**COMMITTEE OF EXPERTS ON THE TRANSPORT OF
DANGEROUS GOODS AND ON THE GLOBALLY
HARMONIZED SYSTEM OF CLASSIFICATION
AND LABELLING OF CHEMICALS**

Sub-Committee of Experts on the Globally
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UPDATING OF THE SECOND REVISED EDITION OF THE GLOBALLY HARMONIZED
SYSTEM OF CLASSIFICATION AND LABELLING OF CHEMICALS (GHS)

Annexes

Report of the ring test and statistical analysis of performance of the guidance on
transformation/dissolution of metals and metal compounds in aqueous media
(Transformation/Dissolution protocol)

Transmitted by the Organization for Economic Co-operation and Development (OECD)

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REPORT OF THE RING TEST AND STATISTICAL ANALYSIS OF PERFORMANCE OF THE
GUIDANCE ON TRANSFORMATION/DISSOLUTION OF METALS AND METAL COMPOUNDS IN
AQUEOUS MEDIA (TRANSFORMATION/DISSOLUTION PROTOCOL)

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No. 87

REPORT OF THE RING TEST AND STATISTICAL ANALYSIS OF PERFORMANCE OF THE GUIDANCE ON TRANSFORMATION/DISSOLUTION OF METALS AND METAL COMPOUNDS IN AQUEOUS MEDIA (TRANSFORMATION/DISSOLUTION PROTOCOL)

IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among **UNEP, ILO, FAO, WHO, UNIDO, UNITAR and OECD**

Environment Directorate

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No. 87, *Report of the Ring Test and Statistical Analysis of Performance of the Guidance on Transformation/Dissolution of*

Metals and Metal Compounds in Aqueous Media (Transformation/ Dissolution Protocol) (2008)

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FOREWORD

The OECD is developing criteria and guidance proposals for classification and labelling in the area of health and environmental hazards, at the request of the United Nations Sub-Committee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals (UNSCEGHS). The *Guidance on Transformation/Dissolution of Metals and Metal Compounds in Aquatic Media* (Transformation/ Dissolution Protocol: abbreviated to T/DP) was published in July 2001 in the OECD Series on Testing and Assessment (OECD, 2001) and as Annex 10 to the United Nations' Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (United Nations, first edition, 2003; first revised edition, 2005; second revised edition, 2007). The harmonized system for classifying chemical substances is established as a hazard-based system. The transformation/dissolution of metals and metal compounds will be affected by a number of factors, not least of which will be the properties of the media with respect to pH, water hardness, temperature, etc. This guidance aims at standardizing the methodology such that the level of total dissolved metal can be related to the loading of the substance. The level of dissolved metal is compared to a set ecotoxicity value to determine the hazard category appropriate for classification. The dissolved metals are not speciated, so that ultimately, results of these experiments can be compared only with the $L(E)C_{50}$ and the NOEC of the ionic form of the parent metals.

The strategy for deriving an aquatic environmental hazard classification of soluble metal species can be found in the early sections of Annex 9 to the United Nations' GHS (United Nations, first edition, 2003; first revised edition, 2005; second revised edition, 2007). The strategy for the use of the T/DP in classifying insoluble metals and metal compounds as hazardous for the aquatic environment is described in section A9.7 of Annex 9 to the GHS.

In January 2002, the OECD Task Force on Harmonisation of Classification and Labelling (HCL) discussed the approaches for the validation, and agreed to establish the Validation Management Group on the Transformation/Dissolution Protocol (VMG). It was also agreed that the validation should address both the reliability and relevance. In December 2002, the UNSCEGHS requested the OECD to complete work on the validation of the Transformation/Dissolution Protocol. The VMG met in January 2003, and developed the plan for the first phase of the validation work, focusing on the reliability aspect of the Protocol, by undertaking intra- and inter-laboratory comparison of transformation/dissolution testing of three to five reference substances. The experimental work (the ring test) was conducted by four laboratories nominated by members of the Task Force on HCL: CANMET (lead laboratory), CIMM, ECVAM and LISEC NV. Dr. Jim Skeaff of CANMET coordinated the experimental work of the participating laboratories. As the T/DP is a general guidance document only, *a procedure for the Transformation/Dissolution of Metals and Sparingly Soluble Metal Compounds* (called SOP in this report) was prepared by CANMET in cooperation with LISEC for use in the experimental work of the participating laboratories. This ring test included 4 chemicals and did not cover the full range of conditions identified in the T/DP that can affect transformation/dissolution. A statistical analysis was conducted by Dr. Reinhard Meister, Technische Fachhochschule (TFH) Berlin, under contract with the OECD, and under the supervision of the VMG.

This document presents the outcome of the results of the ring test and statistical analysis of performance of the T/DP. Chapter 1 summarises the experimental procedure and results of the ring test. Chapters 2-1, 2-2 and 2-3 provide the outcome of the statistical analysis. The T/DP is attached as Annex 1 to this document and an extract from the SOP used by the participating laboratories for the ring test is also attached as Annex 2.

The document was developed by the VMG and overseen by the Task Force on HCL. The VMG had three meetings in 2006 to discuss the results of the experimental work and statistical analysis.The final draft was agreed by the VMG in January 2007 and sent to the Task Force on HCL in February 2007. The VMG met in October 2007 to discuss how to address comments received from members of the Task Force and agreed on the revised final draft. The revised final draft was sent to the Task Force on HCL in January 2008 seeking approval by a written procedure and approved in February 2008.

Ongoing work overseen by the VMG with a view to amend certain features of the T/DP will be taken into account in future VMG recommendations on the reliability and relevance of the T/DP. The VMG supports the declassification of this report which concerns the inter- and intra-laboratory variability and refined statistical analysis of the data from the ring test. Utility and applicability of the T/DP for a defined purpose is not dealt with in this report but will be part of a subsequent report.

This document is published on the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology of the OECD.

TABLE OF CONTENTS

EXECUTIVE SUMMARY

Introduction

1.   The *Guidance on Transformation/Dissolution of Metals and Metal Compounds in Aquatic Media* (Transformation/Dissolution Protocol: abbreviated to T/DP: see Annex 1) was published as an OECD Guidance Document in 2001 and as guidance in Annex to the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (OECD, 2001; United Nations, first edition 2003; first revised edition, 2005; second revised edition, 2007). Acceptance of the T/DP is subject to experimental validation.

Ring Test (Chapter 1)

2.   Four laboratories, CANMET, CIMM, ECVAM and LISEC, agreed to participate in a ring test for the validation of the performance of the T/DP (Chapter 1), with a limited range of compounds and narrowed pH range and limitation in some other conditions (see below). CANMET was the lead laboratory and wrote the procedure used by participating laboratories (called SOP in this report: see Annex 2) for the validation experiments in cooperation with LISEC, and distributed metal samples to the other laboratories. Three metal or metal oxide powders were tested for release of Co, Ni and Cu. An alloy was tested for release of three metals: Fe, Ni and Co. In the case of the alloy, samples were cut from wire. Tests were not double blind. CO_2 was used to control pH.

3.   Dr. Reinhard Meister, Technische Fachhochschule (TFH) Berlin, was engaged by the OECD to perform a statistical analysis (see paragraphs 5-15) under the supervision of the Validation Management Group on the T/DP (VMG) after the experimental phase was completed. The data were such that Dr. Meister was able to perform fairly extensive analyses to determine variability in dissolution among the laboratories. However, the number of replicates was limited for estimating variability.

4.   The T/DP called for experiments at three loadings and three durations for each metal sample. Temperature, loading, pH, were all measured (in most cases, although one laboratory did not measure actual loading at zero time). However, during the ring testing, the four laboratories were not able to follow all T/DP conditions and specifications:

- pH's in the range 5.5 to 8.5 are of importance environmentally. The T/DP notes that the transformation/dissolution tests are to be carried out at a pH that maximizes the concentration of the dissolved metal ions in solution within the prescribed pH range. A pH-range of 6 to 8.5 must be used for the screening test and the 7 day full test, and a range of 5.5 to 8.5 for the 28 day full test. For technical reasons, experimental procedures used two pH target values, 6 and 8. This was agreed by the VMG.

- The T/DP indicates CO_2 or other alternative equivalent buffering methods for pH control. However, experiments used CO_2 only.

- The T/DP calls for determination of dissolution in simulated fresh water and marine water. In addition to the fresh water medium, the use of a standardized marine test medium may also be

considered when the solubility or transformation of the metal compound is expected to be significantly affected by the high chloride content or other unique chemical characteristics of marine waters and when toxicity test data are available on marine species. However, experiments used fresh water only.

Statistical Analysis of the Performance of the T/DP (Chapter 2-1, 2-2 and 2-3)

5.   The statistical analysis aimed at a quantitative assessment of reproducibility (between laboratories) and repeatability (within laboratories) of results. One of the original four collaborating laboratories had to be excluded from the analysis due to obvious discrepancies in the concentration measurements. A rationale for this difference could not be elucidated statistically, while a qualified reasoning to the discrepancies is given in Chapter 1, Section 1.4.

6.   The study conducted by the participating laboratories in the ring test was supplemented by a more **summarizing approach**, enabling a decision on acceptable reliability of results.

7.   The statistical analysis of performance of the T/DP assesses **reproducibility** and **repeatability** numerically, and analyses influence of time, loading, target-pH, and substance on the variability of the data.

8.   Data were provided for three endpoints in time: 24h (loading= 100 mg/L), 7d (loading = 1, 10, 100 mg/L), 28d (loading =1 mg/L); substances used are Cu_2O, Co_3O_4, Ni metal, and an alloy consisting of Fe, Ni, and Co. Metals were in the form of powders, the alloy was in the form of wire segments.

9.   Experimental conditions reported by the laboratories are used to **describe the adherence to the SOP**, and, in addition are used to adjust the results for deviations from the protocol-prescribed values (e.g. target pH, loading) as appropriate.

10.   Data analysis was performed on the **log10 transformation scale**, avoiding most of the problems of inhomogeneity of variances and skewed data distributions. Final results are reported as estimated standard deviations and prediction factors. Retransformation of standard deviations to original scale results in coefficients of variation, which, in combination with a chosen confidence level of 90%, allows estimating the 90% range for a new measurement (one replication) from a randomly chosen laboratory by a prediction factor.

Descriptive results – particular approach

11.   Variance components are provided by substance, loading and target-pH. As these estimates are based on small sample sizes (n=9) their numerical values have **low precision**. Cu_2O, Co_3O_4 and Ni metal powders exhibited concentrations of dissolved metal with a significant dependence on time and loading. For the alloy components, no consistent dependence of concentration on loading or time could be established, probably due to concentrations around or below the limit of detection.

Metals		
	7 days data	prediction factors ranging from **1.2 to 2.5**
	28 days data	prediction factors ranging from **1.7 to 4.9***
	24 hours data	prediction factors ranging from **1.8 to 3.5**
Alloy		prediction factors ranging from **4.0 to 16**.

*) The value of 4.9 results from Ni metal measurements, where limiting concentration is reached within 7 days.

Comments: no clear cut effect of target-pH or loading on variability and prediction factors could be established.

12.　　For reliable and precise estimates of variances, **large sample sizes** are necessary. The particular approach was insufficient for appropriate quantitative assessment of variability. Therefore, Experimental results were combined for an **integrated approach**. Due to the large proportion of measurements at the limit of detection for the alloy compounds only the results for Ni metal and the oxides of Cu and Co were analysed using this integrated approach.

Integrated approach

13.　　For Cu_2O, Co_3O_4 and Ni metal, variability could be estimated from a model analyzing the **7d data** for all loadings of all substances simultaneously. A **common 95% prediction factor** of **1.7** could be derived. **Standard deviations between and within** laboratories are very similar, approximately **0.1 on log10-scale** of concentrations. These standard deviations correspond to a **coefficient of variation of 25%** on the original scale[1].

Conclusion

14.　　Excluding results of one aberrant laboratory, reproducibility (CV=25%) and repeatability (CV=25%) could be assessed reliably[1] for the experimental data on Cu_2O, Co_3O_4 and Ni metal in powder form at pH 6 and 8.

15.　　From a statistical point of view, variability for the measured concentrations of the components of the alloy tested could not be assessed with sufficient precision.

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[1] The T/DP identifies the within- and between-vessels variability as follows: "For a standard set-up of three replicate test vessels and two replicate samples per test vessel at each sampling time, it is reasonable to anticipate that for a constant loading of a substance, tested in a narrow particle size (e.g. 37 - 44 μm) and total surface area range, the within-vessel variation in transformation data should be less than 10% and the between-vessel variation should be less than 20 %."

CHAPTER 1: EXPERIMENTAL PROCEDURE AND RESULTS OF THE RING TEST

1.1 Introduction

16.   The Transformation/Dissolution Protocol (T/DP) (OECD, 2001; United Nations, 2005) establishes standard conditions intended to be representative of those generally occurring in the environment. The laboratory protocol is based on a simple experimental procedure of agitating various quantities of the test substance in a pH buffered aqueous medium, and sampling and analysing the solutions at specific time intervals to determine the concentrations of metal ions in the water. The T/DP calls for a series of tests lasting either 7 or 28 days, in aqueous media at pH values in the range of 6.0 to 8.5 for the 7 day full test and the range of 5.5 to 8.5 for the 28 day full test, and loadings of 1, 10 and 100 mg/L of a metal or sparingly soluble metal compound. For the latter class of substances, the T/DP also provides a 24-hr screening test at a loading of 100 mg/L.

1.2 Experimental

1.2.1 Test Procedure

17.   The work plan which was developed by the Validation Management Group on the T/DP (VMG) and approved by the Task Force on Harmonisation of Classification and Labelling (HCL) is presented in Table 1.1 and provides for testing a metal, two metal compounds and an alloy in seven- and 28-day tests at pH levels of 6 and 8, as well as 24-hr screening tests on the metal compounds. One hundred and twenty-eight tests were conducted, including blanks and replicates. The substances were introduced into the aqueous test medium at loadings of 1, 10 and 100 mg/L and agitated for seven days, while the 1 mg/L loadings were continued for 28 days. In the seven- and 28-day tests for each of the four substances, one blank (see under 0 loading column) was conducted for every three 1, 10 and 100 mg/L loadings, for a total of 24 per substance. For the 24-hr tests on each of the two metal oxides, three blanks (see under 0 loading column) were conducted for every five 100 mg/L loadings, making a total of 16 tests per substance. At target pHs of 6 and 8, yielding the requirement for 3012 analytical determinations. The work plan also provided for 24-hr screening tests at 100 mg/L for the metal oxide powders.

18.   In the T/DP, a pH of 8 is to be attained by the agitation in air of an aqueous medium with the composition of the OECD 203 aquatic toxicity solution (but lacking micronutrients), while pH 6 is to be achieved by equilibrating 10X (10 times) dilute OECD 203 under a flowing atmosphere of 0.5% CO_2, with the balance air.

Metal: Ni powder	Metal compound: Cu$_2$O	Metal compound: Co$_3$O$_4$	Alloy: Ni-Co-Fe
	Screening test (1 day) • pH 6 and 8 • dosing 100 mg/L • 3 replicates	Screening test (1 day) • pH 6 and 8 • dosing 100 mg/L • 3 replicates	
7-day test • pH 6 and 8 • loading 1, 10, 100 mg/L • 3 replicates	7-day test • pH 6 and 8 • loading 1, 10, 100 mg/L • 3 replicates	7-day test • pH 6 and 8 • loading 1, 10, 100 mg/L • 3 replicates	7-day test • pH 6 and 8 • loading 1, 10, 100 mg/L • 3 replicates
28-day test (extended from 7-day test) • pH 6, 8 • dosing 1mg/L • 3 replicates	28-day test (extended from 7-day test) • pH 6, 8 • dosing 1mg/L • 3 replicates	28-day test (extended from 7-day test) • pH 6, 8 • dosing 1mg/L • 3 replicates	28-day test (extended from 7-day test) • pH 6, 8 • dosing 1mg/L • 3 replicates

Table 1.1: Work Plan for the Validation of the OECD Transformation/Dissolution Protocol

Note: This work plan was agreed by the VMG in December 2003

1.2.2 Participating Laboratories and their Quality Assurance and Quality Control

19.	The study was an international collaborative effort among four participating laboratories:

- CANMET-Mining and Mineral Sciences Laboratories, Natural Resources Canada, Ottawa, Canada (lead laboratory);

- Centro de investigaciòn minera y metalurgica (CIMM), Santiago, Chile;

- European Commission Joint Research Centre, Institute for Health and Consumer Protection, European Centre for the Validation of Alternative Methods (ECVAM), Ispra, Italy; and,

- LISEC NV, Genk, Belgium.

20.	The four laboratories operated under quality systems. LISEC is GLP-certified and CIMM closely follows the GLP (Good Laboratory Practice) guidelines (OECD, 1998) with full traceability of analyses. CANMET operates under the ISO 9001:2000 system. As a research institute, ECVAM operates under GLP principles.

21.	In this report, the laboratories have been assigned identification numbers between 1 and 4, although not in the above order.

1.2.3 Test Substances

22.	Substances tested were nickel metal, Ni; cuprous oxide, Cu$_2$O; tricobalt tetraoxide, Co$_3$O$_4$; and an INVAR nickel-cobalt-iron alloy, Nilo K. The first three substances were in powder form while the alloy was provided as cuttings from a 1 mm diameter wire in lengths in the range 1 to 3.5 mm. Details on the substances are presented in Table 1.2. The substances were shipped from the suppliers to the lead laboratory, CANMET, where sub-samples were prepared by the Canadian Certified Reference Material Program (CCRMP) under strict quality control and assurance procedures (details available upon request). The prepared sub-samples of each of the four substances were simultaneously shipped to participating

laboratories. Additionally, CANMET's Analytical Services Group (ASG) made up acidified solutions with known concentrations of the dissolved Ni, Co, Cu and Fe to be measured under the test program. These quality assurance solutions were also shipped to the participating laboratories.

1.2.4 Aqueous Media

23.   Tests were conducted at pH levels of 6 and 8 in the aqueous media for which compositions are presented in Tables 1.3a and 1.3b and recommended in the T/DP.

1.2.5 Test Scheme

24.   The work plan, Table 1.1, calls for seven-day T/D tests to be conducted in triplicate at pH 6 and 8 on the four substances at loadings of 1, 10 and 100 mg/L, with the 1 mg/L loadings extending to 28 days. The two oxides were further subjected to 24-hr screening T/D tests, also in triplicate at the two pH levels, but only at the 100 mg/L loadings. Accordingly, a test scheme, Table 1.4, was developed and approved by the VMG. Under the scheme, samples were collected at 0, 2, 6, 24, 48, 96 and 168 hr for the seven-day tests, and also at 336, 504 and 672 hr for the 1 mg/L tests and blanks for the 28-day tests. Three samples were collected at each time except for 2 and 6 hr when single samples were drawn. Samples from the alloy T/D tests were analysed for Ni, Co and Fe, while the other samples were analysed for single metals only.

