{avg} \pm s_{between}$	CV
chromium (Cr)	dissolution	0.0147 ± 0.0013 mg/L	9 %
	absolute Cr release	0.0734 ± 0.0065 mg/g	9 %
	% mixture released as Cr	0.00734 %	
	% of total available Cr eluted	0.045 %	
iron (Fe)	dissolution	63.8 ± 1.3 mg/L	2 %
	absolute Fe release	319 ± 6 mg/g	2 %
	% mixture released as Fe	31.9 %	
	% of total available Fe eluted	46 %	
nickel (Ni)	dissolution	5.05 ± 0.27 mg/L	5 %
	absolute Ni release	25.2 ± 1.3 mg/g	5 %
	% mixture released as Ni	2.52 %	
	% of total available Ni eluted	24 %	

x_{avg} = average of 3 test vessels (2 samples per test vessel; blank corrected, if the blank values are greater than the reporting limit. Otherwise blank assumed as 0) (N=6).

$s_{between}$ = between vessel standard deviation (N=3).

CV = Coefficient of Variation (%).

Annex 4b:

Summarised results of the average dissolved element concentrations, element release per g test item mixture, element release as a percentage of the nominal mixture loading and the percentage of available element eluted, after 2 hours of extraction of **1 g/L Fe-Cr-Ni Powder Mixture** in simulated gastric fluid at pH 1.5:

Fe-Cr-Ni Powder Mixture: 2-h gastric at pH 1.5 at **1 g/L** loading			
Analyte		$x_{avg} \pm s_{between}$	CV
chromium (Cr)	dissolution	0.0300 ± 0.0010 mg/L	3 %
	absolute Cr release	0.0300 ± 0.0010 mg/g	3 %
	% element release as Cr	0.00300 %	
	% of total available Cr eluted	0.018 %	
iron (Fe)	dissolution	253 ± 7 mg/L	3 %
	absolute Fe release	253 ± 7 mg/g	3 %
	% element release as Fe	25.3 %	
	% of total available Fe eluted	37 %	
nickel (Ni)	dissolution	29.2 ± 1.1 mg/L	4 %
	absolute Ni release	29.2 ± 1.1 mg/g	4 %
	% element release as Ni	2.92 %	
	% of total available Ni eluted	27 %	

x_{avg} = average of 3 test vessels (2 samples per test vessel; blank corrected, if the blank values are greater than the reporting limit. Otherwise blank assumed as 0) (N=6).

$s_{between}$ = between vessel standard deviation (N=3).

CV = Coefficient of Variation (%).

Annex 4c:

Summarised results of the average dissolved element concentrations, element release per g test item mixture, element release as a percentage of the nominal mixture loading and the percentage of available element eluted, after 2 hours of extraction of **2 g/L Fe-Cr-Ni Powder Mixture** in simulated gastric fluid at pH 1.5:

Fe-Cr-Ni Powder Mixture: 2-h gastric at pH 1.5 at **2 g/L** loading			
Analyte		$x_{avg} \pm s_{between}$	CV
chromium (Cr)	dissolution	0.0432 ± 0.0004 mg/L	1 %
	absolute Cr release	0.0216 ± 0.0002 mg/g	1 %
	% element release as Cr	0.00216 %	
	% of total available Cr eluted	0.013 %	
iron (Fe)	dissolution	437 ± 3 mg/L	1 %
	absolute Fe release	219 ± 2 mg/g	1 %
	% element release as Fe	21.9 %	
	% of total available Fe eluted	32 %	
nickel (Ni)	dissolution	40.1 ± 1.1 mg/L	3 %
	absolute Ni release	20.1 ± 0.6 mg/g	3 %
	% element release as Ni	2.01 %	
	% of total available Ni eluted	19 %	

x_{avg} = average of 3 test vessels (2 samples per test vessel; blank corrected, if the blank values are greater than the reporting limit. Otherwise blank assumed as 0) (N=6).

$s_{between}$ = between vessel standard deviation (N=3).

CV = Coefficient of Variation (%).

Annex 5:

A detailed overview of the samples.

Sample no.	Sampling time (h)	pH	Loading (g/L)	Sample treatment	Comment
X02a-130-1	2	1.5	0	filtration (0.2 µm)	Blank replica1 spl1
X02a-130-2	2	1.5	0	filtration (0.2 µm)	Blank replica1 spl2
X02a-130-3	2	1.5	0.2	filtration (0.2 µm)	R0039/01 Reference1 spl1
X02a-130-4	2	1.5	0.2	filtration (0.2 µm)	R0039/01 Reference1 spl2
X02a-130-5	2	1.5	0.2	filtration (0.2 µm)	R0040/02 Reference2 spl1
X02a-130-6	2	1.5	0.2	filtration (0.2 µm)	R0040/02 Reference2 spl2
X02a-130-7	2	1.5	0.2	filtration (0.2 µm)	T0220/01 Reference3 spl1
X02a-130-8	2	1.5	0.2	filtration (0.2 µm)	T0220/01 Reference3 spl2
X02a-130-9	2	1.5	0.2	filtration (0.2 µm)	Fe/Cr/Ni-Mixture replica1 spl1
X02a-130-10	2	1.5	0.2	filtration (0.2 µm)	Fe/Cr/Ni-Mixture replica1 spl2
X02a-130-11	2	1.5	0.2	filtration (0.2 µm)	Fe/Cr/Ni-Mixture replica2 spl1
X02a-130-12	2	1.5	0.2	filtration (0.2 µm)	Fe/Cr/Ni-Mixture replica2 spl2
X02a-130-13	2	1.5	0.2	filtration (0.2 µm)	Fe/Cr/Ni-Mixture replica3 spl1
X02a-130-14	2	1.5	0.2	filtration (0.2 µm)	Fe/Cr/Ni-Mixture replica3 spl2
X02a-130-15	2	1.5	1	filtration (0.2 µm)	Fe/Cr/Ni-Mixture replica1 spl1
X02a-130-16	2	1.5	1	filtration (0.2 µm)	Fe/Cr/Ni-Mixture replica1 spl2
X02a-130-17	2	1.5	1	filtration (0.2 µm)	Fe/Cr/Ni-Mixture replica2 spl1
X02a-130-18	2	1.5	1	filtration (0.2 µm)	Fe/Cr/Ni-Mixture replica2 spl2
X02a-130-19	2	1.5	1	filtration (0.2 µm)	Fe/Cr/Ni-Mixture replica3 spl1
X02a-130-20	2	1.5	1	filtration (0.2 µm)	Fe/Cr/Ni-Mixture replica3 spl2
X02a-130-21	2	1.5	2	filtration (0.2 µm)	Fe/Cr/Ni-Mixture replica1 spl1
X02a-130-22	2	1.5	2	filtration (0.2 µm)	Fe/Cr/Ni-Mixture replica1 spl2
X02a-130-23	2	1.5	2	filtration (0.2 µm)	Fe/Cr/Ni-Mixture replica2 spl1
X02a-130-24	2	1.5	2	filtration (0.2 µm)	Fe/Cr/Ni-Mixture replica2 spl2
X02a-130-25	2	1.5	2	filtration (0.2 µm)	Fe/Cr/Ni-Mixture replica3 spl1
X02a-130-26	2	1.5	2	filtration (0.2 µm)	Fe/Cr/Ni-Mixture replica3 spl2