25.   The scheme also included 24-hr screening tests in which triplicate samples were collected at 0 and 24 hr from each of the five replicates at 100 mg/L and for three replicate controls at each of the test pH values. For each metal oxide, the test scheme of Table 1.1 provides for three blanks and five 100 mg/L loading replicates at each pH. In the entire scheme, the total numbers of samples and determinations are 2,112 and 3,012, respectively.

Table 1.2 : Particulars on Reference Substances for OECD Validation Study.

name	formula or composition	method of production	physical form	weight of reference substance per bottle, g	no. of bottles available at CANMET	composition/analyses, %	particle size analysis	specific surface area, m²/g		supplier	comments
								calculated*	measured**		
cuprous oxide	Cu₂O		powder	50	24	active ingredient: cuprous oxide 94 inert ingredients 6	50% -16.5 m (Microtrac)	6.7	0.5	American Chemet Corp. East Helena, Montana 59635 U.S.A.	LOLO TINT 97 Antifouling ingredient for formulating use; Untreated EPA Registration no. 26883-7 lot no.: 42521 date: Mar. 18/03
nickel metal	Ni	decomposition of nickel carbonyl	powder	10	10	typically 99.9 pure nickel metal	50% -10 m (Microtrac)	0.18	0.43	Inco Limited 145 King Street West, Suite 1500 Toronto, M5H 4B7 Canada	INCO® Type 123 lot # 3472165 date: Apr. 24/97
tricobalt tetraoxide	Co₃O₄	decomposition of cobalt hydroxide	powder	25	10	73 Co min.	median 11 m 10% -6.7 m 90% -17.8 m (Microtrac)		16.2	The Shepard Chemical Co. 4900 Beech St. Cincinnati, Ohio 45212 U.S.A.	product no. 1485 lot: 1019232 date: May/02
Ni-Co-Fe INVAR alloy			wire	3	5	Ni:Fe:Co 29.5:53.0:17.0	1 mm dia. x 1-3.5 mm length; 0.005 - 0.02 g per cutting	0.0007	0.02	EUROFER Rue de Noyer 211 B-1000 Brussels Belgium	Nilo K alloy

* from particle size analysis.
** by N₂ adsorption-desorption (BET method).

Table 1.3a: Composition of Aqueous Test Medium OECD 203 (no micronutrients).

	M.W.	mg/L	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	SO_4^{2-}	Na	K	mol/L
$CaCl_2.2H_2O$	147	293.8		79.95	141.70					0.0020
$MgSO_4.7H_2O$	246	123.3	12.03				48.12			0.00050
$NaHCO_3$	84	64.8				47.06		17.74		0.000771
KCl	74.5	5.8			2.76				3.04	0.000078
totals			12.03	79.95	144.46	47.06	48.12	17.74	3.04	
mmol/L			0.50	2.00	4.08	0.771	2.09	0.45		
hardness as $CaCO_3$		250								

calculated pH of medium when in equilibrium with air (0.033% CO_2): 8.02

Table 1.3b: Composition of 10X Dilute Aqueous Test Medium OECD 203 (no micronutrients).

	M.W.	mg/L	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	SO_4^{2-}	Na	K	mol/L
$CaCl_2.2H_2O$	147	29.38		7.99	14.17					0.0002
$MgSO_4.7H_2O$	246	12.33	1.20				4.81			0.00005
$NaHCO_3$	84	6.48				4.71		1.77		0.000077
KCl	74.5	0.58			0.28				0.30	0.000008
totals			1.20	7.99	14.45	4.71	4.81	1.77	0.30	
mmol/L			0.05	0.20	0.41	0.077	0.21	0.05		
hardness as $CaCO_3$		25.0								

calculated pH of medium when in equilibrium with 0.5% CO_2-balance air: 6.09

The total organic carbon in the media is not to exceed 2 mg/L.
M.W. = molecular weight.

TABLE 1.4: Test Scheme for OECD Validation Study of the Draft T/D Protocol

Tests 1–96 (test time in days):

test no.	0	1	10	100	(1)	(2)	(3)	(4)	pH 6	pH 8	test time, days
1	x				x				x		28
2		x			x				x		28
3			x		x				x		7
4				x	x				x		7
5	x				x				x		7
6		x			x				x		28
7			x		x				x		7
8				x	x				x		7
9	x				x				x		7
10		x			x				x		28
11			x		x				x		7
12				x	x				x		7
13	x					x			x		28
14		x				x			x		28
15			x			x			x		7
16				x		x			x		7
17	x					x			x		7
18		x				x			x		28
19			x			x			x		7
20				x		x			x		7
21	x					x			x		7
22		x				x			x		28
23			x			x			x		7
24				x		x			x		7
25	x						x		x		28
26		x					x		x		28
27			x				x		x		7
28				x			x		x		7
29	x						x		x		7
30		x					x		x		28
31			x				x		x		7
32				x			x		x		7
33	x						x		x		7
34		x					x		x		28
35			x				x		x		7
36				x			x		x		7
37	x							x	x		28
38		x						x	x		28
39			x					x	x		7
40				x				x	x		7
41	x							x	x		7
42		x						x	x		28
43			x					x	x		7
44				x				x	x		7
45	x							x	x		7
46		x						x	x		28
47			x					x	x		7
48				x				x	x		7
49	x				x					x	28
50		x			x					x	28
51			x		x					x	7
52				x	x					x	7
53	x				x					x	7
54		x			x					x	28
55			x		x					x	7
56				x	x					x	7
57	x				x					x	7
58		x			x					x	28
59			x		x					x	7
60				x	x					x	7
61	x					x				x	28
62		x				x				x	28
63			x			x				x	7
64				x		x				x	7
65	x					x				x	7
66		x				x				x	28
67			x			x				x	7
68				x		x				x	7
69	x					x				x	7
70		x				x				x	28
71			x			x				x	7
72				x		x				x	7
73	x						x			x	28
74		x					x			x	28
75			x				x			x	7
76				x			x			x	7
77	x						x			x	7
78		x					x			x	28
79			x				x			x	7
80				x			x			x	7
81	x						x			x	7
82		x					x			x	28
83			x				x			x	7
84				x			x			x	7
85	x							x		x	28
86		x						x		x	28
87			x					x		x	7
88				x				x		x	7
89	x							x		x	7
90		x						x		x	28
91			x					x		x	7
92				x				x		x	7
93	x							x		x	7
94		x						x		x	28
95			x					x		x	7
96				x				x		x	7

Tests 97–128 (test time in hr):

test no.	0	100	(1)	(2)	(3)	(4)	pH 6	pH 8	test time, hr
97		x	x	x			x		24
98	x		x	x			x		24
99		x	x	x			x		24
100	x		x	x			x		24
101		x	x	x			x		24
102	x		x	x			x		24
103		x	x	x			x		24
104		x	x	x			x		24
105		x			x		x		24
106	x				x		x		24
107		x			x		x		24
108	x				x		x		24
109		x			x		x		24
110	x				x		x		24
111		x			x		x		24
112		x			x		x		24
113		x	x	x				x	24
114	x		x	x				x	24
115		x	x	x				x	24
116	x		x	x				x	24
117		x	x	x				x	24
118	x		x	x				x	24
119		x	x	x				x	24
120		x	x	x				x	24
121		x			x			x	24
122	x				x			x	24
123		x			x			x	24
124	x				x			x	24
125		x			x			x	24
126	x				x			x	24
127		x			x			x	24
128		x			x			x	24

Substances (see Table 1.2):
(1) nickel metal (Ni) powder
(2) cuprous oxide (Cu_2O) powder
(3) cobalt oxide (Co_3O_4) powder
(4) INVAR wire cuttings

1.2.6 Methods of Agitation and Temperature Control

26.   The Standard Operating Procedure (SOP: see Annex) calls for orbital shaker and radial impeller agitation of the seven- and 28-day T/D test solutions to be at 100 and 200 r.p.m., respectively. For the screening tests, these rates are to be doubled. The SOP also calls for the temperature of the test solutions to be controlled to ±1.5C° in the range 20°-23°C.

Laboratory 1

27.   For the seven- and 28-day tests, Laboratory 1 used an Invicon type IVC laboratory table shaker with a horizontal displacement amplitude of 5 cm (2") and frequency of 100/min, maintained in a room temperature-controlled in the range 20°-23°C. For the 24-hr tests, the displacement frequency was 200/min. The table shaker had a capacity of 50 kg, or about 40 one-L Schott-Duran jars, and proportionately fewer two-and five-L jars.

Laboratory 2

28.   Laboratory 2 used two custom-manufactured top-loading orbital shakers with a 2.54 cm (1") orbit, each with a capacity of 15 2.5-L Erlenmeyer flasks enclosed in a lucite chamber. The orbital shakers were set at 100 r.p.m. and were situated in a room that was temperature-controlled in the range 20°-23°C.

Laboratory 3

29.   Except for the 1 mg/L loadings of the alloy cuttings and all 24-hr screening tests at pH 6, a top-loading LabLine Model 3530-1 refrigerated environmental shaker with a 2" (5 cm) orbit set at 100 r.p.m. and 21°C, was used. The orbital shaker had a capacity of 24 one-L Schott-Duran jars, which were kept in the dark during testing except during sampling.

30.   For T/D tests with 1 mg/L loadings of the alloy cuttings, 20-L low-density Nalgene polyethylene carboys for pH 8 and two-L reaction kettles for pH 6 were used. All were maintained in a room air-conditioned in the range 20°-23°C. The aqueous medium in the 20L carboys was agitated with molded glass impellers, 10 mm diameter x 50 cm length, with a paddle of diameter 67 mm immersed in the aqueous medium to a depth of ~20 cm. A setting of 200 r.p.m. was sufficient to provide adequate agitation of the aqueous medium. For the 1 mg/L loadings of alloy cuttings at pH 6, the agitation of the aqueous medium in the two-litre reaction kettles was done by radial impellers at 400 r.p.m. A radial impeller was set at 5 cm from the bottom of a reaction kettle and consisted of two fixed 40 mm x 15 mm polypropylene blades on a PVC-coated steel rod 8 mm diameter and 350 mm long. The impellers were driven by either a Caframo RZR 50 or a Heidolph RZR 2021 stirring motor.

31.   For the pH 6 screening tests, solution agitation conditions were similar to those for the 1 mg/L alloy cuttings except that one-L reaction kettles were used instead of two-L. Laboratory 3's orbital shaker could not withstand 200 r.p.m. for sustained periods of time, and so the 24-hr screening tests at pH 8 were done at 100 r.p.m.

Laboratory 4

32.   Laboratory 4 used 3-L Erlenmeyer flasks fitted with a 24/40 ST (standard taper) joint and vertical inlet tube to introduce gas into the space above the aqueous medium. For agitation, they used three Universal Table Shakers, Model 790, ASAL Srl with a horizontal displacement of 5 cm (2"), each table shaker having a capacity of four Erlenmeyer flasks. The frequencies of the seven- and 28-day, and the 24-hr tests were 100/min and 200/min, respectively.

1.2.7 pH Control and Measurement

33.   Thermochemical calculations show that, in the absence of metal, metal compound or alloy additions, the pH of OECD 203 equilibrated in air is 8.02, while pH 6.09 can be achieved by equilibrating 10X (ten times) dilute OECD 203 with an atmosphere comprised of 0.5% CO_2 with the balance air. Accordingly, all four laboratories aimed for a pH of 6 by delivering the CO_2air mixture either into headspace above the test vessels or directly into the 10X dilute OECD 203 medium. The ionic strength of the OECD 203 medium is a moderate 0.0087, while that of the 10X dilute medium is 0.0009, and can be considered low.

34.   For a target pH of 8, the desired variability in pH over a test period is ±0.2 units, with the same variability for the target pH of 6 for the 10X dilute OECD 203, the pH should vary between 5.89 and 6.29.

Laboratory 1

35.   Laboratory 1 used a manifold arrangement similar to that used by Laboratory 3 (see below), and measured solution pH according to their standard procedure.

Laboratory 2

36.   Laboratory 2 used a fan to draw air into the chamber, and, for a target pH of 6, used a rotameter with a teflon ball to adjust the flowrate of pure CO_2 to maintain the pH. In general, a CO_2 flowrate in the range 3-7 L/min was sufficient to maintain pH 6. After a change in CO_2 flowrate, the pH settled into a new value within about 15 min. They measured their pHs with a Fisher Scientific Accumet 25, with temperature measurement capability and automatic temperature correction (resolution: 0.1 pH units; accuracy: ±0.002).

Laboratory 3

37.   Laboratory 3 used a manifold arrangement as per the schematic diagram of Figure 1.1. The manifold consisted of a ¼" x 1/16" tube of rubber latex from which the gas was delivered through 0.023" inside dia. polyethylene tubing, one for each one-L Schott-Duran jar and two for each two-L reaction kettle. They used rotameters to monitor the gas flowrates. To measure the pH, they used either a Radiometer Analytical Red Rod combined electrode with an Acumet AR 50 readout (pH range: -2.000 to +20.000; resolution: 0.1/0.01/0.001; accuracy: ±0.002) or a Schott-Gerade Model N 42 with an Orion 250A readout (pH range: -2.00 to 19.99; resolution: 0.01; accuracy: ±0.02).
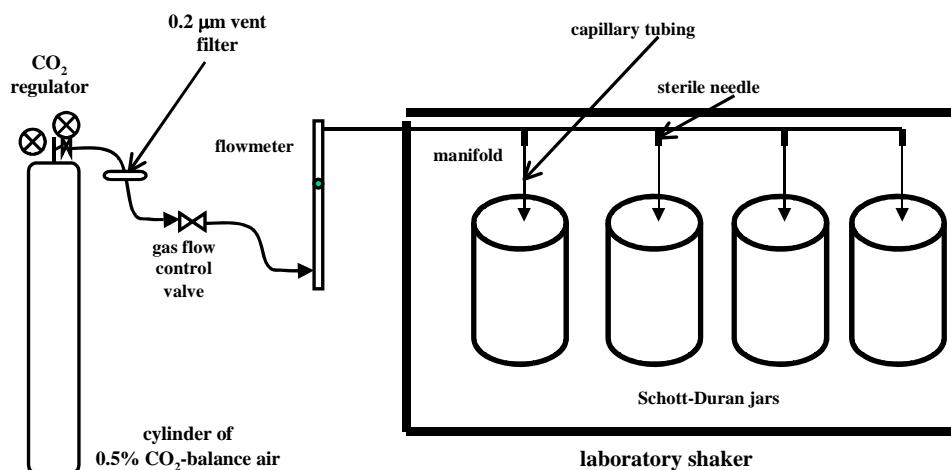
Figure 1.1: Schematic diagram of gas train and Schott-Duran jars.

Laboratory 4

38.   Laboratory 4 used rotameters to meter separate streams of pure CO_2 and air into the Erlenmeyer flasks. They used an Orion 250A pH meter (pH range: -2.00 to 19.99; resolution: 0.01; accuracy: ±0.02) to measure pH.

1.2.8 Procedure

39.   All four laboratories used similar procedures to make the OECD 203 and 10X dilute OECD 203 aqueous media. Volumes of media were made up and held in 10- or 20-L Nalgene carboys a few days or weeks in advance of use, by adding the weighed reagents to the deionized water. Prior to use, the aqueous media were filtered with a 90 mm Pall Life Science Supor©-200 0.2 mm filter or equivalent.

40.   Prior to the start of a set of tests, to condition the aqueous media targeted at pH 6, the gas mixture was passed through or over the solutions in each vessel. For Laboratory 3, the conditioning gas flowrate through the solutions was about 3 L/hr for about 10 hr for each Schott-Duran jar. The corresponding conditioning flowrates for Laboratory 3 for the one- and two-L reaction kettles were ~4 and 9.5 L/hr, respectively. During the Laboratory 3 tests, the flowrate through the aqueous medium was about 2 L/hr for each one-L Schott-Duran jar, and the same as during conditioning for the reaction kettles.

41.   At each sampling time, the laboratories generally followed the sampling procedure as per below, or equivalent:

- measure pH, temperature and dissolved oxygen of test solution;

- use a 15 mL syringe to collect the required number of approximately 12 mL solution samples, either one or three, depending on the sample time;

- filter samples with a 0.8/0.2 mm Acrodisc filter and acidify to pH <2 with 1-2 drops of Fisher Trace Metal grade HNO3 (68-71%);

- at 24 hours and thereafter, replenish the aqueous media with fresh aqueous medium equivalent in volume to those drawn for analysis in order to maintain the surface area loading as constant as possible.

42.   For Laboratories 1, 2 and 3, the determinations of total dissolved cobalt, copper and nickel were by inductively-coupled plasma mass spectrometry (ICP-MS) and for dissolved iron by graphite furnace atomic absorption spectrometry (GFAAS). Laboratory 4 used GFAAS for all determinations.

1.3 Results

1.3.1 Temperature

43.   Almost all of the temperatures measured by the four laboratories fell within the range 20º-23ºC. Minor departures from this range were evident for all laboratories, with Laboratories 1 and 3 having a few sample temperatures lower than 20ºC but none above 23ºC, while Laboratories 2and 4 had some samples above 23ºC but none below 20ºC. These departures include:

- a 0-hr sample at 19.1ºC for one test at Laboratory 3;

- 0-hr samples in the range of 19.7ºC-19.9ºC for four tests at Laboratory 3 and eight tests at Laboratory 1;

- 24- and/or 48-hr samples at 23.1ºC or 23.2ºC for 16 of the seven-day tests at Laboratory 2;

- 24-hr samples at 24.3ºC and 24.8ºC for all of the Laboratory 2's 24-hr screening tests at pH 8 and 6, respectively;

- various samples in the range of 23.1ºC-23.7ºC for 58 of the seven- and 28-day tests at Laboratory 4.

44.   In spite of these departures from the 20º-23ºC range, the temperature in all of the tests was controlled within the required ±1.5C° temperature range, with one series of notable exceptions. The 24-hr screening tests for all the laboratories, at pH 6 for both of the metal oxide powders were in a 4.4C° temperature range: 20.4º-24.8ºC.

1.3.2 Dissolved Oxygen

45.   The measured dissolved oxygen concentrations were always above 7 mg/L. The range of dissolved oxygen for Laboratory 1 was 8.3-13.3 mg/L, for Laboratory 3 7.5-11.9 mg/L, for Laboratory 2 7.38.1 mg/L and for Laboratory 4 7.5-9.4 mg/L. The calculated thermochemical equilibrium value for in both the pH 8 media and the dilute pH 6 media with 0.5% CO_2-balance air is 8.8 mg/L.