Annex 6:

Analysis of the dissolved element concentrations at the *Test Site,* WLN

- Analytical Report 17R07555.X02a-130 (8 pages)

WLN Business B.V.
Rijksstraatweg 85
9756 AD Glimmen
telefoon (050) 402 21 21
telefax (050) 409 42 74

e-mail klantenservice@wln.nl
website www.wln.nl

Analytical Report 17R07555.X02a-130

(22/09/2017)

Project: **17R0152/26**

Determination of chromium, iron and nickel in Simulated Gastric Fluid: ECTX Study X02a-130

Submitted to:

ECTX bvba
(Ecotoxicology & Biodegradation)
Havenstraat 46/0.01
B-3500 Hasselt, Belgium

WLN Business B.V., Rijksstraatweg 85, NL-9851 AD Glimmen, The Netherlands
(ISO 17025 Accreditation certificate n° L075 from 12/11/1993, valid until 01/12/2017)

Scientist: **Jan Bonnema** Management: **Jeroen van Nuil / Harm de Kleine**

Analyst (ICP-MS): **Koos Kasper**

Quality Control Manager: **Jeroen Boekenoogen**

WLN Report 17R07555.X02a-130

CONTENTS

	CONFIDENTIALITY AND RETENTION PERIODS, DATA AND MATERIALS	3
	DISCLAIMER	3
1.	INTRODUCTION	4
2.	ANALYTICAL METHODOLOGY	4
3.	DEVIATIONS FROM THE SOP IN RELATION TO THE EXECUTED ANALYTICAL METHOD	5
4.	COMMENTS CONCERNING THE SAMPLES	5
5.	COMMENTS CONCERNING THE ANALYSIS	5
6.	RESULTS	6
7.	REFERENCES	7
	APPENDIX	8

CONFIDENTIALITY AND RETENTION PERIODS, DATA AND MATERIALS

WLN supplies data and results to the client only. Information to third parties will only take place after receiving a written consent from the client. Samples will be stored under controlled conditions until the research has been fully completed. This period can be extended on the clients' request. All information regarding the analyses or/and research, including rough analysis data, will be kept over a period of at least 5 years. A copy of the raw analysis data will be handed to the study director of ECTX after finalisation of the analytical report.

DISCLAIMER

Any checks, opinions and interpretations in the report fall outside of the RvA accreditation. The results, if relevant, only concern the analysed items.

Any determination and/or reference made in this report with respect to any specific commercial product, process or service by trade name, trademark, manufacturer or otherwise shall be considered to be opinion; WLN makes no, and does not intend to make any, representations or implied warranties of merchantability or fitness for a particular purpose nor is it intended to endorse, recommend or favour any specific commercial product, process or service. The views and opinions of authors expressed herein do not necessarily state or reflect those of WLN and may not be used for advertising or product endorsement purposes.

1. INTRODUCTION

At the request of ECTX, we have determined the concentrations of chromium (**Cr**), iron (**Fe**) and nickel (**Ni**) in Simulated Gastric Fluid solutions (0.07N HCl) that they sent to us for analysis. The data may be a component of the dossiers submitted to the REACH (Registration, Evaluation and Authorisation of Chemicals) registry, an environmental protection measure within the framework of the European Union (EU), reporting provisions of which producers, importers, users and distributors are obligated to meet in order to maintain access to their EU markets.

In this report, we present the analyses for **Cr**, **Fe** and **Ni** in ECTX samples of study X02a-130 as determined by the analytical laboratory of WLN. The sampling was performed by ECTX.

2. ANALYTICAL METHODOLOGY

The analyses of chromium, iron and nickel is performed according to our Standard Operating Procedure "WLN-CM.W.11.1: Determination of the metal content in water by means of an inductively coupled plasma and mass-spectrometry", which is conform NEN-EN-ISO 17294-2:2016 Water quality - Application of inductively coupled plasma mass spectrometry (ICP-MS) - Part 2: "Determination of selected elements including uranium isotopes" and which was valid at the time when the analyses were performed.

Waterlaboratorium Noord (WLN) used a Thermo Fisher Scientific iCAP Q ICP-MS (inductively-coupled plasma-mass spectrometer) to determine the total dissolved concentrations for the elements chromium (**Cr**), iron (**Fe**) and nickel (**Ni**) in the as-received test and blank matrix solutions. To prepare blanks, calibration and control solutions/dilutions we always used de-ionized water (d.i. H_2O) at a purity level of 18 MΩ.

The ICP-MS was calibrated at the start of each run. Recalibration was performed every 25 samples and again at the end of the run. These recalibrations were also used for instrument drift corrections.

The linearity (2x calibration level) and the factors of interference were determined and checked after the initial calibration.

The calibration standard solution used was a custom made multi-element standard (iV inorganic Ventures).

Matrix corrections were performed by the online addition of an internal standard.

Each run also contained at least one blank procedure.

All measurements were checked for "carry over" as also the RSD of the three-integrations of each measurement.

For each measurement the 'drift' of the internal standard was checked.

The calibrations were checked by means of an independent control standard (another supplier, either SCP Science or J.T. Baker), which was prepared using single element standards.

All measured values of the control standards were evaluated against the control charts. These values were expected to be within 2 sigma levels of the control.

The last control sample value was transferred to the WLN laboratory information management system (LIMS).

Table 1: Operating conditions for Thermo Fisher Scientific iCAP Q ICP-MS

- Instrument: **Thermo Fisher Scientific iCAP Q ICP-MS (serial no.: 730R, WLN no.: 730000 / serial no.: 731R, WLN no.: 731000).**
- Forward power: **1550** W
- Cool gas flow rate: **14.00** L/min
- Auxiliary gas flow rate: **0.80** L/min
- Number of replicates analyses: **1 (average of 3 integrations)**
- Isotope monitored: **^{52}Cr, ^{56}Fe and ^{60}Ni**

Table 2: Performance characteristics - Measured parameters accredited for drinking water, groundwater, surface water

- Reporting limit: **0.5 µg/L** for Cr and Ni; and **10 µg/L** for Fe (= according to our SOP)
- Repeatability: **≤10%** (at the reporting limits)
- Reproducibility: **≤10%** (at the reporting limits)
- Accuracy: **90-110%** (at the reporting limits)

The operating conditions of each run are described in a performance report which is printed and archived.

All data are handled and stored according to our quality system.

3. DEVIATIONS FROM THE SOP IN RELATION TO THE EXECUTED ANALYTICAL METHOD

On request of the study director, raw data instead of rounded data (according to our SOP) were reported as also values below the validated reporting limit. For values below the validated reporting limits an accuracy between 90-110% cannot be guaranteed.

4. COMMENTS CONCERNING THE SAMPLES

None.

5. COMMENTS CONCERNING THE ANALYSIS

None.

6. RESULTS

The Table of analytical determinations are in the Appendix as follows:

- □ Annex 1: Concentrations of Cr, Fe and and Ni in sample solution X02a-130-1 to X02a-130-26 (in µg/L).

7. REFERENCES

a) WLN-CM.W.11.1 Determination of the metal content in water by means of an inductively coupled plasma and mass-spectrometry.

b) NEN-EN-ISO 17294-2:2016 : Water quality - Application of inductively coupled plasma mass spectrometry (ICP-MS) - Part 2: Determination of selected elements including uranium isotopes.

APPENDIX

Annex 1: Concentrations of Cr, Fe and Ni in sample solution X02a-130-1 to X02a-130-26 (in µg/L).

Parameter	Description	Standard	
Procedure	Accreditation		
Cr	Chromium	WLN-CM.W.11.1	RD
Fe	Iron	WLN-CM.W.11.1	RD
Ni	Nickel	WLN-CM.W.11.1	RD

Sample Number	Description (ECTX)	Sampling Date	Date of Analysis	Cr (µg/L)	Fe (µg/L)	Ni (µg/L)
1748109	X02a-130-1	19-09-2017	21-09-2017	0.7	5	0.3
1748110	X02a-130-2	19-09-2017	21-09-2017	0.7	4	0.3
1748111	X02a-130-3	19-09-2017	21-09-2017	0.8	85854	0.5
1748112	X02a-130-4	19-09-2017	21-09-2017	0.7	82525	0.3
1748113	X02a-130-5	19-09-2017	21-09-2017	20.7	5	0.5
1748114	X02a-130-6	19-09-2017	21-09-2017	20.9	4	0.4
1748115	X02a-130-7	19-09-2017	21-09-2017	0.9	7	32340
1748116	X02a-130-8	19-09-2017	21-09-2017	0.8	6	32753
1748117	X02a-130-9	19-09-2017	21-09-2017	16.8	62755	4800
1748118	X02a-130-10	19-09-2017	21-09-2017	17.0	61903	4672
1748119	X02a-130-11	19-09-2017	21-09-2017	14.6	64258	5186
1748120	X02a-130-12	19-09-2017	21-09-2017	14.6	64735	5204
1748121	X02a-130-13	19-09-2017	21-09-2017	14.8	64547	5228
1748122	X02a-130-14	19-09-2017	21-09-2017	14.7	64585	5182
1748123	X02a-130-15	19-09-2017	21-09-2017	29.7	238277	27908
1748124	X02a-130-16	19-09-2017	21-09-2017	29.8	250430	28256
1748125	X02a-130-17	19-09-2017	21-09-2017	31.0	252895	29938
1748126	X02a-130-18	19-09-2017	21-09-2017	30.7	259516	30574
1748127	X02a-130-19	19-09-2017	21-09-2017	31.7	256962	29424
1748128	X02a-130-20	19-09-2017	21-09-2017	31.6	257708	29203
1748129	X02a-130-21	19-09-2017	21-09-2017	43.5	441711	40643
1748130	X02a-130-22	19-09-2017	21-09-2017	43.4	428390	39602
1748131	X02a-130-23	19-09-2017	21-09-2017	44.3	441333	41611
1748132	X02a-130-24	19-09-2017	21-09-2017	44.1	431630	40958
1748133	X02a-130-25	19-09-2017	21-09-2017	43.7	444260	39695
1748134	X02a-130-26	19-09-2017	21-09-2017	44.4	437379	38366

RD: raw data, not rounded as mentioned in our standard operating procedures

Annex 7:

Certificate of Analysis of the **Chromium powder** (Sigma-Aldrich, 2 pages).

SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, Saint Louis, MO 63103, USA
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:
Chromium – powder, ≥99% trace metals basis, –325 mesh

Cr

Product Number:	**266299**
Batch Number:	**MKBP5427V**
Brand:	ALDRICH
CAS Number:	7440-47-3
MDL Number:	MFCD00010944
Formula:	Cr
Formula Weight:	52.00 g/mol
Quality Release Date:	10 JUN 2013

Test	Specification	Result
Appearance (Color) Grey	Conforms to Requirements	Grey
Appearance (Form)	Powder	Powder
X-Ray Diffraction	Conforms to Structure	Conforms
Size	≤ 45.0 micron	42.0 micron
Trace Metal Analysis	≤ 10000.0 ppm	7853.5 ppm
Aluminum (Al)		1993.1 ppm
Boron (B)		21.2 ppm
Barium (Ba)		2.3 ppm
Calcium (Ca)		15.0 ppm
Cobalt (Co)		2.0 ppm
Copper (Cu)		14.3 ppm
Iron (Fe)		5168.7 ppm
Potassium (K)		24.4 ppm
Lithium (Li)		0.9 ppm
Magnesium (Mg)		41.3 ppm
Manganese (Mn)		157.1 ppm
Sodium (Na)		11.3 ppm
Nickel (Ni)		87.1 ppm
Titanium (Ti)		90.6 ppm
Vanadium (V)		96.0 ppm
Zinc (Zn)		128.2 ppm
Purity > = 99% Based On Trace Metals Analysis	Conforms	Conforms

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Version Number: 1

Page 1 of 2

SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, Saint Louis, MO 63103, USA
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Number: **266299**
Batch Number: **MKBP5427V**

Jamie Gleason, Manager
Quality Control
Milwaukee, Wisconsin US

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Version Number: 1

Annex 8:

Certificate of Analysis of the specific surface (N-BET) analysis, the particle size distribution (PSD) analysis of the **Chromium powder** (Umicore analytical Competence Center, 2 pages).