1.3.3 Reported Limits of Detection (LOD) and Limits of Quantification (LOQ)

46.   In several instances, data from the four laboratories were reported as "<LOQ" (limit of quantification) or "<LOD" (limit of detection) where it was necessary to have numerical values. Table 1.5 presents the laboratories' LODs and LOQs. The replacement values used in the analysis (the limit values divided by 2) are also presented in Table 1.5. Unlike other laboratories, Laboratory 4 did not report data

for 48 hr, and so to enable plotting, the 48-hr dissolved metal values of Laboratory 4 have been interpolated.

		Laboratory 1		Laboratory 2		Laboratory 3		Laboratory 4	
		µg/L							
		LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ
Ni		1.47	6	0.07	0.23		*		0.2
Cu		0.70	2	0.03	0.10		*		0.2
Co		0.46	1	0.026	0.09		*		0.2
Fe		31.70	90	2.6	8.66		*		0.2
Ni		0.735	3	0.035	0.117		*		0.1
Cu		0.35	1	0.015	0.050		*		0.1
Co		0.23	0.5	0.013	0.043		*		0.1
Fe		15.85	45	1.3	4.3		*		0.1

*: variable

Table 1.5 Reported Limits of Detection (LOD) and Limits of Quantification (LOQ) for the Four Laboratories

1.3.4 Transformation/Dissolution Data

47.	Figures 1.2, 1.3 and 1.4 present the four laboratories' Transformation/Dissolution Data for Cu_2O powder, Co_3O_4 powder and Ni metal powder at each loading and pH. The average dissolved metal concentrations, Me(aq), are plotted as a function of time for each loading and pH. To enable plotting, Laboratory 4's 48-hr dissolved metal values have been interpolated. Data from the alloy tests are excluded from the plots.

Cu_2O Powder (Figure 1.2a; 1.2b)

48.	For the cuprous oxide powder at pH 6 and a loading of 1 mg/L, Laboratories 1, 2 and 3 found significant levels of dissolution, which yielded 168 and 672 hr average dissolved copper concentrations of 131 and 391 µg/L, respectively for which the coefficient of variance, CV or $\sigma_\%$=44 and 42, while Laboratory 4 measured only 1 µg/L or less at both times. For the 10 and 100 mg/L loadings at seven days at pH 6, Laboratory 4 measured significantly greater copper concentrations than the other three. Similar patterns were evident for Cu_2O powder at pH 8.

Co_3O_4 Powder (Figure 1.3a; 1.3b)

49.	The dissolution data for the tricobalt tetraoxide powder presented a contrast to the cuprous oxide powder. At pH 6, the reported average 168 and 672 hr dissolved cobalt concentrations among all four laboratories for the 1 mg/L loading were 3.32 and 17.5 µg/L, respectively ($\sigma_\%$=22 and 34). Moreover, the average seven-day cobalt concentrations at 10 and 100 mg/L loadings were 33.5 and 169 µg/L, respectively ($\sigma_\%$=39 and 37). These values of $\sigma_\%$ indicate good data reproducibility among the four laboratories and illustrate the reliability of the T/DP. However, for pH 8, Laboratory 4 reported cobalt concentrations that were considerably greater than those reported by the other three at each loading.

Ni Metal Powder (Figure 1.4a; 1.4b)

50. Among Laboratories 1, 2 and 3, the 1 mg/L loadings of nickel metal powder at pH 6 delivered an average of 4.4 μg/L at 672 hr, although with $\sigma_\% = 44$. For Laboratory 4, the average value was 12-fold higher, ~52 μg/L. The 10 and 100 mg/L loadings from Laboratories 1, 2 and 3 presented 168 hr averages of 53 and 560 μg/L, respectively, with corresponding $\sigma_\%$ values of 12 and 18, respectively, while Laboratory 4 measured an average of 2,980 μg/L. Similar results among Laboratories 1, 2 and 3 were reported for pH 8, although the dissolved nickel concentrations were about 30% lower than at pH 6. However, Laboratory 4 reported nickel concentrations at pH 8 close to those they measured at pH 6.

Nilo K Alloy Cuttings

51. For every loading and test duration, and both pH 6 and 8, the average dissolved concentrations of nickel, cobalt and iron among Laboratories 1, 2 and 3 were less than 15 μg/L. The low concentrations were sometimes associated with elevated $\sigma_\%$s, as high as 80%. These relatively high $\sigma_\%$s are a feature that was common to many of the measurements of low concentrations of metals in this study. Laboratory 4 reported nickel and iron concentrations as high as ~1,235 μg/L at seven days and 100 mg/L loadings.

52. The alloy data are extremely low values, often near or below the limit of detection, therefore valid statistical estimation failed. Alloy data more suitable for estimation of within and between laboratories' variability would be needed for proper evaluation of alloys.

1.4 Discrepancies in Laboratory 4 Test data

53. Laboratory 4 had to be excluded from the statistical analysis due to obvious discrepancies in the concentration measurements (See paragraph 81). Variations in the CO_2 flowrate used by Laboratory 4 to establish pH 6 has been suggested as a possible cause of the discrepancies between their results and those of the other three laboratories. It should be noted also that another of the characteristics that hindered inclusion of Laboratory 4 was the fact that 23 of the 43 most obvious discrepancies between Laboratory 4's results and those of the other three laboratories occurred at pH 8, which the possible variability of the CO_2 flowrate cannot explain. Moreover, variable CO_2 flowrates cannot explain the near-zero concentrations of dissolved copper that Laboratory 4 reported for the 1 mg/L loadings of Cu_2O at pH 6 and 8, while the other three labs reported Cu concentrations in the ranges ~180-600 and 40-100 μg/L, respectively. Copper is mentioned as an example but discrepancies are also noted for other metals.

54. These discrepancies were not limited to a single or few measurements. Nor were they limited to the measurement of a single metal or metal loading.

55. While Laboratory 4 measured near-zero copper concentrations, the other three laboratories identified significant levels of dissolution with an average dissolved concentration of 131 and 391 μg/l for the 7 day and 28 day test, respectively (target pH 6 and loading of 1 mg Cu_2O/l). Similar pattern was shown for target pH 8 (see Paragraph 47 and Figure 1.2a)[1].

56. For the loadings of 10 and 100 mg Cu_2O/L and target pH of 6 and 8, the Laboratory 4 that showed obvious discrepancies with other laboratories reported significantly greater copper concentrations with these loadings than the other three laboratories. And while the other three reported a significant

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[1] Already back in 2001, the European Copper Institute reported T/D test data on Cu_2O following the T/D protocol. The report revealed for the target pH of 6 and a loading of 1 mg/l a measured copper concentration of 236 mg/l after 7 days (European Copper Institute (ECI), 2001). Hence, the ECI results are very much in line with the results reported from three of the four laboratories in the validation ring test.

difference in copper concentrations between the tests performed under the two target pHs the fourth laboratory could not show this clear difference (see Figure 1.2 a and 1.2.b)[1].

57.   Even if there are no obvious reasons at this time as to why there should be such considerable discrepancies between one laboratory and the other three laboratories, and a thorough investigation was not done to investigate this further, avoiding results like those of Laboratory 4 is considered not to be a statistical issue. Therefore, Laboratory 4 was excluded from the statistical analysis in this report.

58.   From a statistical point of view, variability for the measured concentrations of the components of the alloy tested could not be assessed with sufficient precision.

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[1] A linear regression of dissolved copper concentration, between pH 6 and 8 was studied and reported by the European Copper Institute, with thermodynamic calculation as supportive evidence (European Copper Institute (ECI), 2001).

Figure 1.2a: The Four Laboratories' T/D Data for Cu₂O Powder

Figure 1.2b: The Four Laboratories' T/D Data for Cu₂O Powder

Figure 1.3a: The Four Laboratories' T/D Data for Co$_3$O$_4$ Powder

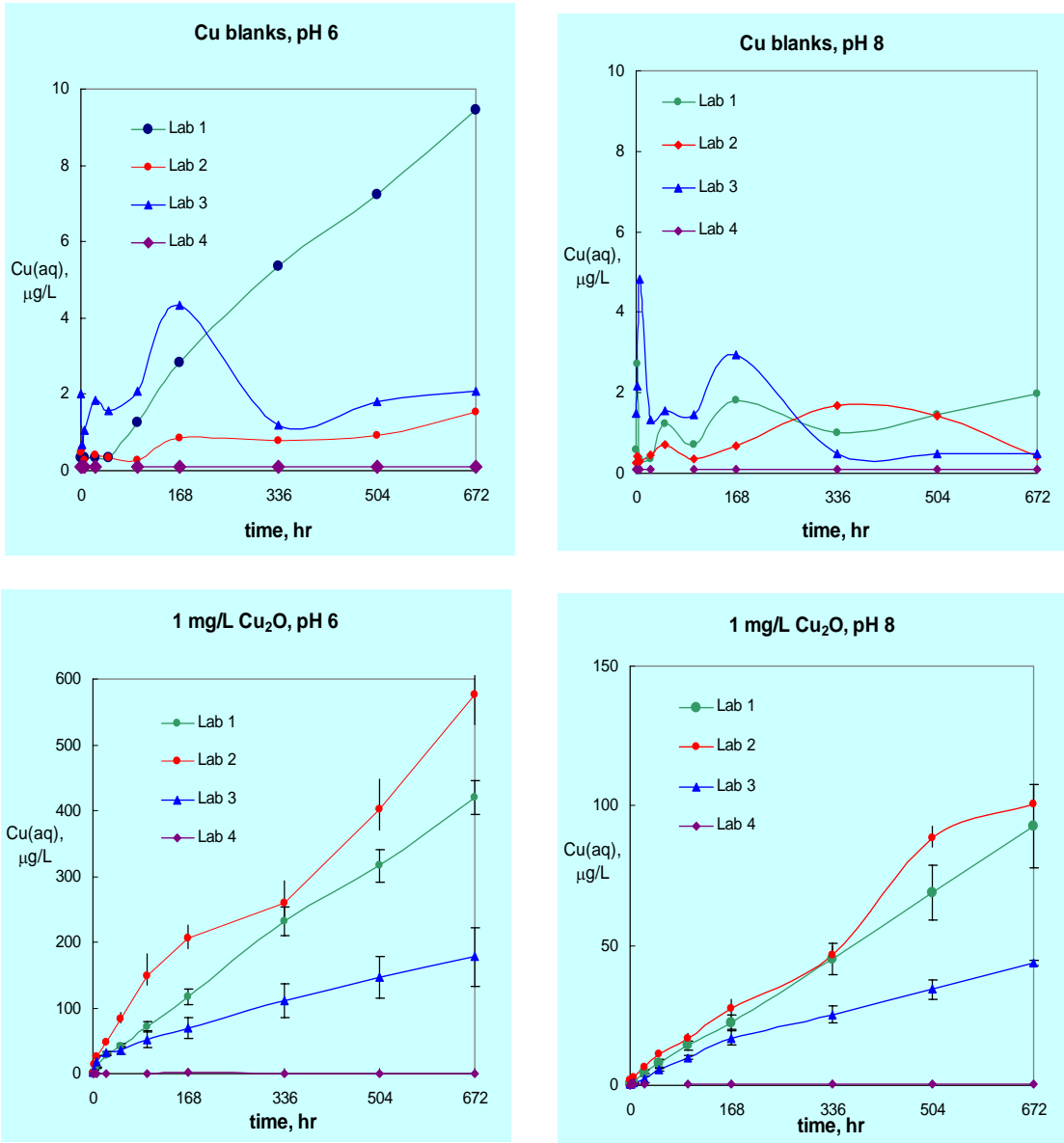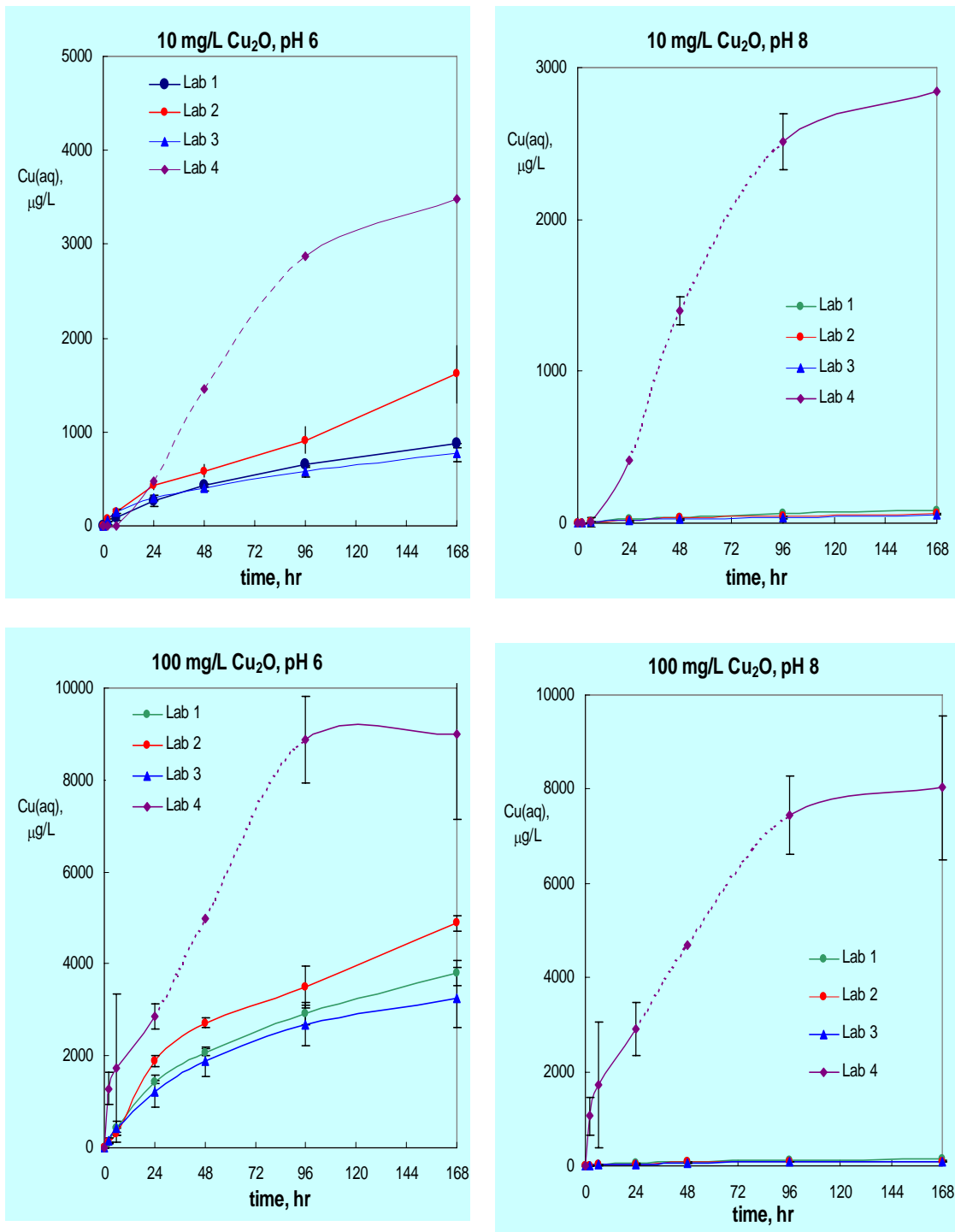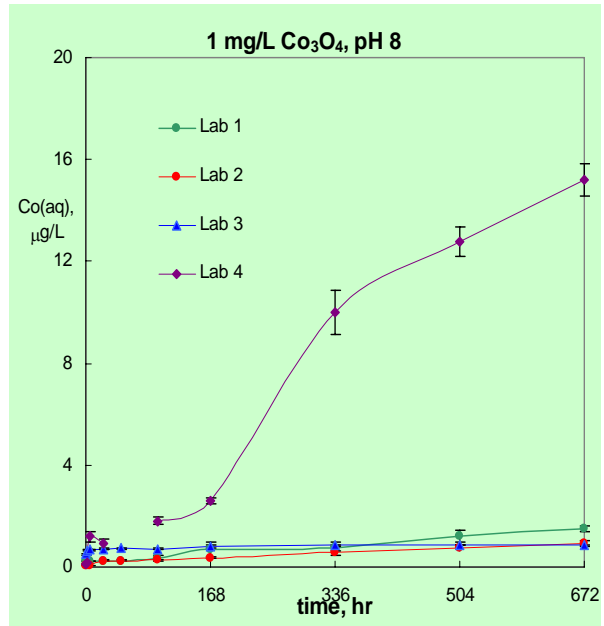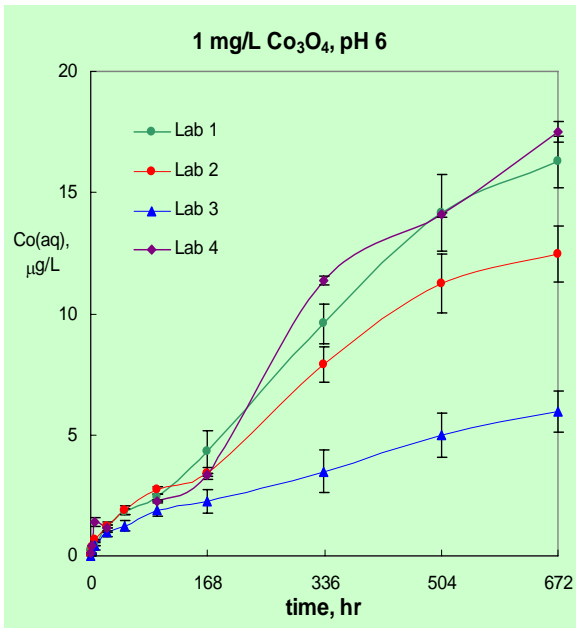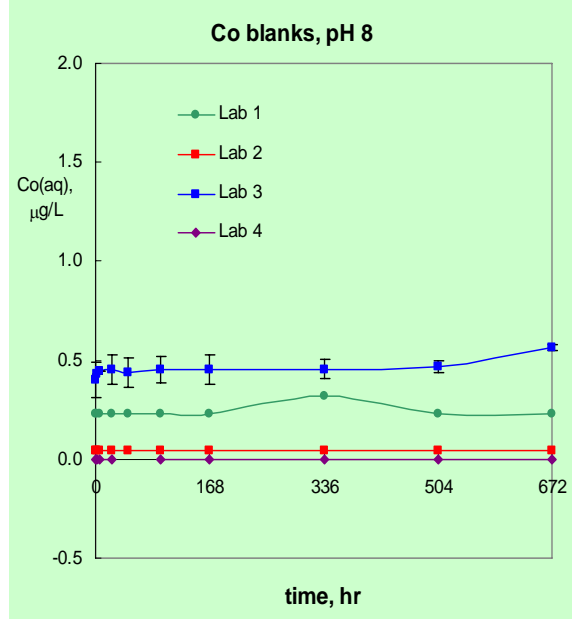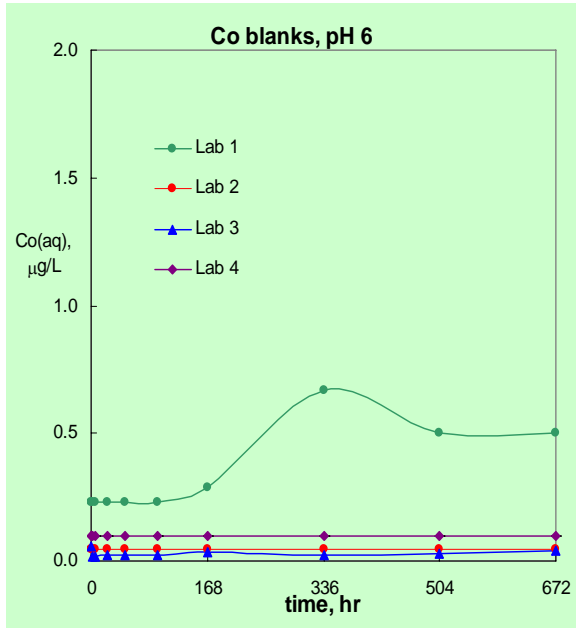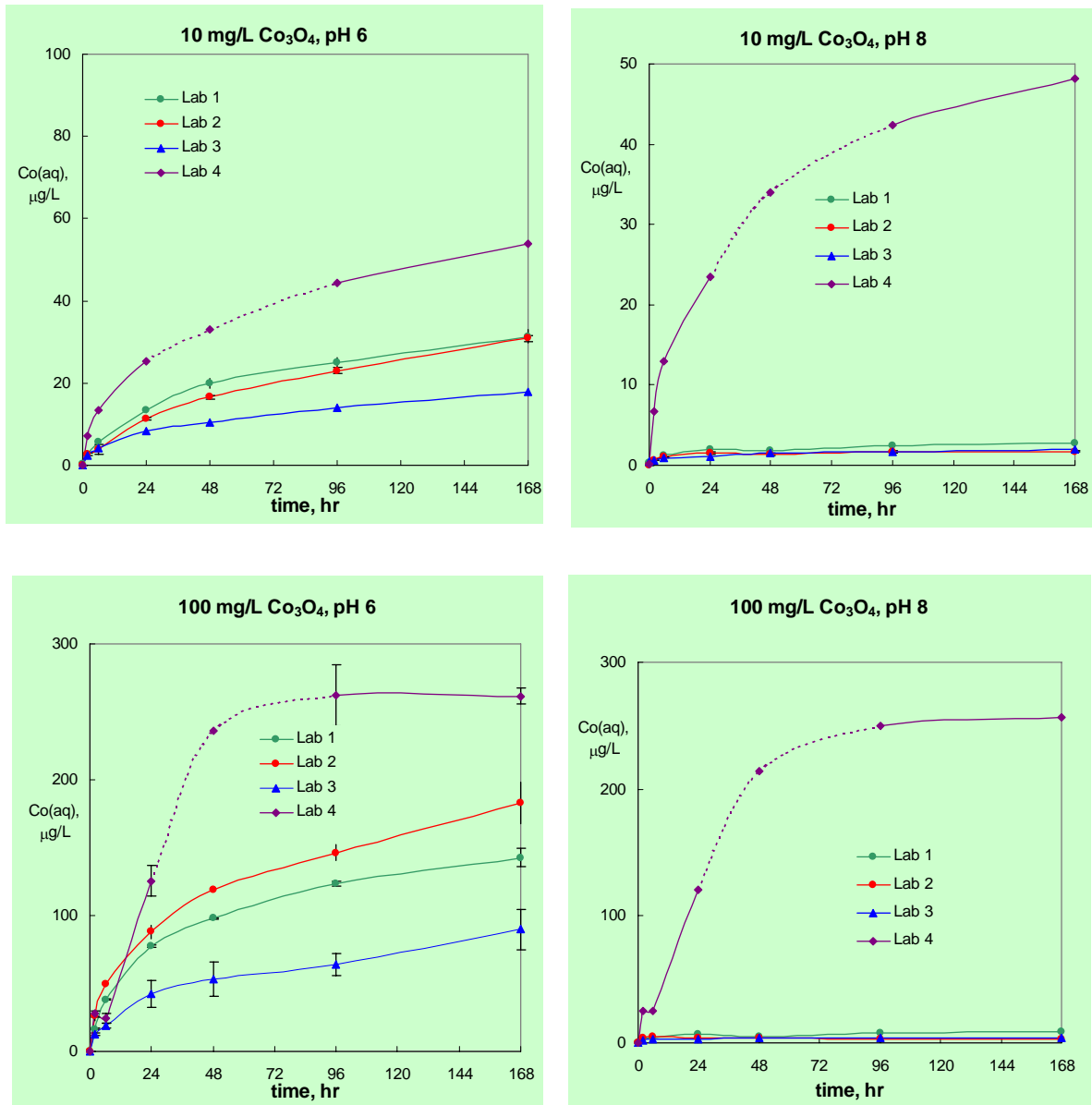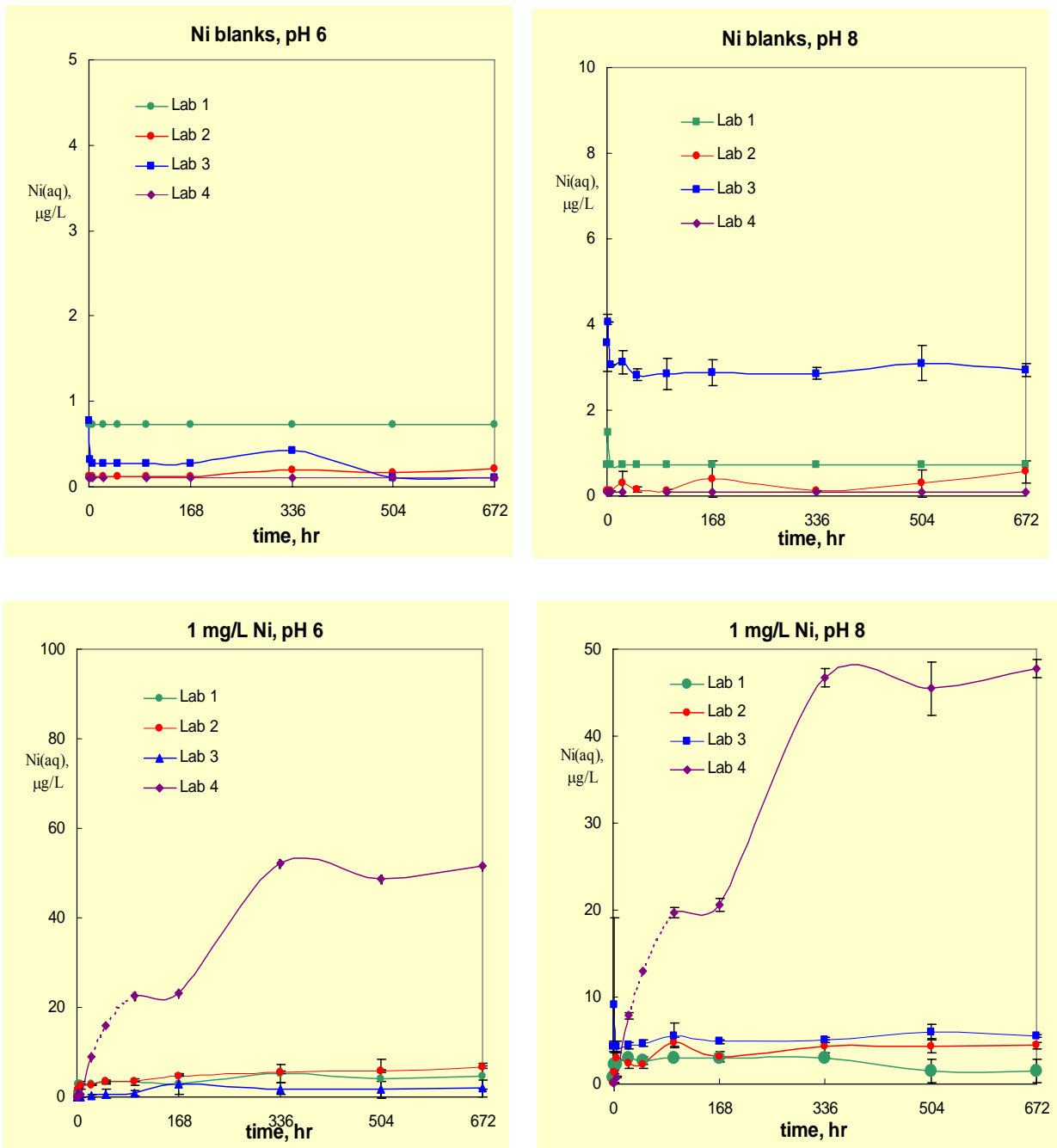**Figure 1.3b: The Four Laboratories' T/D Data for Co$_3$O$_4$ Powder**