Umicore
Analytical Competence Center
Watertorenstraat 33
B-2250 OLEN

CoA 2017-0107

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CERTIFICATE OF ANALYSIS ***Page No. 1 of 2***

Certificate Date	**03/10/2017**	Certificate No.	Lab LIMS No.	Your Ref. / Order No.
Date of Receipt	**29/09/2017**	**2017-0107**	**B-2017-0053150**	**Tel from 18/09/2017**

Supplier:
Tony Brouwers
ECTX bvba
Havenstraat 46/0.01
3500 Hasselt
Belgium

Sample Identification	R0040/01 Chromium powder SA 266299

Analysis result of BET and PSD analysis:

BET: 0.08 m²/g

Particle size distribution: on Page 2

Abstract Tests & Conditions:

- **BET analysis:**
  The determination of the specific surface, expressed in m²/g, is based on the adsorption of N2 molecules on the surface of powder particles. At a relative pressure of 30% and at liquid N2 temperature, the amount of adsorbed N2 atoms is related to the total available surface of all powder particles.
  The used equipment to measure the resulting pressure drop around a certain mass of powder is the 'Tristar 3000' from Micromeritics with measuring range: 0.02-215 m²/g.
- **PSD analysis:**
  Particle size distribution on sample as such using Sympatec Helos laser diffraction. Laser diffraction is an optical measuring process, where the diffraction pattern caused by the presence of particles in the path of a laser beam is measured. The dispersion of the particles in the laser beam is realised by compressed air on dry powder.
  The diameter of the diffraction ring generated is inversely proportional to the size of the particles present, while the distribution of the light energy in the diffraction pattern contains the information on their quantity.

Certificate signed by

☒ Eddy Boydens
Lab Manager

Liesbet Jongen
Lab Manager

Marc Sterckx
Lab Manager

Signed:



The results of analyses in this report are only valid for the tested sample(s).
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Registered Office Broekstraat 31 Rue du Marais, B-1000 Brussels, Belgium

Umicore
Analytical Competence Center
Watertorenstraat 33
B-2250 OLEN

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CERTIFICATE OF ANALYSIS

Particle size distribution

Sympatec GmbH
System-Partikel-Technik

HELOS Particle Size Analysis
WINDOX 5

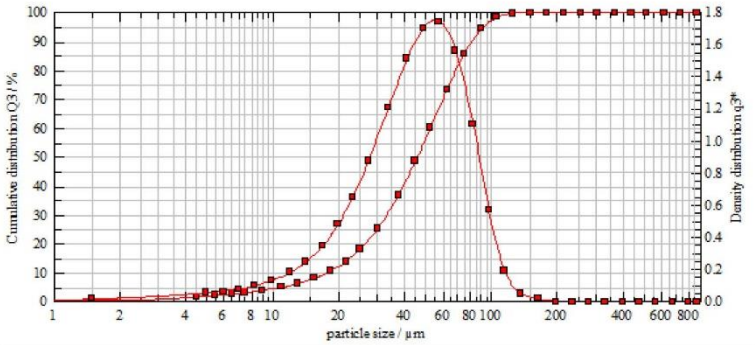
UMICORE

HELOS (H1920) & RODOS, R5: 0.5/4.5...875µm **2017-10-04, 14:15:13**,311

Divers B-2017-0053150 R0040-01

x_1 = 3,08 µm	x_{16} = 23,27 µm	x_{95} = 92,88 µm
x_2 = 5,51 µm	x_{50} = 45,91 µm	x_{98} = 104,75 µm
x_5 = 11,23 µm	x_{84} = 73,47 µm	x_{99} = 117,86 µm
x_{10} = 17,72 µm	x_{90} = 82,69 µm	x_{100} = 180,00 µm

Optische concentratie: 7,98 %



comment:
Chromium powder SA 266299

user parameters:
Gebruiker: jvdv
Identifier: B-2017-0053150 R0040-01
Parameter 3:

cumulative distribution

x_0/µm	Q_3/%	x_0/µm	Q_3/%	x_0/µm	Q_3/%	x_0/µm	Q_3/%
4,50	1,55	18,50	10,70	75,00	85,51	305,00	100,00
5,50	2,00	21,50	13,85	90,00	94,27	365,00	100,00
6,50	2,46	25,00	18,10	105,00	98,06	435,00	100,00
7,50	2,94	30,00	25,05	125,00	99,52	515,00	100,00
9,00	3,71	37,50	36,69	150,00	99,88	615,00	100,00
11,00	4,85	45,00	48,63	180,00	100,00	735,00	100,00
13,00	6,15	52,50	59,99	215,00	100,00	875,00	100,00
15,50	8,03	62,50	73,16	255,00	100,00		

evaluation: WINDOX 5.7.1.0, *HRLD*
revalidation:
reference measurement: 10-04 14:14:56
contamination: 0,00 %

Measurement duration
Measurement duration: 1,00 s

C_{opt}= 7,98 %

trigger condition: opt conc 2%
time base: 100,00 ms
start: c.opt >= 2,1%
valid: always
stop: 5s c.opt <= 1,9% or 99s real time

dispersing method: 3.0bar;100%;1.0mm;delay20s
cascade: 0
pressure: 3,04 bar, vacuum: 98,00 mbar
revolution: 100,00 %
doser: VIBRI, feed rate: 100,00 %

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Annex 9:

Certificate of Analysis of the **Iron powder** (Sigma-Aldrich, 1 page).

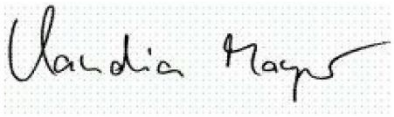
SIGMA-ALDRICH®

3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sial.com Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:	IRON powder, –325mesh, 97 %
Product Number:	209309
Batch Number:	STBG7186
Brand:	Aldrich
CAS Number:	7439-89-6
Formula:	Fe
Formula Weight:	55.85
Quality Release Date:	07 DEC 2016

TEST	SPECIFICATION	RESULT
APPEARANCE (COLOR)	GREY	GREY
APPEARANCE (FORM)	POWDER	POWDER
THIOSULFATE TITRATION	96.5 - 103.5 %	98.5 %
PARTICLE SIZE	-325 MESH	CONFIRMED
X-RAY DIFFRACTION	CONFORMS TO STRUCTURE	CONFORMS



Claudia Mayer
Manager Quality Control
Steinheim, Germany

Sigma-Aldrich warrants that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Annex 10:

Certificate of Analysis of the specific surface (N-BET) analysis, the particle size distribution (PSD) analysis of the **Iron powder** (Umicore analytical Competence Center, 2 pages).

Umicore
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Watertorenstraat 33
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CERTIFICATE OF ANALYSIS ***Page No. 1 of 2***

Certificate Date	**03/10/2017**	Certificate No.	Lab LIMS No.	Your Ref. / Order No.
Date of Receipt	**29/09/2017**	**2017-0106**	**B-2017-0053149**	**Tel from 18/09/2017**

Supplier:
Tony Brouwers
ECTX bvba
Havenstraat 46/0.01
3500 Hasselt
Belgium

Sample Identification	R0039/01 Iron powder SA 209309

Analysis result of BET and PSD analysis:

BET: 0.30 m²/g

Particle size distribution: on Page 2

Abstract Tests & Conditions:

- **BET analysis:**
  The determination of the specific surface, expressed in m²/g, is based on the adsorption of N2 molecules on the surface of powder particles. At a relative pressure of 30% and at liquid N2 temperature, the amount of adsorbed N2 atoms is related to the total available surface of all powder particles.
  The used equipment to measure the resulting pressure drop around a certain mass of powder is the 'Tristar 3000' from Micromeritics with measuring range: 0.02-215 m²/g.
- **PSD analysis:**
  Particle size distribution on sample as such using Sympatec Helos laser diffraction. Laser diffraction is an optical measuring process, where the diffraction pattern caused by the presence of particles in the path of a laser beam is measured. The dispersion of the particles in the laser beam is realised by compressed air on dry powder.
  The diameter of the diffraction ring generated is inversely proportional to the size of the particles present, while the distribution of the light energy in the diffraction pattern contains the information on their quantity.

Certificate signed by

☒ Eddy Boydens
Lab Manager

Liesbet Jongen
Lab Manager

Marc Sterckx
Lab Manager

Signed:



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CoA 2017-0106

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Research & Development

CERTIFICATE OF ANALYSIS

Particle size distribution

Sympatec GmbH
System-Partikel-Technik

HELOS Particle Size Analysis
WINDOX 5

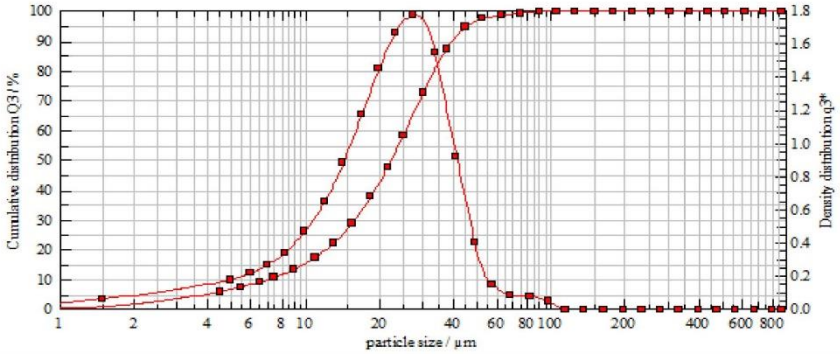
UMICORE

HELOS (H1920) & RODOS, R5: 0.5/4.5...875µm **2017-10-04, 14:02:09,441**

Divers B-2017-0053149 R0039-01

x_1 = 1,18 µm	x_{16} = 10,31 µm	x_{95} = 46,15 µm
x_2 = 1,86 µm	x_{50} = 22,34 µm	x_{98} = 58,79 µm
x_5 = 3,91 µm	x_{84} = 35,85 µm	x_{99} = 73,48 µm
x_{10} = 7,12 µm	x_{90} = 40,28 µm	x_{100} = 105,00 µm

Optische concentratie: 13,13 %



comment:
Iron powder SA 209309

user parameters:
Gebruiker: jvdv
Identifier: B-2017-0053149 R0039-01
Parameter 3:

cumulative distribution

x_0/µm	Q_3/%	x_0/µm	Q_3/%	x_0/µm	Q_3/%	x_0/µm	Q_3/%
4,50	5,86	18,50	37,87	75,00	99,08	305,00	100,00
5,50	7,41	21,50	47,38	90,00	99,70	365,00	100,00
6,50	8,98	25,00	58,30	105,00	100,00	435,00	100,00
7,50	10,63	30,00	72,33	125,00	100,00	515,00	100,00
9,00	13,33	37,50	87,30	150,00	100,00	615,00	100,00
11,00	17,42	45,00	94,59	180,00	100,00	735,00	100,00
13,00	22,13	52,50	97,29	215,00	100,00	875,00	100,00
15,50	28,84	62,50	98,42	255,00	100,00		

evaluation: WINDOX 5.7.1.0, *HRLD*
revalidation:
reference measurement: 10-04 14:01:53
contamination: 0,00 %

Measurement duration
Measurement duration: 1,31 s

C_{opt}= 13,13 %

trigger condition: opt conc 2%
time base: 100,00 ms
start: c.opt >= 2,1%
valid: always
stop: 5s c.opt <= 1,9% or 99s real time

dispersing method: 3.0bar;100%;1.0mm;delay20s
cascade: 0
pressure: 3,03 bar, vacuum: 99,00 mbar
revolution: 100,00 %
doser: VIBRI, feed rate: 100,00 %

UMICORE n.v.
Analytical Competence Center Olen
Watertorenstraat 33,
B – 2250 Olen,
BELGIUM

Tel +32-14 24 51 49
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VAT (BE) 0 401 574 852
Bank 210-0053806-23
HR Turnhout 64 124
Registered Office Broekstraat 31 Rue du Marais, B-1000 Brussels, Belgium

Annex 11:

Certificate of Analysis of the **Nickel powder** (PIXE Test Report - Elemental Analysis, Inc., 4 pages).

Element Analysis Corporation
101 Venture Ct. Ste. B-1
Lexington, KY 40510
Tel: 1-800-282-PIXE(7493)
Email Technical: PIXE@IGLOU.COM

Date: 12/19/01
Run: 267 267
Job: 8701-01
Name: Particle Technology Labs
Project: Kelli Hubert
Target: Nickel Powder