Figure 1.4a: The Four Laboratories' T/D Data for Ni metal Powder
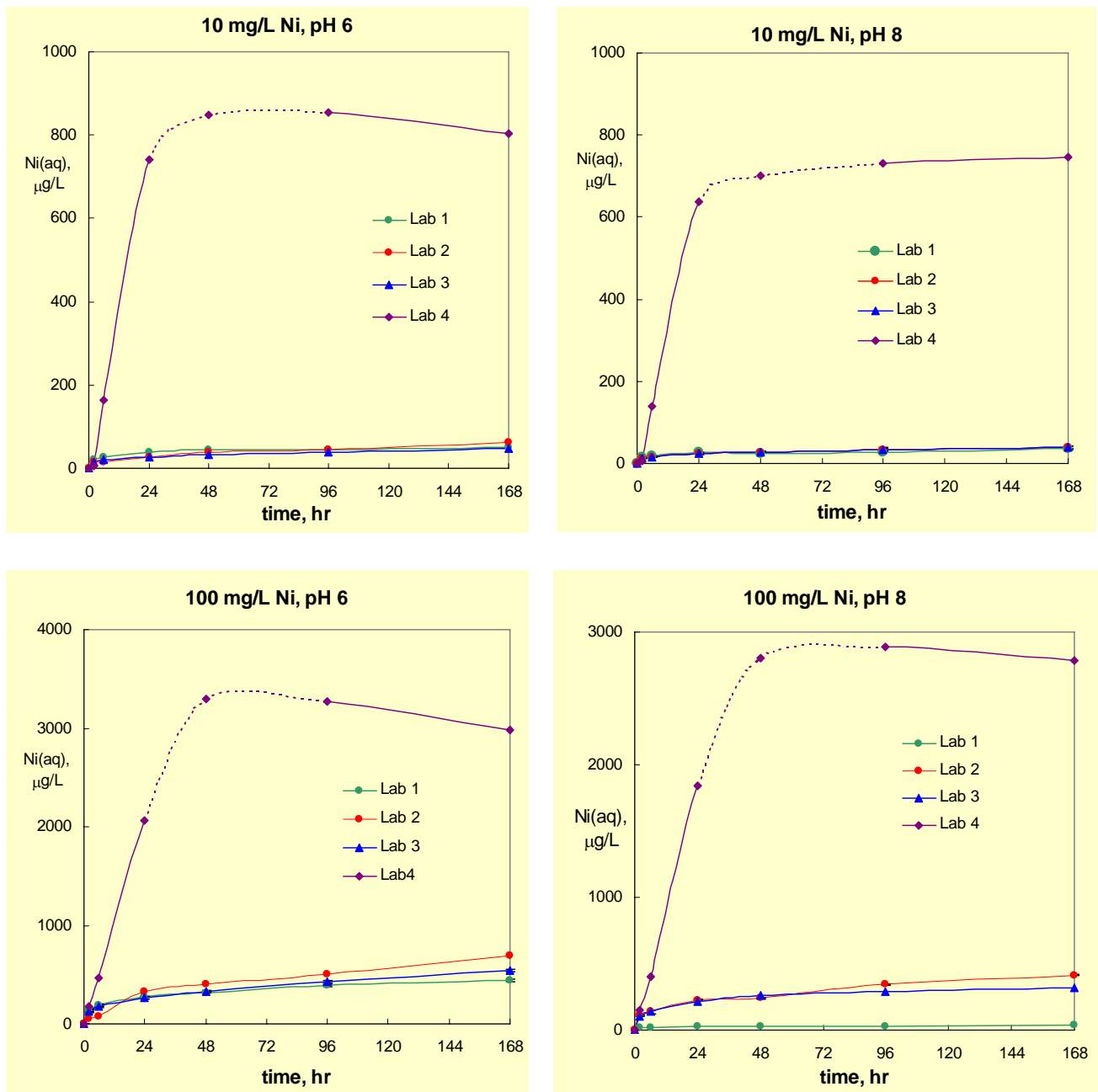
Figure 1.4b: The Four Laboratories' T/D Data for Ni metal Powder

CHAPTER 2-1: INTRODUCTION TO STATISTICAL ANALYSIS

59.   Determination of concentrations of "sparingly soluble substances" is a challenge. However, determination has to be reliable, when used for assessment of the ecotoxic potential of substances. Therefore, a validation study had been undertaken by the participating laboratories with the aim of investigating the performance of the T/DP. The results were submitted to the VMG in July 2005 as a draft report. In a review of the draft report, Dr. Reinhard Meister, Technische Fachhochschule (TFH) Berlin, was contracted by the OECD to provide statistical expertise of the validation. Dr Meister claimed with respect to the validation aspect: "The study data appears as result of very well done experiments. However, a more general overview of results could make such a decision easier." Discussion about this proposal led the VMG to the proposal of a reanalysis of the study results and Dr Meister was requested to provide an assessment of the study results concerning validity of the protocol in a summarizing way.

60.   Chapters 2-1, 2-2 and 2-3 present a statistical analysis of the results of the ring test for the validation of performance of the T/DP which was conducted by Dr. Meister under the supervision of the VMG. This reevaluation is performed in order to get a more comparative view of the study results. For the validation process, variability of experimental results within and between laboratories has to be judged according to several points of view. Questions relevant for this judgement are given below:

- Do results provide evidence of transformation/dissolution for a given experiment?

- Is there a clear-cut dependence of measured concentrations on loading?

- Do the results reflect the experimental conditions, e.g. target-pH?

61.   The data is presented (see Chapter 2-2) in a way that evidence about these questions can be drawn without special statistical training and without formal calculations. A more methodological approach is given in Chapter 2-3.

62.   In case of positive answers to all of the above questions, it makes sense to calculate measures of variability within and between laboratories. To this end decisions and definitions are needed addressing the following problems:

- How should the distribution of data of a single T/DP experiment be characterized?

- How should variability be assessed for this type of distribution?

- How can variability be expressed in the original scale of measurement?

63.   It was decided to use a log10-transformation of the concentrations. This transformation successfully yields rather homogeneous variances, and so the usual linear models methods can be applied (see Chapter 2-3).

64.   Details about measures of variability are given in Chapter 2-2. The use of prediction factors characterizing the variability on the originals scale is explained there as well.

65.   For the descriptive part of the report given in Chapter 2-2, two views of the data are provided. In a first step means and standard deviations of the log10-transformed data at each experimental level given by time, loading, target-pH, substance, laboratory are calculated and presented.

66.   The second presentation shows all raw data for a fixed time of measurement (1 week, 1 day, 4 weeks) in a systematic way. The implementation of Cleveland's trellis-display (Cleveland, 1993; Becker, et al., 1996) in the packages lattice (Sarker 2006) and nmle (Pinheiro and Bates, 2000; Pinheiro et al., 2006) of the statistical software system R (R Development Core Team, 2006) is used. Trellis displays arrange single plots in a table-like way. A lattice representing aspects of the experimental design is illed with panels showing for example the dependence of measurements on loading (see e.g. figure 2-2.1).

67.   This approach shall enable the reader to conceive structure and similarities of the data under different conditions.

68.   The last of Chapter 2-3 consists of an integrated approach to assess reproducibility and repeatability for all metals (1 week data) and all loadings. The role of possibly confounding covariates such as difference between actual pH and target pH and temperature is studied and used to achieve adjusted variance estimates.

69.   The concept of writing this report is part of the result, and will, therefore, be sketched below. Analyzing reproducibility needs reproducible computations. Handling large sets of data, dozens of tables and figures enables any author to include lots of errors. Some of the methods might be too special for an easy description of the procedures. Several authors in the field of applied statistics have therefore agreed upon a view that says: the program-code, containing all algorithms used, is the documentation. The generation of the final document has to be included in this process.

70.   This report has been generated using techniques of reproducible research, in order to achieve as much as transparency as possible in linking original data, statistical computations and results reported. The approach makes use of the R statistical software (R Development Core Team, 2006), and the technique describe in Leisch (2002 a; b).

Outline of Results

71.   The descriptive part of the report (Chapter 2-2) demonstrates that the results of three of the labs were in all consistent, showing all effects of pH-level and loading expected. The way of presentation explained in detail the need for excluding the results of Laboratory 4.

72.   In contrary to the findings for powders, the alloy data appear much more scattered. Neither pH dependence nor loading-dependence of concentrations measured can be demonstrated.

73.   Actual values for the protocol-prescribed experimental conditions are described in addition.

74.   In Chapter 2-3, the more methodological part of this report, measures of within and between laboratory variability are provided. These measures, estimated on a log10 transformed scale correspond to coefficients of variation in the region of 25% for powders of Ni metal, Co_3O_4, and Cu_2O. The computations for the alloy components result in coefficients of variation of several hundred percent. Therefore, the alloy data have not been analyzed further.

75.   In an integrated approach, it has been possible to analyze concentrations of all powders, and all loadings simultaneously. The resulting overall estimates of within and between lab standard deviations appear stable and resemble the individually estimated values.

76.   Prediction factors have been provided for giving an impression of possible statistically sound decision making using results from the T/DP. An overall prediction factor of 1.7 is derived for the 1-week data for powders, allowing computation of limits and prediction intervals.

77.   Adjustment for covariates – here temperature – improved the estimated variability slightly. However, the unadjusted data itself seems rather consistent on the log10 scale.

78.   Concerning the experimental conditions, accurate reporting, and, if possible, better control could perhaps improve reproducibility; refinement of the SOP might achieve this. In the present data, deviations from target did not have large influence on the results.

CHAPTER 2-2: THE T/DP AND ACTUAL EXPERIMENTAL CONDITIONS

2-2.1 Description of one-week experiments

79.   According to the protocol, the experiments had to be performed at two levels of pH. The body of data contains information on several aspects of the actual experimental conditions. The following tables, and, even more important, graphical displays shall enable the reader to get an overview of results and actual experimental conditions. Deviations from the protocol can be compared between labs. The data is displayed either versus the *loading* or versus *target-pH*.