Element Name	Energy (keV)	Det. Limit 95% Conf.	Concentration Mass	Error
* O	----------		0.001 % ±	
Na	1.041	0.131 %		
Mg	1.254	422.300 ppm		
Al	1.485	204.000 ppm		
Si	1.740	137.000 ppm		
P	2.014	103.600 ppm		
S	2.308	91.780 ppm		
Cl	2.623	79.680 ppm		
K	3.314	44.690 ppm		
Ca	3.692	41.460 ppm		
Sc	4.091	45.400 ppm		
Ti	4.511	44.010 ppm		
V	4.952	41.840 ppm		
Cr	5.415	47.950 ppm		
Mn	5.899	75.600 ppm		
Fe	6.404	100.300 ppm		
Co	6.930	119.600 ppm		
Nickel	7.478	154.200 ppm	99.999 % ±	1.000 %
Cu	8.048	369.300 ppm		
Zn	8.639	118.700 ppm		
Ga	9.250	70.640 ppm		
Ge	9.887	88.120 ppm		
As	10.544	55.260 ppm		
Se	11.222	77.310 ppm		
Br	11.924	130.200 ppm		
Rb	13.395	133.700 ppm		
Sr	14.165	179.500 ppm		
Y	1.922	263.900 ppm		
Zr	2.043	234.300 ppm		
Nb	2.166	218.800 ppm		
Mo	2.294	207.000 ppm		
Tc	2.424	177.500 ppm		
Ru	2.559	179.000 ppm		
Rh	2.697	154.500 ppm		
Pd	2.839	133.800 ppm		
Ag	2.984	123.800 ppm		
Cd	3.133	122.700 ppm		
In	3.286	128.400 ppm		
Sn	3.444	121.900 ppm		
Sb	3.604	122.500 ppm		
Te	3.768	106.700 ppm		
I	3.937	114.200 ppm		
Cs	4.288	122.600 ppm		
Ba	4.466	134.300 ppm		
La	4.648	124.700 ppm		
Ce	4.841	120.300 ppm		
Pr	5.034	137.000 ppm		
Nd	5.230	144.800 ppm		
Pm	5.431	160.700 ppm		
Sm	5.632	319.200 ppm		
Eu	5.841	263.800 ppm		
Gd	6.050	191.900 ppm		
Tb	6.271	220.400 ppm		
Dy	6.492	270.900 ppm		
Ho	6.725	234.100 ppm		
Er	6.945	316.000 ppm		
Tm	7.182	610.100 ppm		
Yb	7.416	0.776 %		
Lu	7.655	0.262 %		
Hf	7.899	486.000 ppm		
Ta	8.149	0.269 %		
W	8.398	0.530 %		
Re	8.652	312.000 ppm		

* It should be noted that the low atomic number elements (C,O,H,N,etc) represent an unanalyzed component to the sample. Please see interpretation sheet for more information.

Element Analysis Corporation
101 Venture Ct. Ste. B-1
Lexington, KY 40510
Tel: 1-800-282-PIXE(7493)
Email Technical: PIXE@IGLOU.COM

Date: 12/19/01
Run: 267 267
Job: 8701-01
Name: Particle Technology Labs
Project: Kelli Hubert
Target: Nickel Powder

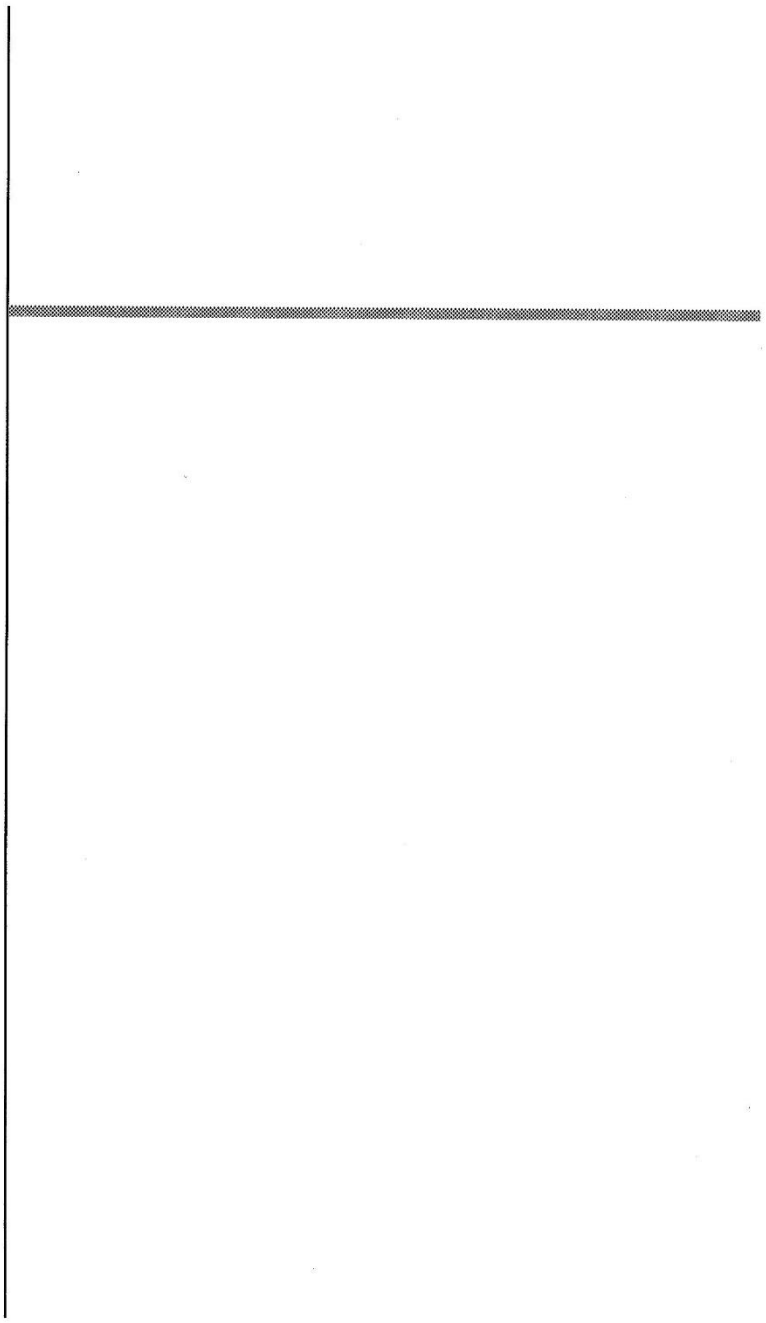
Element Name	Energy (keV)	Det. Limit 95% Conf.	Concentration Mass	Error
Os	8.911	228.900 ppm		
Ir	9.174	212.500 ppm		
Pt	9.443	179.100 ppm		
Au	9.712	191.300 ppm		
Hg	9.989	208.600 ppm		
Tl	10.267	195.200 ppm		
Pb	10.551	145.300 ppm		
Bi	10.838	226.200 ppm		
Th	12.968	306.900 ppm		
U	13.616	385.400 ppm		