2-2.1.1 Concentrations of metal dissolved: 1-week results

80.   A tabular form of these data is provided by

	lab	load	target.ph	Co.alloy	Co3O4	Cu2O	Fe.alloy	Ni.alloy	Ni.metal
1	lab1	1	6	-0.64(0)	0.61(0.12)	2.06(0.04)	1.2(0)	-0.13(0)	0.48(0)
2	lab2	1	6	-0.73(0.58)	0.53(0.03)	2.31(0.04)	0.35(0.07)	-0.6(0.55)	0.67(0.01)
3	lab3	1	6	-1.1(0.11)	0.34(0.09)	1.83(0.11)	0.45(0.06)	0.26(0.05)	0.33(0.39)
4	lab4	1	6	0.95(0)	0.52(0.01)	0.01(0.02)	-1(0)	1.09(0.04)	1.37(0)
5	lab1	1	8	-0.46(0.31)	-0.23(0.12)	1.35(0.05)	1.2(0)	-0.13(0)	0.48(0)
6	lab2	1	8	-1.37(0)	-0.47(0.04)	1.44(0.05)	0.67(0.06)	-0.84(0.13)	0.49(0.08)
7	lab3	1	8	-0.33(0.03)	-0.1(0.02)	1.22(0.07)	0.41(0.29)	0.61(0.04)	0.69(0.02)
8	lab4	1	8	0.58(0.02)	0.41(0.02)	-0.29(0.24)	0.95(0.02)	0.76(0.02)	1.31(0.02)
9	lab1	10	6	-0.64(0)	1.5(0.02)	2.94(0.02)	1.2(0)	-0.13(0)	1.71(0.02)
10	lab2	10	6	-0.52(0.73)	1.49(0.01)	3.2(0.09)	0.51(0.18)	0.09(0.14)	1.79(0.04)
11	lab3	10	6	-0.77(0.49)	1.25(0.03)	2.89(0.05)	0.14(0.37)	-0.04(0.2)	1.67(0.02)
12	lab4	10	6	2.24(0.06)	1.73(0.01)	3.54(0)	-1(0)	2.38(0.03)	2.9(0.05)
13	lab1	10	8	-0.64(0)	0.45(0.03)	1.93(0.1)	1.2(0)	0.54(0.59)	1.55(0.01)
14	lab2	10	8	-1.14(0.39)	0.23(0.02)	1.83(0.05)	0.82(0.31)	-0.92(0)	1.58(0.03)
15	lab3	10	8	0(0.19)	0.27(0.06)	1.76(0.02)	-0.3(0)	0.71(0.03)	1.49(0.06)
16	lab4	10	8	1.85(0.04)	1.68(0.04)	3.45(0.03)	2.51(0.02)	2.32(0.02)	2.87(0.02)
17	lab1	100	6	0.64(0.16)	2.15(0.02)	3.58(0.03)	1.2(0)	0.52(0.57)	2.65(0.01)
18	lab2	100	6	1.21(0.11)	2.26(0.04)	3.69(0.01)	0.43(0.28)	1.47(0.11)	2.84(0.02)
19	lab3	100	6	0.53(0.1)	1.95(0.07)	3.51(0.08)	0.17(0.48)	0.84(0.12)	2.73(0.01)
20	lab4	100	6	2.62(0.06)	2.42(0.01)	3.95(0.09)	2.61(0.24)	3.08(0.14)	3.47(0.01)
21	lab1	100	8	0.86(0.49)	0.93(0.07)	2.21(0.03)	1.2(0)	1.07(0.53)	2.62(0)
22	lab2	100	8	0.31(0.15)	0.5(0.01)	1.95(0.01)	0.32(0.27)	0.55(0.15)	2.62(0)
23	lab3	100	8	0.31(0.21)	0.56(0.12)	2.01(0.02)	-0.3(0)	0.85(0.14)	2.5(0.04)
24	lab4	100	8	2.58(0.02)	2.41(0.02)	3.9(0.08)	3.08(0.01)	2.89(0.01)	3.44(0.01)

Table 2-2.1: Concentrations of metal dissolved by labs and conditions Mean(SD) of log10(concentration)

81.   In addition a graph is provided showing all measured concentrations by *lab, target-pH, substance*, and *loading*. For the rest of this description, data from Laboratory 4 will no longer be analysed, due to the apparent discrepancies in the results reported by this laboratory. The clear cut effect of *target-pH* is missing in the Laboratory 4 data, and the range of measured concentrations is in most cases far from the other labs.
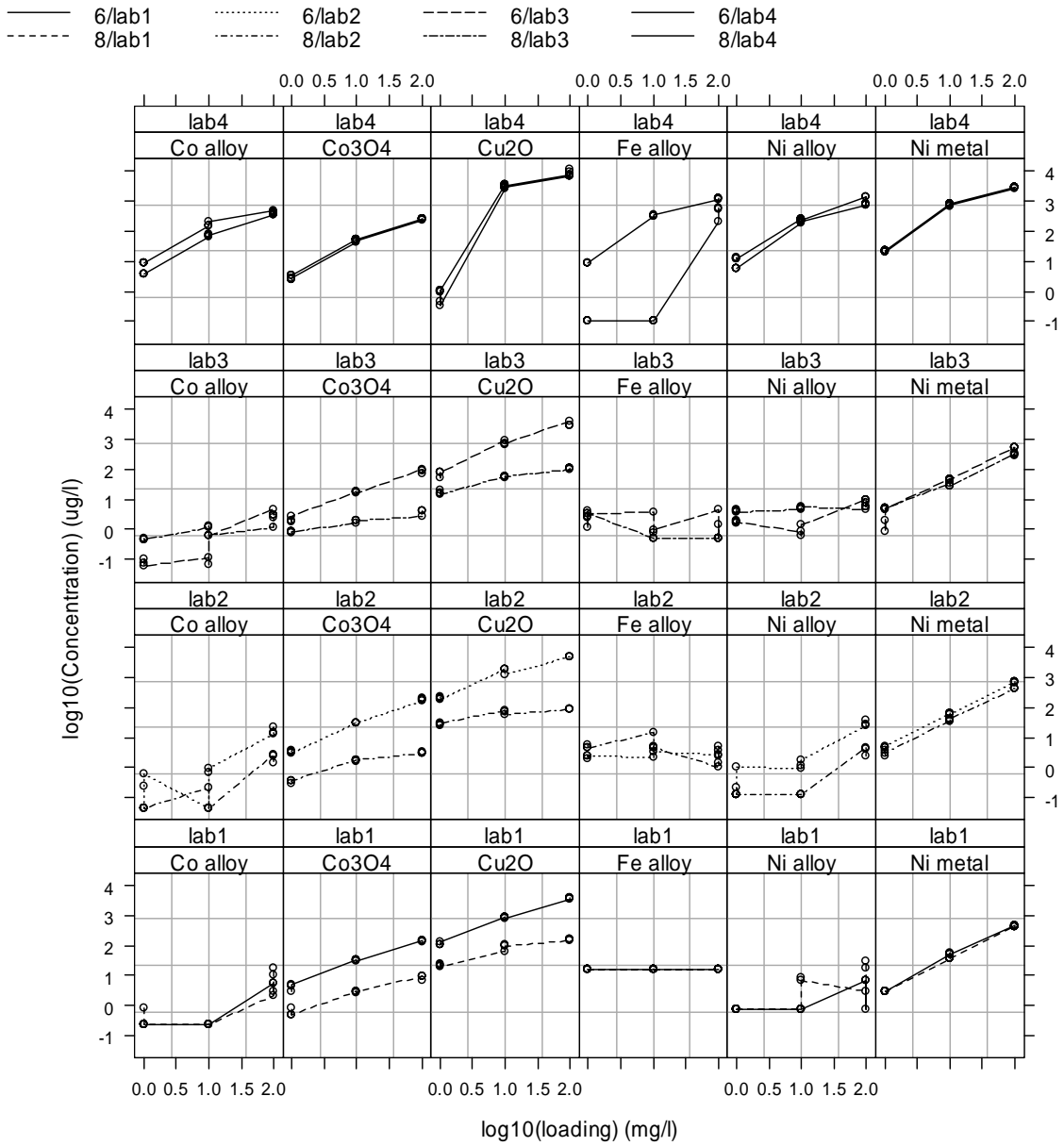
Figure 2-2.1: Concentrations of dissolved substances by lab and loading

2-2.1.2 pH-level: 1-week data

82.   One gets an overview of the observed differences in *pH* by the following graph:
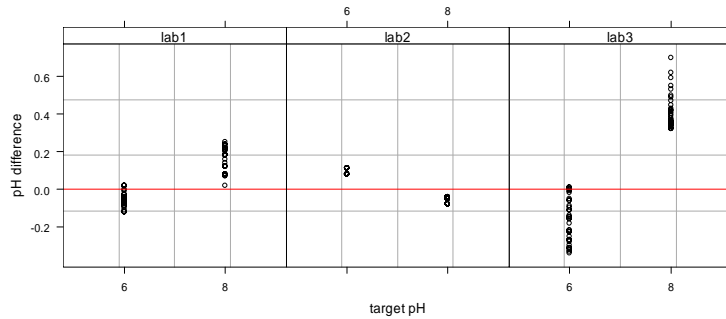
Figure 2-2.2: pH-difference by lab and target-pH
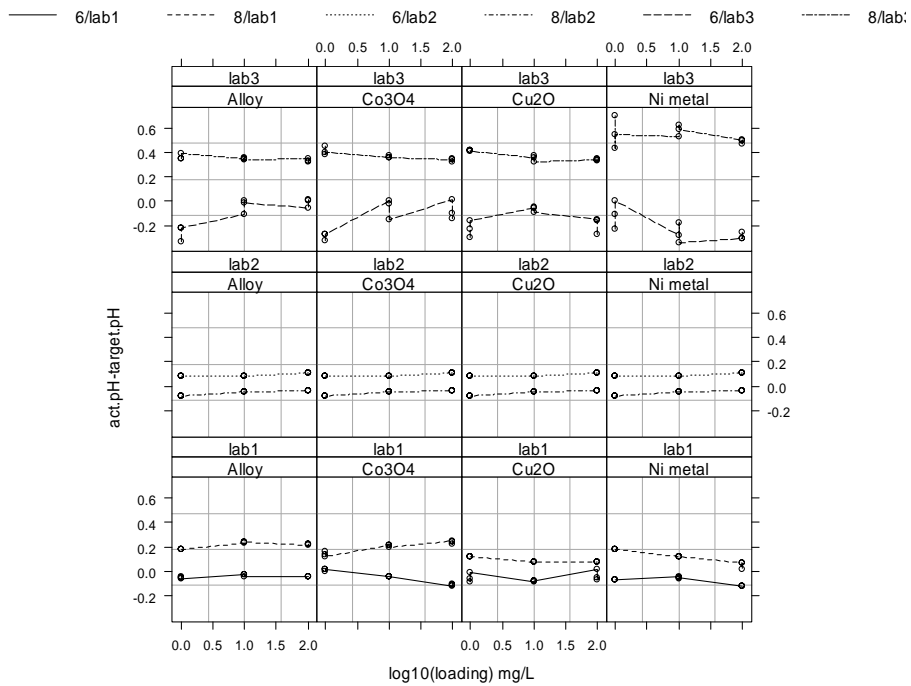
Figure 2-2.3: pH-difference by lab and loading

2-2.1.3 *Actual loading: 1-week data*

83.   One gets an overview of the observed differences in loading by the following graph:
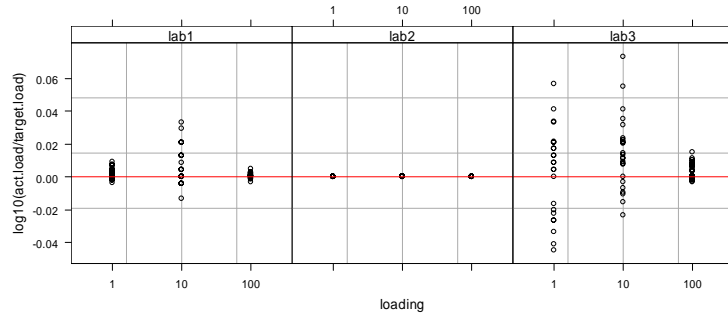
Figure 2-2.4: log10(act.load/target.load) by lab and loading
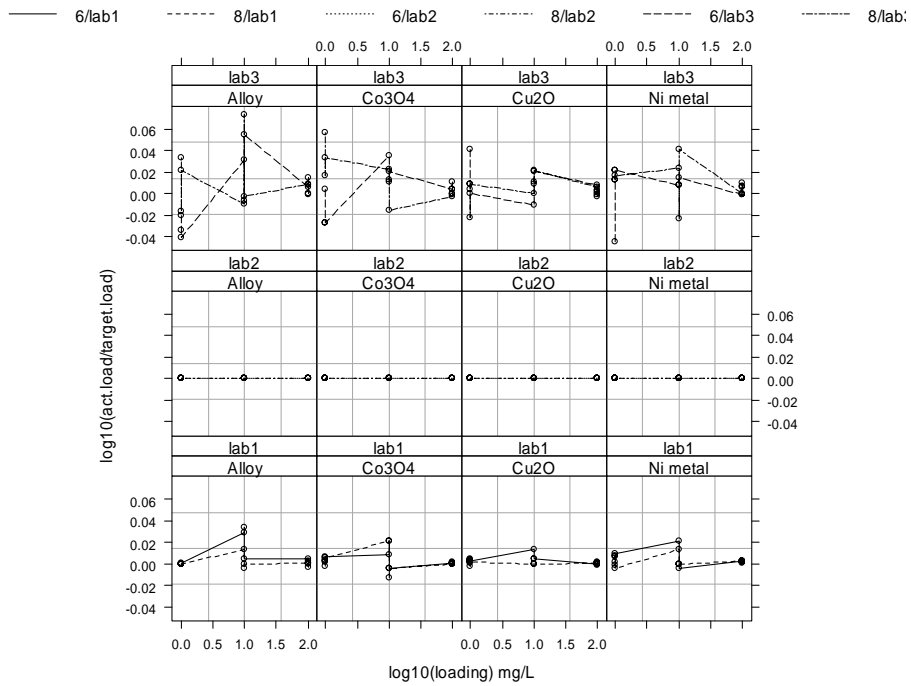
Figure 2-2.5: log10(act.load/target.load) by lab and loading

2-2.1.4 Temperature: 1-week data

84.   One gets an overview of the observed differences in *temperature* by the following graph:
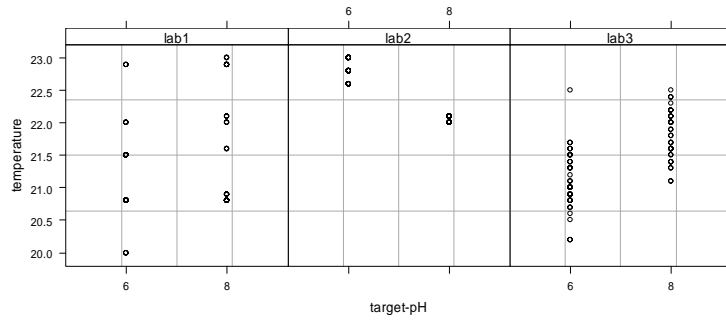
Figure 2-2.6: Temperature by lab and target-pH.
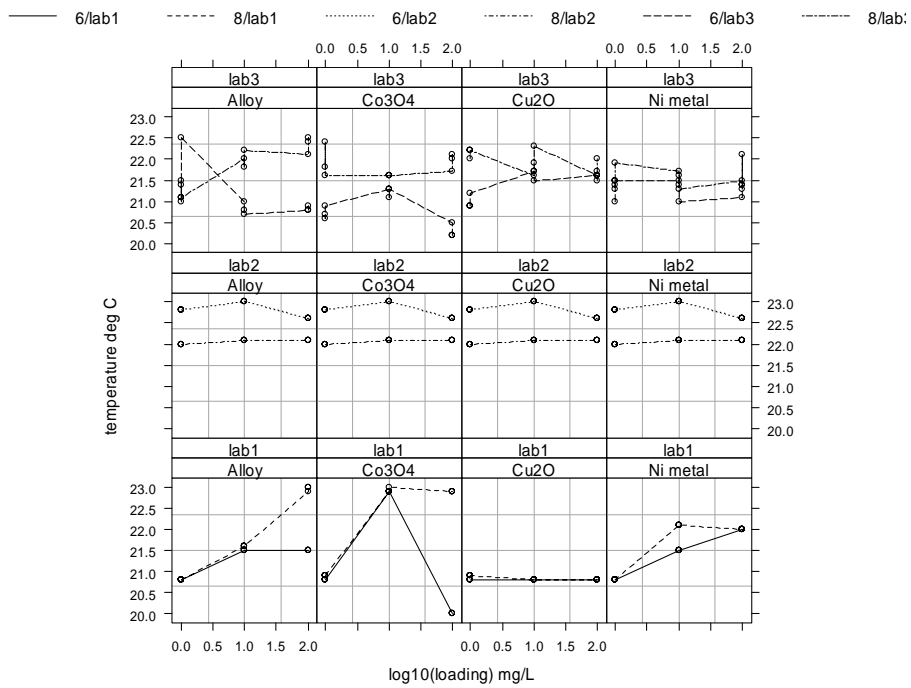
Figure 2-2.7: Temperature by lab and loading

2-2.1.5 Oxygen dissolved: 1-week data

85.   One gets an overview of the observed differences in *oxy.dis* by the following graph:
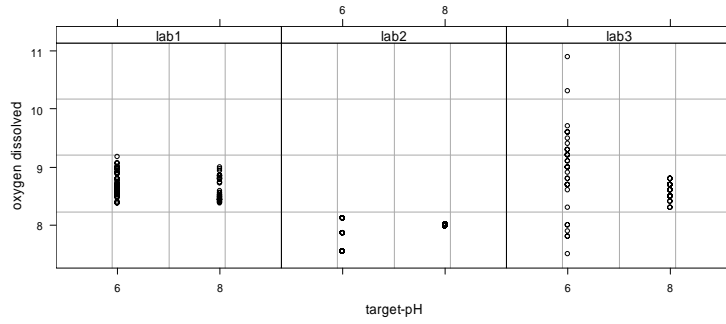
Figure 2-2.8: Oxygen dissolved by lab and target-pH
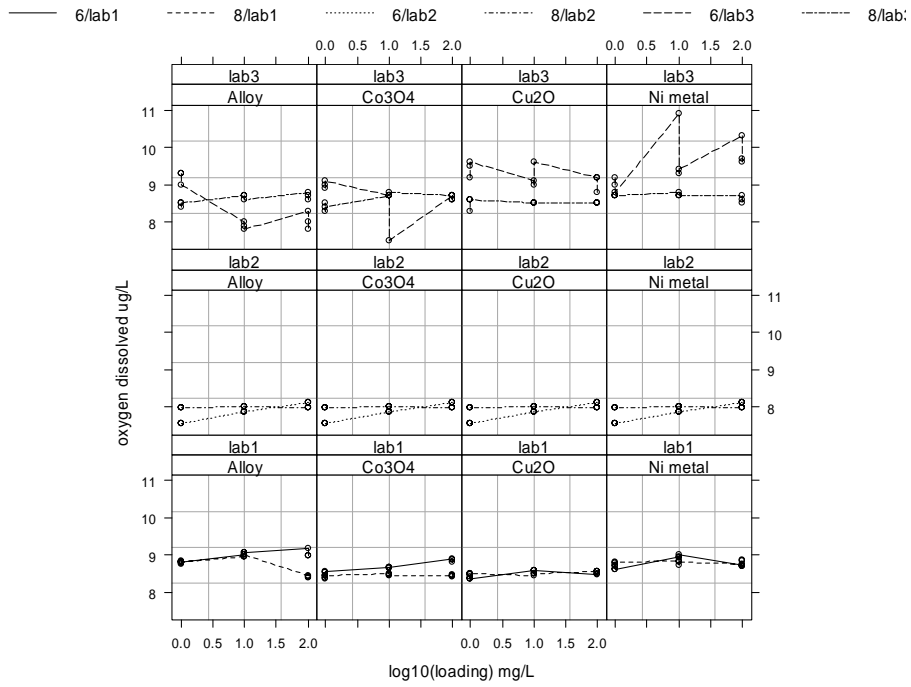
Figure 2-2.9: Oxygen dissolved by lab and loading

2-2.2 Description of short term screening

86.   The short term screening consists of measurements after 24h experimental time. Only loadings of 100 mg/L are under study. Graphs showing all measurements by lab and substance are provided, and, in addition a condensed plot, where the agreement between labs can be assessed directly.

2-2.2.1 Concentrations of metal dissolved: 24h results
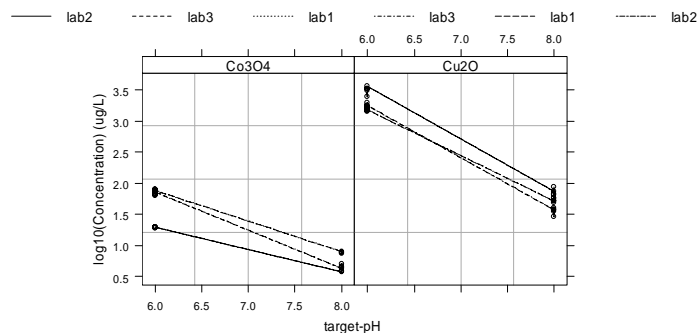
Figure 2-2.10: 24h data: concentrations of dissolved substances by target-pH
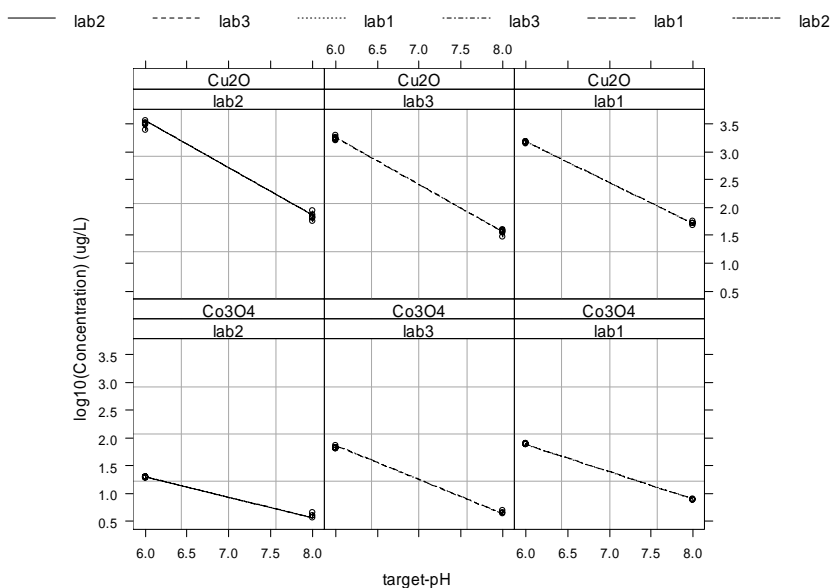
Figure 2-2.11: 24h data: concentrations of dissolved substances by lab and target-pH

2-2.3 Description of long-term tests

87.   The long-term tests consist of measurements after 4 weeks experimental time. Only loadings of 1 mg/L are under study. Graphs showing all measurements by lab and substance are provided, and, in addition a condensed plot, where the agreement between labs can be assessed directly.

2-2.3.1 Concentrations of metal dissolved: 4-weeks results
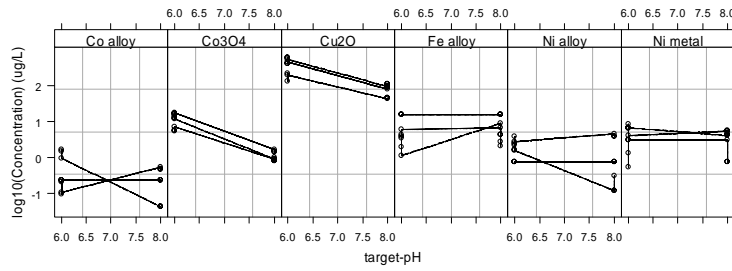
Figure 2-2.12: 672h data: concentrations of dissolved substances by target-pH
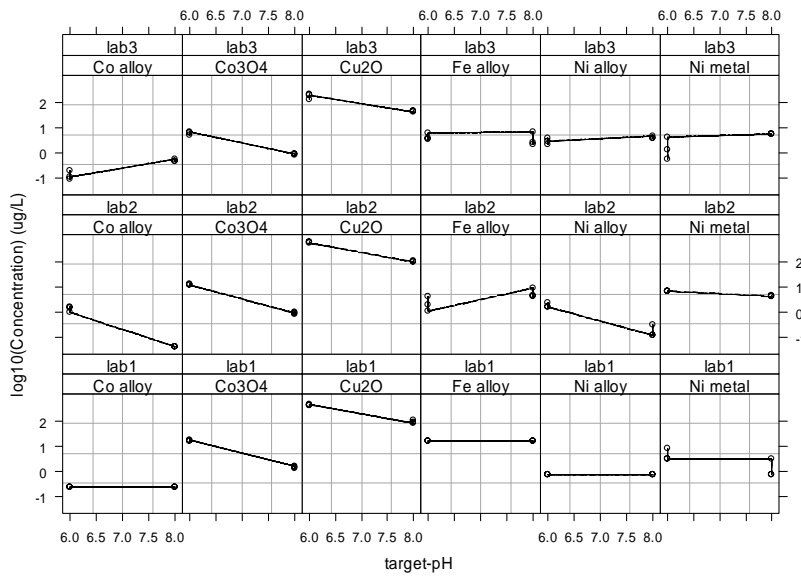
Figure 2-2.13: 672h data: concentrations of dissolved substances by lab and target-pH

CHAPTER 2-3: ASSESSING REPRODUCIBILITY AND REPEATABILITY

2-3.1 Measures for variability within and between laboratories

88.　　In the following sections estimates for of the standard deviations within and between laboratories, based on log10 transformed concentrations are provided. Results are given for each endpoint in time, target-pH, loading, and substance. The combined standard deviations correspond to the variability in values of a determination of a randomly selected laboratory. The basic formula is

$$s_{combined} = \sqrt{s_{between}^2 + s_{within}^2}$$

89.　　Estimates of standard deviations are calculated from a one-way analysis of variance decomposition of sum of squares, using laboratory as factor (see e.g. Searle 1987, p 490). For the case of 3 labs and 3 tests per lab it holds true, that

$$s_{within} = \sqrt{MS(error)}, \; and \; s_{between} = \sqrt{(MS(lab) - MS(residuals))/3} \, .$$

90.　　The computations use this ANOVA-based method. The method can fail, returning *negative* estimates of the between lab variance, if by chance very similar means between labs occur with accompanying considerable within lab variance. This case did not happen but once for the actual data. From the combined standard deviation a prediction interval can be derived giving a realistic picture of reproducibility of results. Such an interval is computed as usual

$$\hat{\mu} \pm z_{1-\alpha/2} \times s_{combined}$$

tacitly assuming approximate normally distributed data. For the log10 transformed concentrations this assumption seems not totally unrealistic, so the derived interval makes sense. It is very easy to retransform such an interval on log10-scale back to the original scale. The antilog-transform now gives us the desired results. One consequence of this retransformation is very useful: the uncertainty is characterized now by a factor $F = F(1-\alpha/2)$ and this factor can be interpreted without needing the corresponding mean. For the value of $\alpha/2 = 5\%$ the following definition and notations used:

$$F.95 = antilog(z_{1-0.05} \times s_{combined}) \, .$$

The following example shall illustrate all computations:

Example: 1 week data, Ni metal, target-pH=6 loading=1mg/L

```
        logC lab
61  1.697151 lab1
68  1.731257 lab1
75  1.710932 lab1
612 1.810757 lab2
681 1.817234 lab2
752 1.737688 lab2
613 1.684033 lab3
684 1.644082 lab3
753 1.668017 lab3


Analysis of Variance Table

Response: logC


         Df     Sum Sq   Mean Sq F value    Pr(>F)
lab       2 0.0231447 0.0115724  13.100  0.006469 **
Residuals 6 0.0053001 0.0008834
---
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

sd.between sd.within sd.combined
0.05969086 0.02972130 0.06668099

F.between F.within F.combined
1.147337  1.070832 1.165953


     uncertainty
F.95    1.287302
```

91.   The above results can be found in the first line of tables 2-3.7 and 2-3.8.

2-3.1.1 Inter laboratory analysis: 24h results

All experiments run with 100 mg/L loading

	lab1	lab2	lab3	sd.between	sd.within	sd.combined	m
6 Cu2O	3.17	3.50	3.25	0.17	0.04	0.18	3.31
6 Co3O4	1.89	1.29	1.82	0.33	0.02	0.33	1.67
8 Cu2O	1.72	1.84	1.55	0.14	0.05	0.15	1.70
8 Co3O4	0.89	0.61	0.66	0.15	0.03	0.15	0.72

Table 2-3.1: 24h results: standard deviations between labs, within labs, combined, and means on log10-scale

	F.between	F.within	F.combined	F0.95	lower	geom.mean	upper
6 Cu2O	1.48	1.10	1.50	1.95	1038.0	2022.2	3939.5
6 Co3O4	2.13	1.04	2.14	3.48	13.3	46.3	161.3
8 Cu2O	1.39	1.13	1.42	1.77	28.5	50.5	89.7
8 Co3O4	1.41	1.08	1.42	1.78	2.9	5.2	9.3

Table 2-3.2: 24h results: prediction factors and 90% prediction interval on orig. scale

2-3.1.2 Inter laboratory analysis: 4-week results

All experiments run with 1 mg/L loading

	lab1	lab2	lab3	sd.between	sd.within	sd.combined	m
6 Ni metal	0.62	0.82	0.15	0.30	0.29	0.42	0.53
6 Cu2O	2.62	2.76	2.23	0.27	0.07	0.28	2.54
6 Co3O4	1.21	1.09	0.77	0.23	0.05	0.23	1.03
6 Ni alloy	-0.13	0.26	0.45	0.29	0.09	0.31	0.19
6 Co alloy	-0.64	0.12	-0.90	0.52	0.12	0.54	-0.47
6 Fe alloy	1.20	0.32	0.62	0.43	0.19	0.47	0.71
8 Ni metal	0.07	0.64	0.74	0.34	0.20	0.40	0.48
8 Cu2O	1.96	2.00	1.64	0.20	0.04	0.20	1.87
8 Co3O4	0.16	-0.05	-0.08	0.13	0.04	0.14	0.01
8 Ni alloy	-0.13	-0.79	0.60	0.69	0.13	0.70	-0.11
8 Co alloy	-0.64	-1.37	-0.32	0.54	0.02	0.54	-0.77
8 Fe alloy	1.20	0.74	0.52	0.33	0.19	0.38	0.82

Table 2-3.3: 4-week results: standard deviations between labs, within labs, combined, and means on log10-scale

	F.between	F.within	F.combined	F0.95	lower	geom.mean	upper
6 Ni metal	2.02	1.94	2.62	4.88	0.7	3.4	16.5
6 Cu2O	1.86	1.18	1.90	2.86	120.7	345.6	989.8
6 Co3O4	1.69	1.11	1.70	2.40	4.4	10.6	25.4
6 Ni alloy	1.96	1.23	2.02	3.18	0.5	1.6	4.9
6 Co alloy	3.34	1.33	3.45	7.67	0.0	0.3	2.6
6 Fe alloy	2.71	1.54	2.97	5.99	0.9	5.2	31.0
8 Ni metal	2.20	1.60	2.51	4.53	0.7	3.1	13.8
8 Cu2O	1.57	1.10	1.59	2.14	34.5	74.0	158.3
8 Co3O4	1.35	1.10	1.37	1.68	0.6	1.0	1.7
8 Ni alloy	4.88	1.36	5.03	14.25	0.1	0.8	11.1
8 Co alloy	3.45	1.05	3.46	7.69	0.0	0.2	1.3
8 Fe alloy	2.13	1.54	2.39	4.20	1.6	6.6	27.8

Table 2-3.4: 4-week results: prediction factors and 90% prediction interval on orig. scale

2-3.1.3 Inter laboratory analysis: 1-week results

Results for 1 mg/L loading

	lab1	lab2	lab3	sd.between	sd.within	sd.combined	m
6 Ni metal	0.48	0.67	0.33	0.11	0.23	0.25	0.49
6 Cu2O	2.06	2.31	1.83	0.24	0.07	0.25	2.07
6 Co3O4	0.61	0.53	0.34	0.13	0.09	0.16	0.49
6 Ni alloy	-0.13	-0.60	0.26	0.39	0.32	0.50	-0.16
6 Co alloy	-0.64	-0.73	-1.10	0.14	0.34	0.37	-0.82
6 Fe alloy	1.20	0.35	0.45	0.47	0.05	0.47	0.66
8 Ni metal	0.48	0.49	0.69	0.11	0.05	0.12	0.55
8 Cu2O	1.35	1.44	1.22	0.10	0.06	0.12	1.34
8 Co3O4	-0.23	-0.47	-0.10	0.19	0.07	0.20	-0.27
8 Ni alloy	-0.13	-0.84	0.61	0.73	0.08	0.73	-0.12
8 Co alloy	-0.46	-1.37	-0.33	0.55	0.18	0.58	-0.72
8 Fe alloy	1.20	0.67	0.41	0.39	0.17	0.43	0.76

Table 2-3.5: 1-week results, loading=1mg/L: standard deviations between labs, within labs, combined, and means on log10-scale

	F.between	F.within	F.combined	F0.95	lower	geom.mean	upper
6 Ni metal	1.30	1.69	1.80	2.62	1.2	3.1	8.2
6 Cu2O	1.73	1.18	1.77	2.55	45.8	117.0	298.8
6 Co3O4	1.34	1.23	1.43	1.80	1.7	3.1	5.6
6 Ni alloy	2.44	2.09	3.18	6.71	0.1	0.7	4.6
6 Co alloy	1.38	2.21	2.35	4.07	0.0	0.2	0.6
6 Fe alloy	2.92	1.13	2.94	5.90	0.8	4.6	27.2
8 Ni metal	1.30	1.12	1.33	1.60	2.2	3.6	5.7
8 Cu2O	1.27	1.14	1.31	1.55	14.0	21.7	33.7
8 Co3O4	1.53	1.18	1.58	2.13	0.3	0.5	1.1
8 Ni alloy	5.32	1.20	5.37	15.89	0.0	0.8	12.0
8 Co alloy	3.59	1.52	3.84	9.14	0.0	0.2	1.7
8 Fe alloy	2.46	1.48	2.67	5.04	1.1	5.7	29.0

Table 2-3.6: 1-week results, loading=1mg/L: prediction factors and 90% prediction interval on orig. scale

Results for 10 mg/L loading

	lab1	lab2	lab3	sd.between	sd.within	sd.combined	m
6 Ni metal	1.71	1.79	1.67	0.06	0.03	0.07	1.72
6 Cu2O	2.94	3.20	2.89	0.17	0.06	0.18	3.01
6 Co3O4	1.50	1.49	1.25	0.14	0.02	0.14	1.41
6 Ni alloy	-0.13	0.09	-0.04	0.08	0.14	0.16	-0.03
6 Co alloy	-0.64	-0.52	-0.77	0.00	0.51	0.51	-0.64
6 Fe alloy	1.20	0.51	0.14	0.52	0.24	0.57	0.62
8 Ni metal	1.55	1.58	1.49	0.04	0.04	0.05	1.54
8 Cu2O	1.93	1.83	1.76	0.08	0.07	0.10	1.84
8 Co3O4	0.45	0.23	0.27	0.11	0.04	0.12	0.32
8 Ni alloy	0.54	-0.92	0.71	0.87	0.34	0.94	0.11
8 Co alloy	-0.64	-1.14	-0.00	0.55	0.25	0.61	-0.59
8 Fe alloy	1.20	0.82	-0.30	0.77	0.18	0.79	0.57

Table 2-3.7: 1-week results, loading=10mg/L: standard deviations between labs, within labs, combined, and means on log10-scale

	F.between	F.within	F.combined	F0.95	lower	geom.mean	upper
6 Ni metal	1.15	1.07	1.17	1.29	41.0	52.8	67.9
6 Cu2O	1.46	1.15	1.50	1.95	525.9	1025.1	1998.3
6 Co3O4	1.38	1.06	1.39	1.71	15.1	25.8	44.2
6 Ni alloy	1.19	1.38	1.44	1.82	0.5	0.9	1.7
6 Co alloy	1.00	3.23	3.23	6.89	0.0	0.2	1.6
6 Fe alloy	3.31	1.72	3.73	8.70	0.5	4.1	36.1
8 Ni metal	1.10	1.09	1.13	1.23	28.3	34.9	42.9
8 Cu2O	1.19	1.16	1.26	1.47	47.2	69.2	101.6
8 Co3O4	1.29	1.10	1.32	1.57	1.3	2.1	3.3
8 Ni alloy	7.50	2.18	8.67	34.91	0.0	1.3	44.9
8 Co alloy	3.58	1.77	4.05	9.97	0.0	0.3	2.5
8 Fe alloy	5.93	1.51	6.21	20.17	0.2	3.7	75.2