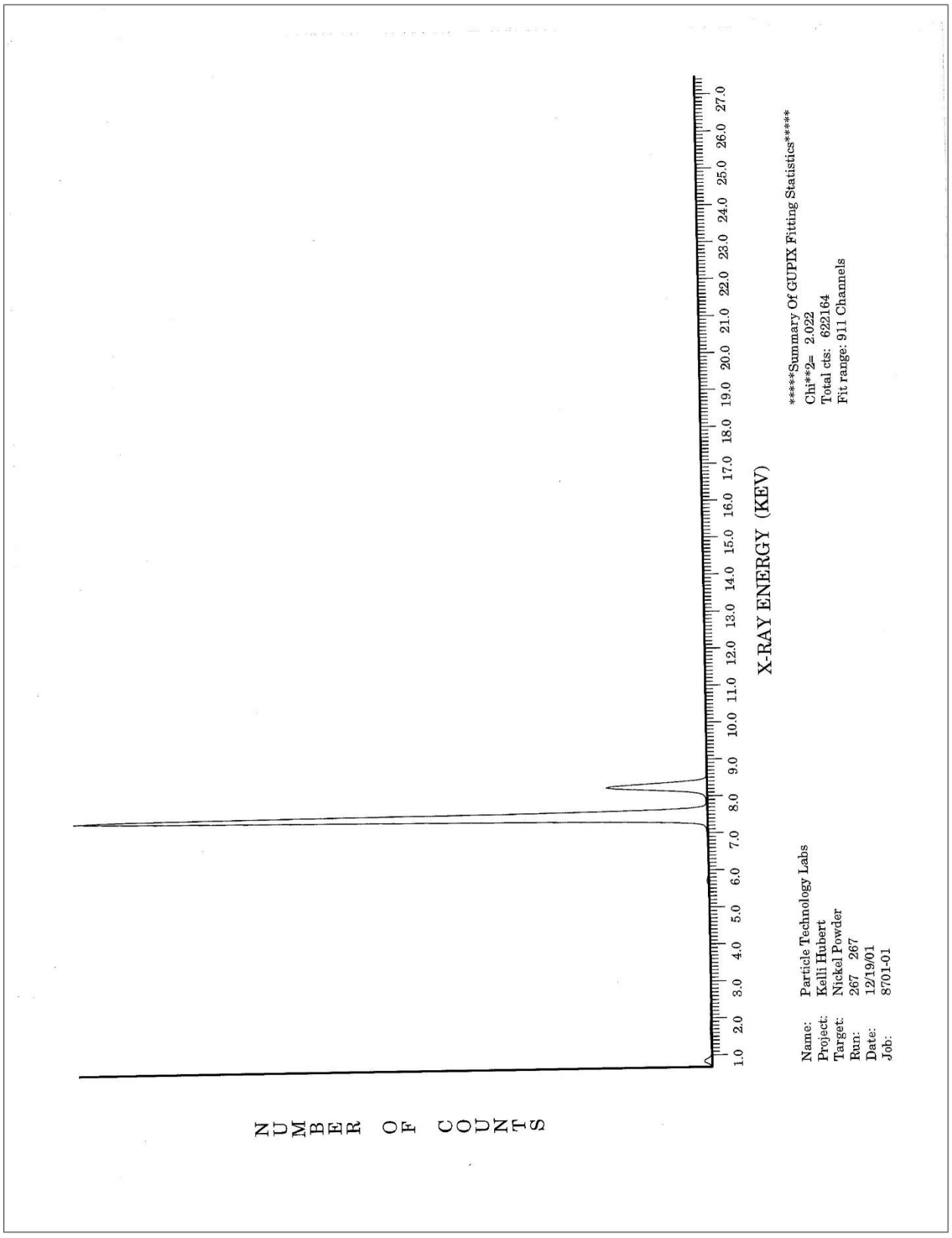
Name: Particle Technology Labs
Project: Kelli Hubert
Target: Nickel Powder

Run: 267 267
Date: 12/19/01
Job: 8701-01

Histogram: Square Root Scaled
Normalization: Internal

Na	[0.131	%]	
Mg	[422.300	ppm]	
Al	[204.000	ppm]	
Si	[137.000	ppm]	
P	[103.600	ppm]	
S	[91.780	ppm]	
Cl	[79.680	ppm]	
K	[44.690	ppm]	
Ca	[41.460	ppm]	
Sc	[45.400	ppm]	
Ti	[44.010	ppm]	
V	[41.840	ppm]	
Cr	[47.950	ppm]	
Mn	[75.600	ppm]	
Fe	[100.300	ppm]	
Co	[119.600	ppm]	
Nickel	99.999	% ±	1.000
Cu	[369.300	ppm]	
Zn	[118.700	ppm]	
Ga	[70.640	ppm]	
Ge	[88.120	ppm]	
As	[55.260	ppm]	
Se	[77.310	ppm]	
Br	[130.200	ppm]	
Rb	[133.700	ppm]	
Sr	[179.500	ppm]	
Y	[263.900	ppm]	
Zr	[234.300	ppm]	
Nb	[218.800	ppm]	
Mo	[207.000	ppm]	
Tc	[177.500	ppm]	
Ru	[179.000	ppm]	
Rh	[154.500	ppm]	
Pd	[133.800	ppm]	
Ag	[123.800	ppm]	
Cd	[122.700	ppm]	
In	[128.400	ppm]	
Sn	[121.900	ppm]	
Sb	[122.500	ppm]	
Te	[106.700	ppm]	
I	[114.200	ppm]	
Cs	[122.600	ppm]	
Ba	[134.300	ppm]	
La	[124.700	ppm]	
Ce	[120.300	ppm]	
Pr	[137.000	ppm]	
Nd	[144.800	ppm]	
Pm	[160.700	ppm]	
Sm	[319.200	ppm]	
Eu	[263.800	ppm]	
Gd	[191.900	ppm]	
Tb	[220.400	ppm]	
Dy	[270.900	ppm]	
Ho	[234.100	ppm]	
Er	[316.000	ppm]	
Tm	[610.100	ppm]	
Yb	[0.776	%]	
Lu	[0.262	%]	
Hf	[486.000	ppm]	
Ta	[0.269	%]	
W	[0.530	%]	
Re	[312.000	ppm]	
Os	[228.900	ppm]	
Ir	[212.500	ppm]	
Pt	[179.100	ppm]	
Au	[191.300	ppm]	
Hg	[208.600	ppm]	
Tl	[195.200	ppm]	
Pb	[145.300	ppm]	
Bi	[226.200	ppm]	
Th	[306.900	ppm]	
U	[385.400	ppm]	




NUMBER OF COUNTS
1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0 16.0 17.0 18.0 19.0 20.0 21.0 22.0 23.0 24.0 25.0 26.0 27.0
X-RAY ENERGY (KEV)
*****Summary Of GUPIX Fitting Statistics*****
Chi**2= 2.022
Total cts: 622164
Fit range: 911 Channels
Name: Particle Technology Labs
Project: Kelli Hubert
Target: Nickel Powder
Run: 267 267
Date: 12/19/01
Job: 8701-01

Annex 12:

Laboratory report of the particle size distribution analysis of the **Nickel powder** (Particle Technology Labs, 1 page).