Table 2-3.8: 1-week results, loading=10mg/L: prediction factors and 90% prediction interval on orig. scale

Results for 100 mg/L loading

	lab1	lab2	lab3	sd.between	sd.within	sd.combined	m
6 Ni metal	2.65	2.84	2.73	0.10	0.01	0.10	2.74
6 Cu2O	3.58	3.69	3.51	0.09	0.05	0.10	3.59
6 Co3O4	2.15	2.26	1.95	0.16	0.05	0.16	2.12
6 Ni alloy	0.52	1.47	0.84	0.44	0.34	0.56	0.94
6 Co alloy	0.64	1.21	0.53	0.36	0.13	0.38	0.79
6 Fe alloy	1.20	0.43	0.17	0.50	0.32	0.60	0.60
8 Ni metal	2.62	2.62	2.50	0.07	0.02	0.07	2.58
8 Cu2O	2.21	1.95	2.01	0.13	0.02	0.13	2.06
8 Co3O4	0.93	0.50	0.56	0.23	0.08	0.24	0.66
8 Ni alloy	1.07	0.55	0.85	0.18	0.33	0.38	0.82
8 Co alloy	0.86	0.31	0.31	0.26	0.32	0.41	0.50
8 Fe alloy	1.20	0.32	-0.30	0.75	0.16	0.77	0.41

Table 2-3.9: 1-week results, loading=100mg/L: standard deviations between labs, within labs, combined, and means on log10-scale

	F.between	F.within	F.combined	F0.95	lower	geom.mean	upper
6 Ni metal	1.25	1.03	1.25	1.45	380.7	550.6	796.2
6 Cu2O	1.22	1.13	1.26	1.46	2672.4	3910.6	5722.6
6 Co3O4	1.43	1.12	1.46	1.85	71.3	132.1	244.9
6 Ni alloy	2.75	2.19	3.60	8.22	1.1	8.7	71.7
6 Co alloy	2.27	1.34	2.39	4.18	1.5	6.2	25.9
6 Fe alloy	3.19	2.08	3.94	9.54	0.4	4.0	38.0
8 Ni metal	1.18	1.06	1.19	1.33	285.9	379.7	504.1
8 Cu2O	1.36	1.05	1.36	1.67	68.6	114.2	190.2
8 Co3O4	1.68	1.20	1.74	2.48	1.8	4.6	11.4
8 Ni alloy	1.53	2.14	2.39	4.19	1.6	6.6	27.8
8 Co alloy	1.81	2.10	2.58	4.76	0.7	3.1	15.0
8 Fe alloy	5.60	1.44	5.82	18.13	0.1	2.6	46.3

Table 2-3.10: 1-week results, loading=100mg/L: prediction factors and 90% prediction interval on orig. scale

2-3.2 Overview of results on reproducibility

2-3.2.1 Metals

	F0.95.l1	F0.95.l10	F0.95.l100	geom.l1	geom.l10	geom.l100
6 Ni metal	2.62	1.29	1.45	3.1	52.8	550.6
6 Cu2O	2.55	1.95	1.46	117.0	1025.1	3910.6
6 Co3O4	1.80	1.71	1.85	3.1	25.8	132.1
8 Ni metal	1.60	1.23	1.33	3.6	34.9	379.7
8 Cu2O	1.55	1.47	1.67	21.7	69.2	114.2
8 Co3O4	2.13	1.57	2.48	0.5	2.1	4.6

Table 2-3.11: Metals, 1-week results, loadings =1, 10, 100mg/L: prediction factors and geom. means on orig. scale

	F0.95.1w	F0.95.4w	geom.1w	geom.4w
6 Ni metal	2.62	4.88	3.1	3.4
6 Cu2O	2.55	2.86	117.0	345.6
6 Co3O4	1.80	2.40	3.1	10.6
8 Ni metal	1.60	4.53	3.6	3.1
8 Cu2O	1.55	2.14	21.7	74.0
8 Co3O4	2.13	1.68	0.5	1.0

Table 2-3.12: Metals, loading 1 mg/L, time =1, 4 weeks: prediction factors and geom. means on orig. scale

	F0.95.1d	F0.95.1w	geom.1d	geom.1w
6 Cu2O	1.95	1.46	2022.2	3910.6
6 Co3O4	3.48	1.85	46.3	132.1
8 Cu2O	1.77	1.33	50.5	379.7
8 Co3O4	1.78	1.67	5.2	114.2

Table 2-3.13: Metals, loading 100 mg/L, time =1 day, 1 week: prediction factors and geom. means on orig. scale

2-3.2.2 Alloy

	F0.95.l1	F0.95.l10	F0.95.l100	geom.l1	geom.l10	geom.l100
6 Ni alloy	6.71	1.82	8.22	0.7	0.9	8.7
6 Co alloy	4.07	6.89	4.18	0.2	0.2	6.2
6 Fe alloy	5.90	8.70	9.54	4.6	4.1	4.0
8 Ni alloy	15.89	34.91	4.19	0.8	1.3	6.6
8 Co alloy	9.14	9.97	4.76	0.2	0.3	3.1
8 Fe alloy	5.04	20.17	18.13	5.7	3.7	2.6

Table 2-3.14: Alloy, 1-week results, loadings =1, 10, 100mg/L: prediction factors and geom. means on orig. scale

	F0.95.1w	F0.95.4w	geom.1w	geom.4w
6 Ni alloy	6.71	3.18	0.7	1.6
6 Co alloy	4.07	7.67	0.2	0.3
6 Fe alloy	5.90	5.99	4.6	5.2
8 Ni alloy	15.89	14.25	0.8	0.8
8 Co alloy	9.14	7.69	0.2	0.2
8 Fe alloy	5.04	4.20	5.7	6.6

Table 2-3.15: Alloy, loading 1 mg/L, time =1, 4 weeks: prediction factors and geom. means on orig. scale

2-3.3 Estimating variability – integrated approach for Ni metal, Co$_3$O$_4$, Cu$_2$O

2-3.3.1 Within and between laboratory variance – estimates without adjustment of covariates

92.   When estimating between-laboratory standard deviations, one has to be aware of the fact, that the estimated values have a large inherent random fluctuation. This is due to the small number of replicates, when considering only one combination of experimental conditions. So it was decided to perform an integrated analysis including all measurements, modeling the effect of substance, lab, and loading. From this linear-mixed-model approach one gets more reliable estimates of the variance components. The estimation is based on the function lme of the R-package nmle (Pinheiro and Bates, 2000; Pinheiro et al., 2006).

Results for target-pH 6

	standard dev.
between	0.133
within	0.116

Table 2-3.16: Estimated standard deviations between and within laboratories for target-pH 6, integrated approach for Ni metal, Co3O4, Cu2O, not adjusted for covariates.

Results for target-pH 8

	standard dev.
between	0.090
within	0.109

Table 2-3.17: Estimated standard deviations between and within laboratories for target-pH 8, integrated approach for Ni metal, Co3O4, Cu2O, not adjusted for covariates.

2-3.3.2 Exploratory analysis – improvement of reproducibility by adjusting for covariates
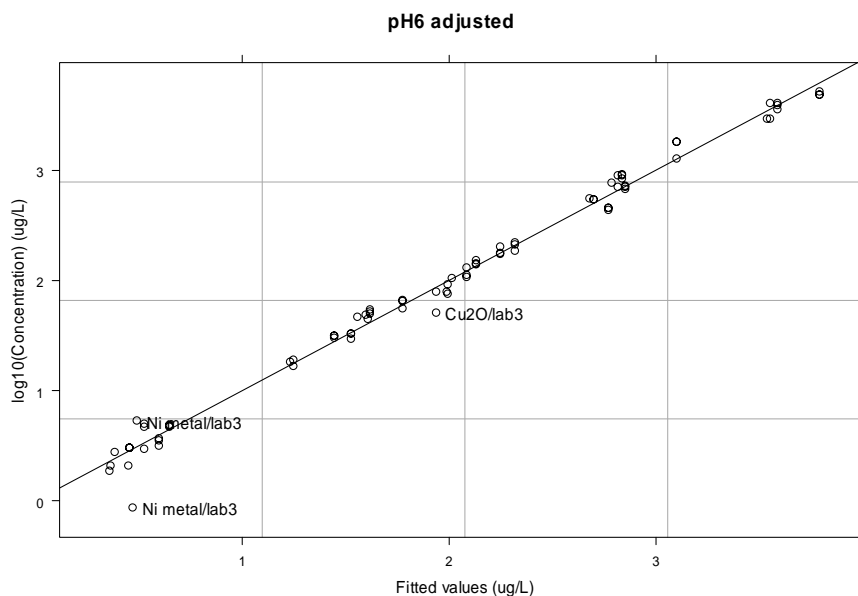
Results for target-pH 6

Figure 2-3.1: Observed and fitted values for target pH 6 results. Concentrations adjusted for log10(load), substance, temperature

93.   Estimated regression coefficients and significance of covariates can be read from the following table.

	Value	Std.Error	DF	t-value	p-value
(Intercept)	-1.227	0.438	66	-2.805	0.0066
log10(load)	0.833	0.026	66	31.834	0.0000
temp	0.080	0.020	66	4.018	0.0002
log10(load):subCu2O	-0.081	0.037	66	-2.185	0.0324
log10(load):subNi metal	0.277	0.038	66	7.388	0.0000
subCu2O:temp	0.075	0.003	66	22.933	0.0000
subNi metal:temp	0.001	0.003	66	0.180	0.8575

Table 2-3.18: Regression coefficients of covariables used for adjustment, target-pH =6

94.   For the computations a mixed model is used with different coefficients per substance for loading and temperature including laboratory within substance as random effect. For this model one gets the following estimates of within and between lab standard-deviation:

	standard dev.
between	0.062
within	0.105

Table 2-3.19: Estimated standard deviations between and within laboratories for target-pH 6, integrated approach for Ni metal, Co3O4, Cu2O, adjusting for temp, sub, and loading
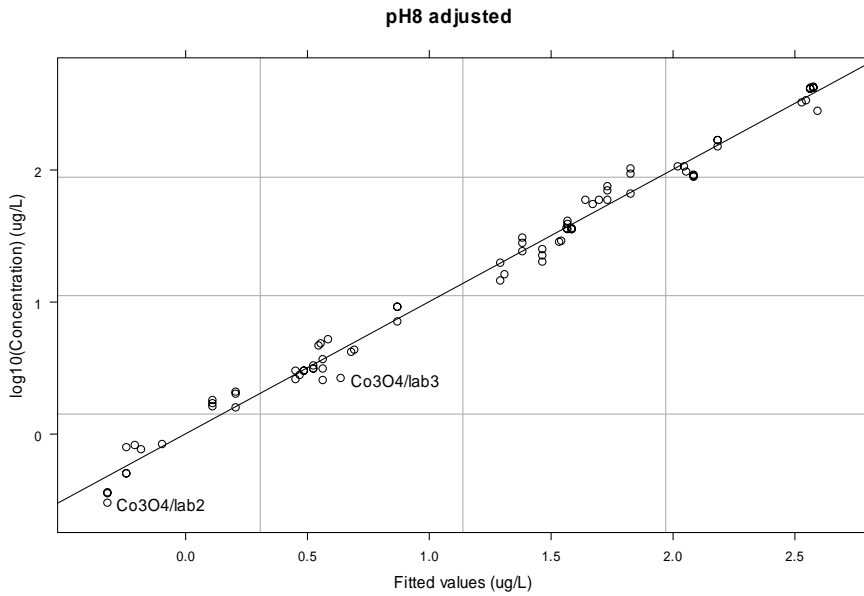
Results for target-pH 8

pH8 adjusted

Figure 2-3.2: Observed and fitted values for target pH 8 results. Concentrations adjusted for log10(load), substance, temperature

	Value	Std.Error	DF	t-value	p-value
(Intercept)	-0.426	2.136	66	-0.200	0.8424
log10(load)	0.416	0.027	66	15.630	0.0000
temp	0.139	0.037	66	3.724	0.0004
log10(load):subCu2O	-0.060	0.036	66	-1.695	0.0948
log10(load):subNi metal	0.580	0.037	66	15.588	0.0000
subCu2O:temp	-0.221	0.080	66	-2.768	0.0073
subNi metal:temp	-0.058	0.065	66	-0.900	0.3713

Table 2-3.20: Regression coefficients of covariates used for adjustment, target-pH = 8

95.   For this model one gets the following estimates of within and between lab standard-deviation:

	standard dev.
between	0.084
within	0.098

Table 2-3.21: Estimated standard deviations between and within laboratories for target-pH 8, integrated approach for Ni metal, Co3O4, Cu2O, adjusting for temp, sub, and loading

2-3.3.3 Checking assumptions

96.   Before summarizing the results of the integrated approach for assessing variability within the T/DP framework, one has to investigate the appropriateness of the model. There are two questions to be answered:
   1. Does residual variance depend on expected value, or can the assumption of homogenous variances be adopted?
   2. Can the error terms be considered as being normally distributed?

97.   For answering question 1 a display of the residuals versus the fitted values has been prepared. These plots shall provide a more detailed view of the residuals than figures 2-3.1 and 2-3.2.
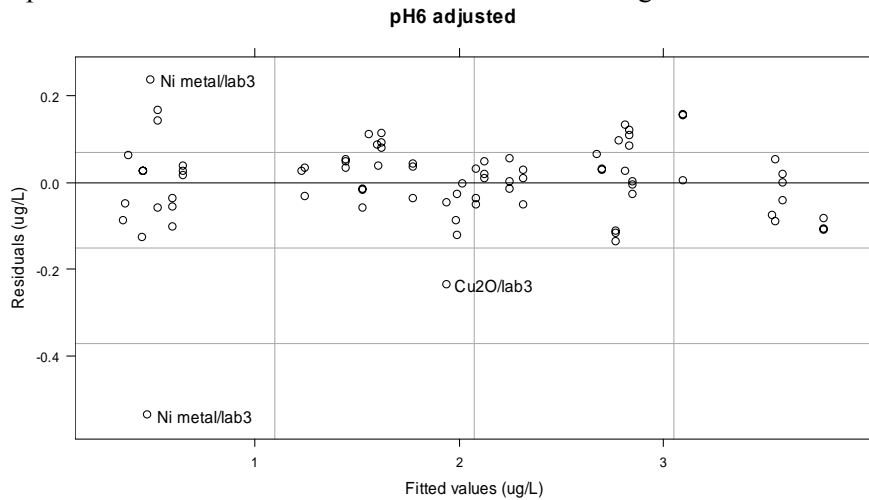
pH6 adjusted

Figure 2-3.3: Plot of residuals versus fitted from adjusted model, target-pH=6. Check for homogeneity of variance.
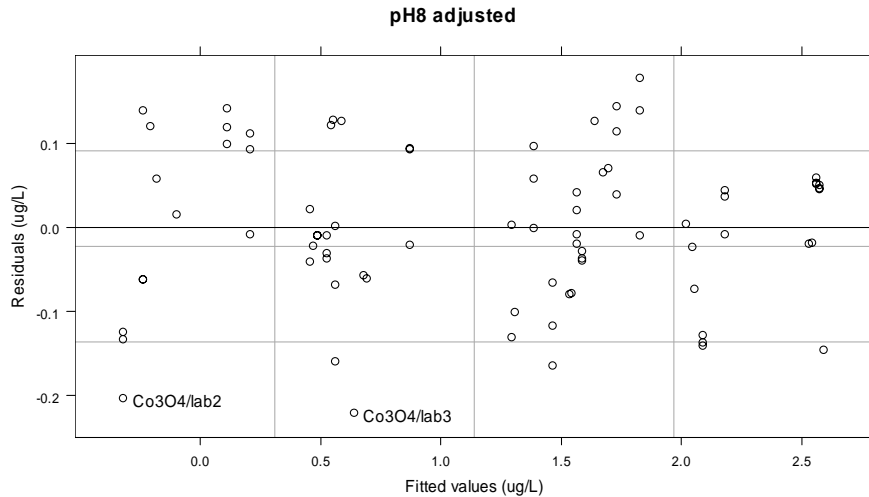
pH8 adjusted

Figure 2-3.4: Plot of residuals versus fitted from adjusted model, target-pH=8. Check for homogeneity of variance.

98.   Question 2 is handled using Q-Q plots of the data. It is common practice in applied statistics to investigate model assumptions in this way. When estimating random effects, normality is more crucial compared to fixed effects models. Therefore, Q-Q plots of the residuals have been prepared. Residuals are computed as differences between observed and fitted values. From the displays of the observed values

versus the fitted, given in figures 2-3.1 and 2-3.2, it could already been concluded, that there is a very good fit of the model over a broad range of concentrations measured. The two residual plots given below demonstrate, that there is no striking evidence against the normality assumption for residuals. This can be seen from the linear relation between standardized residuals and corresponding normal quantiles. There are several outliers, pointing to problems of measurement near the limit of detection, but these outliers don't have large influence on the final results. Therefore, no special outlier handling was performed. The outliers marked in the graph stem from the low 1mg/L loading. It is already obvious from the display of the raw data given in figure 2-2.1 that some of the low-loading tests show larger variability than all others.

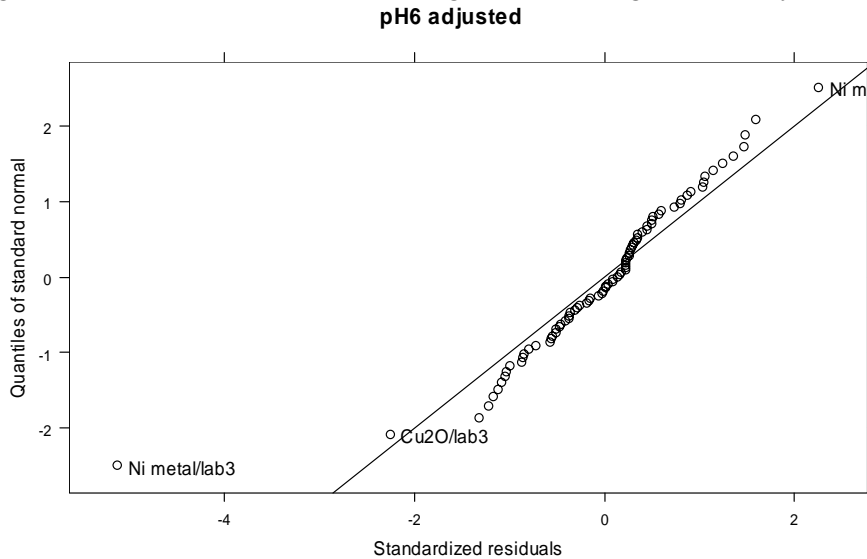**Figure 2-3.5: Q-Q plot of residuals from adjusted model, target-pH=6. Check for normality assumption.**
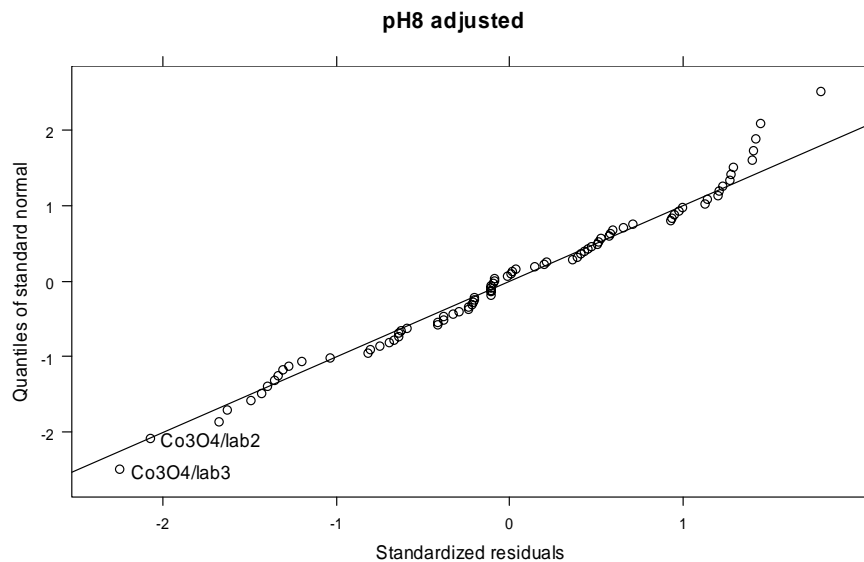

Figure 2-3.6: Q-Q plot of residuals from adjusted model, target-pH=8. Check for normality assumption.