MALVERN INSTRUMENTS MASTERSIZER

Result: Histogram Report

Sample Details

Sample ID: N36-PTL
Sample File: 4968
Sample Path: A:\
Sample Notes: NiPERA Nickel Powder SNP-122
Carrier/Dispersant: Canola Oil
Ultrasonic: 1 Minute
Particle Technology Labs

Run Number: 2
Record Number: 3
Analyst: AG
PTL: N36-PTL

Measured: Thu Nov 15 2001 11:37AM
Analysed: Thu Mar 14 2002 10:40AM
Result Source: Analysed

System Details

Range Lens: 300RF mm
Presentation: 3_NIPOW
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.9800, 0.1000); Dispersant R.I. = 1.4712]

Sampler: MS1

Obscuration: 22.1 %
Residual: 2.090 %

Result Statistics

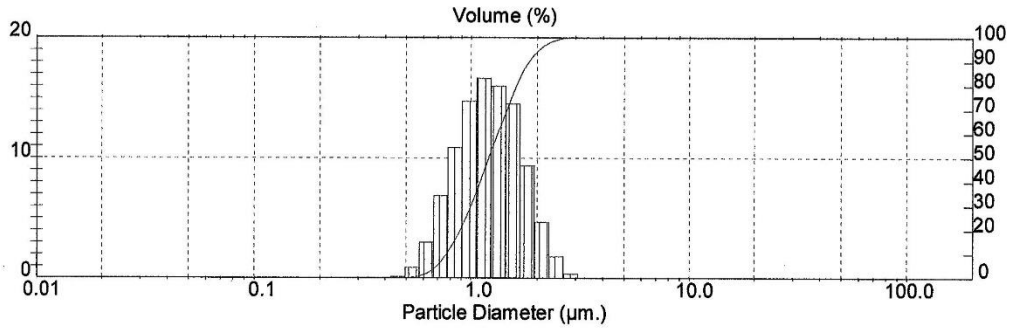
Distribution Type: Volume
Mean Diameters:
D [4, 3] = 1.26 um

Concentration = 0.0029 %Vol
D (v, 0.1) = 0.77 um
D [3, 2] = 1.13 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 1.20 um
Span = 8.916E-01

Specific S.A. = 5.3068 sq. m / g
D (v, 0.9) = 1.84 um
Uniformity = 2.826E-01

Size (um)	Volume Under %	Size (um)	Volume Under %	Size (um)	Volume Under %	Size (um)	Volume Under %
0.055	0.00	0.635	2.60	7.31	100.00	84.15	100.00
0.061	0.00	0.700	5.57	8.06	100.00	92.79	100.00
0.067	0.00	0.772	10.26	8.89	100.00	102.3	100.00
0.074	0.00	0.851	16.56	9.80	100.00	112.8	100.00
0.082	0.00	0.938	24.53	10.81	100.00	124.4	100.00
0.090	0.00	1.03	34.04	11.91	100.00	137.2	100.00
0.099	0.00	1.14	44.54	13.14	100.00	151.3	100.00
0.109	0.00	1.26	55.19	14.49	100.00	166.8	100.00
0.121	0.00	1.39	65.45	15.97	100.00	183.9	100.00
0.133	0.00	1.53	75.39	17.62	100.00	202.8	100.00
0.147	0.00	1.69	84.20	19.42	100.00	223.6	100.00
0.162	0.00	1.86	90.70	21.42	100.00	246.6	100.00
0.178	0.00	2.05	95.03	23.62	100.00	271.9	100.00
0.196	0.00	2.26	97.69	26.04	100.00	299.8	100.00
0.217	0.00	2.49	99.14	28.72	100.00	330.6	100.00
0.239	0.00	2.75	99.80	31.66	100.00	364.6	100.00
0.263	0.00	3.03	99.99	34.92	100.00	402.0	100.00
0.290	0.00	3.34	100.00	38.50	100.00	443.3	100.00
0.320	0.00	3.69	100.00	42.45	100.00	488.8	100.00
0.353	0.00	4.07	100.00	46.81	100.00	539.0	100.00
0.389	0.00	4.48	100.00	51.62	100.00	594.3	100.00
0.429	0.00	4.94	100.00	56.92	100.00	655.4	100.00
0.473	0.08	5.45	100.00	62.76	100.00	722.7	100.00
0.522	0.31	6.01	100.00	69.21	100.00	796.9	100.00
0.576	1.05	6.63	100.00	76.32	100.00	878.7	100.00



Malvern Instruments Ltd.
Malvern, UK
Tel:=+[44] (0)1684-892456 Fax:+[44] (0)1684-892789

Mastersizer S long bed Ver. 2.18
Serial Number:

p. 2
14 Mar 02 10:41

Annex 13:

Laboratory report of the specific surface (N-BET) analysis of the **Nickel powder** (Particle Technology Labs, 2 pages).

Date: 11/27/2001 Page 1

Particle Technology Labs, Ltd.
Quantachrome Autosorb Automated Gas Sorption System Report
Autosorb for Windows® Version 1.16

Sample ID	N36-PTL				
Description	NIPERA				
Comments	4968 N36-PTL Nickel Powder SNP-122				
Sample Weight	1.3649 g				
Adsorbate	NITROGEN	Outgas Temp	200.0 °C	Operator	ED
Cross-Sec Area	16.2 Å²/molecule	Outgas Time	5.0 hrs	Analysis Time	41.5 min
NonIdeality	6.580E-05	P/Po Toler	4	End of Run	11/19/01 07:13PM
Molecular Wt	28.0134 g/mol	Equil Time	2	File Name	AS0B1501.RAW
Station #	1	Bath Temp.	0.00		

MULTIPOINT BET

P/Po	Volume [cc/g] STP	1/(W((Po/P)-1))
1.1300e-01	0.6301	1.618E+02
1.6300e-01	0.6814	2.287E+02
2.1300e-01	0.7327	2.956E+02

Area = 2.582E+00 m²/g

Slope = 1.338E+03

Y - Intercept = 1.059E+01

Correlation Coefficient = 1.000000

C = 1.273E+02

Date: 11/27/2001

Particle Technology Labs, Ltd.
Quantachrome Autosorb Automated Gas Sorption System Report
Autosorb for Windows® Version 1.16

Sample ID	N36-PTL				
Description	NIPERA				
Comments	4968 N36-PTL Nickel Powder SNP-122				
Sample Weight	1.3649 g				
Adsorbate	NITROGEN	**Outgas Temp**	200.0 °C	**Operator**	ED
Cross-Sec Area	16.2 Å²/molecule	**Outgas Time**	5.0 hrs	**Analysis Time**	41.5 min
NonIdeality	6.580E-05	**P/Po Toler**	4	**End of Run**	11/19/01 07:13PM
Molecular Wt	28.0134 g/mol	**Equil Time**	2	**File Name**	AS0B1501.RAW
Station #	1	**Bath Temp.**	0.00		

A —○— BF —○—