99.   Similar to the Q-Q plot for the target-pH 6 residuals, the normality assumption is met. Outliers appear even nearer the expected range, and do not influence the results at all.

2-3.4 Summary of integrated approach

100.   The integrated approach presented above shows that the variability of the data is rather homogenous in log10-scale over a range of nearly 4 orders of magnitude and between the substances under study. A review of the model features and an assessment of the results will be given below.

Selection of Experiments

101.   The overview in Section 2-3.2 shows that variability of alloy results is extremely high. In addition the alloy data do not provide evidence for effects of loading and target-pH level, clearly demonstrated with all metal-compounds under investigation. Therefore, integrated analysis is restricted to the metal powder and the metal oxide powders only.

Statistical Models

102.   Linear mixed models are the framework of the integrated analysis. First a simple linear model is fitted with log10 (loading), substance and the interaction as fixed effects and the lab within each substance as random effect. The estimated variance components from this model are the basis for the assessment of reproducibility and repeatability. Confidence bounds for this components are not reported for two reasons: the within-lab component is not too large and reasonable, confidence interval show the same, however for the between-lab component there are not enough degrees of freedom, and, therefore, uninformative wide intervals result.

103.   In addition the simple model is expanded, including covariates such as the difference of actual and target pH in order to check, whether a smaller variability can be achieved by adjustment for these covariates. The estimates from this adjusted analysis can be regarded as best results achievable under perfect control of experimental conditions.

104.   This adjustment has to be interpreted with care. One has to keep in mind, that the actual experimental conditions included in the analysis are just observational results, more or less randomly deviating from the values prescribed by the protocol. Therefore, accidental overfitting without any real background, cannot be excluded. For the data at hand the actual values are mostly well within the range specified by the T/DP. A second aspect concerns possibly different reporting of the experimental conditions by the different labs. The actual loading could not be used as a covariate, therefore, as Laboratory 2 reported always perfect matching of nominal and actual loadings.

Results

105.   **Loading:** Retransforming the model-equation gives $C \sim L^{\beta}$, C denoting concentration and L loading, with substance-specific coefficients β. For Nickel metal one gets β-coefficients approximately equal to 1 for both levels of target-pH. For Co_3O_4 and Cu_2O coefficients are found, slightly below 1 for a target-pH of 6, whereas the oxide-concentration measured is no longer proportional to loading for target-pH 8: β-coefficients near 0.4 are estimated.

106.   **Covariates:** Only temperature remains as covariate after a model-selection procedure. Deviations of actual pH from target value show no significant influence on the concentrations measured.

107.   **Variability:** For the estimated standard deviations based on the log10 transformed data similar values near 0.1 are found within and between laboratories (see tables 2-3.16 and 2-3.17). The antilog-transformation of the estimated standard deviations gives $10^{0.1}=1.25$. It provides a measure for the variability of the data on original scale. This factor corresponds to a 25% coefficient of variation for

repeatability and pure between lab reproducibility. As one multiplies and divides by this factor, the lower deviations are only 20%. The combined standard deviation of 0.14 corresponds to a factor of 1.38. Using this combined standard-deviation in combination with a 95% quantile of the normal distribution, one arrives at a 95% prediction factor of 1.70. This factor can be used to derive one-sided 95% confidence bounds for results from of replicate experiment of a randomly chosen laboratory, or for a 90% prediction interval.

108. **Variability adjusted:** The estimated variance components are slightly smaller for the adjusted approach. Taking the most favorable values from table 2-3.19 for target-pH 6, standard deviations of 0.1 within and of 0.06 between can be used, arriving at factors of 1.25 and 1.15 respectively. The 95% prediction factor for this most favorable case would be 1.55.

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[1] The first edition of the GHS was approved by the UN Sub-Committee of Experts on the GHS in December 2002 and published in 2003. The first revised edition was published in 2005 and the second revised edition in 2007. Transformation/Dissolution Protocol is attached to the first edition as Annex 9 and to the first and second revised edition as Annex 10.

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ANNEX 1: GUIDANCE ON TRANSFORMATION/DISSOLUTION OF METALS AND METAL COMPOUNDS IN AQUEOUS MEDIA

NOTE FOR READERS:

This guidance was published in July 2001 as the *Guidance Document on Transformation/Dissolution of Metals and Metal Compounds in Aqueous Media* (OECD Series on Testing and Assessment No. 29, 2001) and as Annex to the United Nations' Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (United Nations, first edition, 2003; first revised edition, 2005; second revised edition, 2007). This guidance is attached to the first edition of the GHS as Annex 9 and to the first and second revised edition as Annex 10

GUIDANCE ON TRANSFORMATION/DISSOLUTION OF METALS AND METAL COMPOUNDS IN AQUEOUS MEDIA[1]

A10.1   Introduction

A10.1.1   This Test Guidance is designed to determine the rate and extent to which metals and sparingly soluble metal compounds can produce soluble available ionic and other metal-bearing species in aqueous media under a set of standard laboratory conditions representative of those generally occurring in the environment. Once determined, this information can be used to evaluate the short term and long term aquatic toxicity of the metal or sparingly soluble metal compound from which the soluble species came. This Test Guidance is the outcome of an international effort under the OECD to develop an approach for the toxicity testing and data interpretation of metals and sparingly soluble inorganic metal compounds (SSIMs) (reference 1, this annex and section A9.7 of Annex 9). As a result of recent meetings and discussions held within the OECD and EU, the experimental work on several metals and metal compounds upon which this Test Guidance is based has been conducted and reported (references 5 to 11, this annex).

A10.1.2 The evaluation of the short term and long term aquatic toxicity of metals and sparingly soluble metal compounds is to be accomplished by comparison of (a) the concentration of the metal ion in solution, produced during transformation or dissolution in a standard aqueous medium with (b) appropriate standard ecotoxicity data as determined with the soluble metal salt (acute and chronic values). This document gives guidance for performing the transformation/dissolution tests. The strategy to derive an environmental hazard classification using the results of the dissolution/transformation protocol is not within the scope of this Guidance document and can be found in Annex 9, section A9.7.

A10.1.3   For this Test Guidance, the transformations of metals and sparingly soluble metal compounds are, within the context of the test, defined and characterized as follows:

(a)   metals, M^0, in their elemental state are not soluble in water but may transform to yield the available form. This means that a metal in the elemental state may react with the media to form soluble cationic or anionic products, and in the process the metal will oxidize, or transform, from the neutral or zero oxidation state to a higher one;

(b)   in a simple metal compound, such as an oxide or sulphide, the metal already exists in an oxidized state, so that further metal oxidation is unlikely to occur when the compound is introduced into an aqueous medium. However, while oxidization state may not change, interaction with the media may yield more soluble forms. A sparingly soluble metal compound can be considered as one for which a solubility product can be calculated, and which will yield small amount of the available form by dissolution. However, it should be recognized that the final solution concentration may be influenced by a number of factors, including the solubility product of some metal compounds precipitated during the transformation/dissolution test, e.g. aluminium hydroxide.

A10.2   Principles

A10.2.1.   This Test Guidance is intended to be a standard laboratory transformation/ dissolution protocol based on a simple experimental procedure of agitating various quantities of the test substance in a pH buffered aqueous medium, and sampling and analysing the solutions at specific time intervals to determine the concentrations of dissolved metal ions in the water. Two different types of tests are described in the text below:

A10.2.2 *Screening transformation/dissolution test – sparingly soluble metal compounds*

A10.2.2.1 For sparingly soluble metal compounds, the maximum concentration of total dissolved metal can be determined by the solubility limit of the metal compound or from a screening transformation/dissolution test. The intent of the screening test, performed at a single loading, is to identify those compounds which undergo either dissolution or rapid transformation such that their ecotoxicity potential is indistinguishable from soluble forms.

A10.2.2.2 Sparingly soluble metal compounds, having the smallest representative particle size on the market are introduced into the aqueous medium at a single loading of 100 mg/l. Such dissolution as will occur is achieved by agitation during a 24 hours period. After 24 hours agitation, the dissolved metal ion concentration is measured.

A10.2.3 *Full transformation/dissolution test - metals and sparingly soluble metal compounds*

A10.2.3.1 The full transformation/dissolution test is intended to determine level of the dissolution or transformation of metals and metal compounds after a certain time period at different loadings of the aqueous phase. Normally massive forms and/or powders are introduced into the aqueous medium at three different loadings: 1, 10 and 100 mg/l. A single loading of 100 mg/l may be used if a significant release of dissolved metal species is not anticipated. Transformation/dissolution is accomplished by standardized agitation, without causing abrasion of the particles. The short term transformation/dissolution endpoints are based on the dissolved metal ion concentrations obtained after a 7 days transformation/dissolution period. The long term transformation/dissolution endpoint is obtained during a 28 days transformation/dissolution test, using a single load of 1 mg/l.

A10.2.3.2 As pH has a significant influence on transformation/dissolution both the screening test and the full test should in principle be carried out at a pH that maximizes the concentration of the dissolved metal ions in solution. With reference to the conditions generally found in the environment a pH range of 6 to 8.5 must be used, except for the 28 day full test where the pH range of 5.5 to 8.5 should be used in order to take into consideration possible long term effects on acidic lakes.

A10.2.3.3 As in addition the surface area of the particles in the test sample has an important influence on the rate and extent of transformation/dissolution, powders are tested at the smallest representative particle size as placed on the market, while massives are tested at a particle size representative of normal handling and use. A default diameter value of 1 mm should be used in absence of this information. For massive metals, this default may only be exceeded when sufficiently justified. The specific surface area should be determined in order to characterize and compare similar samples.

A10.3 **Applicability of the test**

This test applies to all metals and sparingly soluble inorganic metal compounds. Exceptions, such as certain water reactive metals, should be justified.

A10.4 **Information on the test substance**

Substances as placed on the market should be used in the transformation/dissolution tests. In order to allow for correct interpretation of the test results, it is important to obtain the following information on the test substance(s):

(a) substance name, formula and use on the market;

(b) physical-chemical method of preparation;

(c) identification of the batch used for testing;

(d) chemical characterization: overall purity (%) and specific impurities (% or ppm);

(e) density (g/cm^3) or specific gravity;

(f) measured specific surface area (m^2/g)- measured by BET N$_2$ adsorption-desorption or equivalent technique;

(g) storage, expiration date;

(h) known solubility data and solubility products;

(i) hazard identification and safe handling precautions;

(j) material Safety Data Sheets (MSDS) or equivalent.

A10.5   Description of the test method

A10.5.1 *Apparatus and reagents*

A10.5.1.1   The following apparatus and reagents are necessary for performing tests:

(a) Pre-cleaned and acid rinsed closed glass sample bottles (A10.5.1.2);

(b) transformation /dissolution medium (ISO 6341) (A10.5.1.3);

(c) test solution buffering facilities (A10.5.1.4);

(d) agitation equipment: orbital shaker, radial impeller, laboratory shaker or equivalent (A10.5.1.5);

(e) appropriate filters (e.g.0.2 µm Acrodisc) or centrifuge for solids-liquid separation (A10.5.1.7);

(f) means to control the temperature of the reaction vessels to + 2 °C within the temperature range of 20 °C to 25 °C, such as a temperature controlled cabinet or a water bath;

(g) syringes and/or automatic pipettes;

(h) pH meter showing acceptable results within + 0.2 pH units;

(i) dissolved oxygen meter, with temperature reading capability;

(j) thermometer or thermocouple; and

(k) analytical equipment for metal analysis (e.g. atomic adsorption spectrometry, inductively cou-pled axial plasma spectrometry).

A10.5.1.2   All glass test vessels must be carefully cleaned by standard laboratory practices, acid-cleaned (e.g. HCl) and subsequently rinsed with de-ionized water. The test vessel volume and configuration (one- or two-litre reaction kettles) should be sufficient to hold 1 or 2 *l* of aqueous medium without overflow during the agitation specified. If air buffering is used (tests carried out at pH 8), it is advised to increase the air buffering capacity of the medium by increasing the headspace/liquid ratio (e.g. 1 *l* medium in 2.8 *l* flasks).

A10.5.1.3   A reconstituted standard water based on ISO 6341 should be used[2], as the standard transformation/dissolution medium. The medium should be sterilized by filtration (0.2 µm) before use in the

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[2]   *For hazard classification purposes the results of the dissolution/transformation protocol are compared with existing ecotoxicity data for metals and metal compounds. However, for purposes such as data validation, there might be cases where it may be appropriate to use the aqueous medium from a completed transformation test directly in an*

tests. The chemical composition of the standard transformation/dissolution medium (for tests carried out at pH 8) is as follows:

$NaHCO_3$:	65.7 mg/l
KCl:	5.75 mg/l
$CaCl_2.2H_2O$:	294 mg/l
$MgSO_4.7H_2O$:	123 mg/l

For tests carried out at lower pH values, adjusted chemical compositions are given in A10.5.1.7.

A10.5.1.4   The concentration of total organic carbon in the medium should not exceed 2.0 mg/l.

A10.5.1.5   In addition to the fresh water medium, the use of a standardized marine test medium may also be considered when the solubility or transformation of the metal compound is expected to be significantly affected by the high chloride content or other unique chemical characteristics of marine waters and when toxicity test data are available on marine species. When marine waters are considered, the chemical composition of the standard marine medium is as follows:

NaF:	3mg/l
$SrCl_2.6H_2O$:	20 mg/l
H_3BO_3:	30 mg/l
KBr:	100 mg/l
KCl:	700 mg/l
$CaCl_2.2H2O$:	1.47g/l
Na_2SO_4:	4.0 g/l
$MgCl_2.6H2O$:	10.78 g/l
NaCl:	23.5 g/l
$Na_2SiO_3.9H2O$:	20 mg/l
$NaHCO_3$:	200 mg/l

The salinity should be 34 ± 0.5 g/kg and the pH should be 8.0 ± 0.2. The reconstituted salt water should also be stripped of trace metals (from ASTM E 729-96).

A10.5.1.6   The transformation/dissolution tests are to be carried out at a pH that maximizes the concentration of the dissolved metal ions in solution within the prescribed pH range. A pH-range of 6 to 8.5 must be used for the screening test and the 7 day full test, and a range of 5.5 to 8.5 for the 28 day full test (A10.2.3.2).

A10.5.1.7   Buffering at pH 8 may be established by equilibrium with air, in which the concentration of CO_2 provides a natural buffering capacity sufficient to maintain the pH within an average of ± 0.2 pH units over a period of one week (reference 7, Annex 10). An increase in the headspace/liquid ratio can be used to improve the air buffering capacity of the medium.

For pH adjustment and buffering down to pH 7 and 6, Table A10.1 shows the recommended chemical compositions of the media, as well as the CO_2 concentrations in air to be passed through the headspace, and the calculated pH values under these conditions.

OECD 202 and 203 daphnia and fish ecotoxicity test. If the CaCl₂.2H₂O and MgSO₄.7H₂O concentrations of the transformation medium are reduced to one-fifth of the ISO 6341 medium, the completed transformation medium can also be used (upon the addition of micronutrients) in an OECD 201 algae ecotoxicity test.

Table A10.1: Recommended chemical composition of testing medium

Chemical composition of medium	$NaHCO_3$	6.5 mg/l	12.6 mg/l
	KCl	0.58 mg/l	2.32 mg/l
	$CaCl_2.2H_2O$	29.4 mg/l	117.6 mg/l
	$MgSO_4.7H_2O$	12.3 mg/l	49.2 mg/l
CO_2 concentration (balance is air) in test vessel		0.50%	0.10%
Calculated pH		6.09	7.07

NOTE: The pH values were calculated using the FACT (Facility for the Analysis of Chemical Thermodynamics) System (http://www.crct.polymtl.ca/fact/fact.htm).

A10.5.1.8   Alternative equivalent buffering methods may be used if the influence of the applied buffer on the chemical speciation and transformation rate of the dissolved metal fraction would be minimal.

A10.5.1.9   During the full transformation/dissolution tests, agitation should be used which is sufficient to maintain the flow of aqueous medium over the test substance while maintaining the integrity of the surface of the test substance and of any solid reaction product coatings formed during the test. For 1 *l* of aqueous medium, this may be accomplished by the use of:

(a)   a radial impeller set at 200 r.p.m., with blades deployed 5 cm from the bottom of a 1 *l* reaction kettle. The radial impellers consist of two fixed polypropylene blades of dimensions 40 mm width by 15 mm height on a PVC-coated steel rod 8 mm diameter and 350 mm long; or

(b)   a 1.0 to 3.0 *l* flask capped with a rubber stopper and placed on an orbital or laboratory shaker set at 100 r.p.m.

Other methods of gentle agitation may be used provided they meet the criteria of surface integrity and homogeneous solution.

A10.5.1.10   The choice of solids-liquid separation method depends on whether adsorption of soluble metal ions on filters occurs and whether or not a suspension is generated by the agitation prescribed in A10.5.1.9, which will in turn depend on particle size distributions and particle density. For solids of density greater than approximately 6 g/cm^3 and particle size ranges as low as 50% < 8 μm, experience has shown that the gentle agitation methods prescribed in A10.5.1.9 are unlikely to result in suspensions. Hence, filtration of a sample through e.g. a 25 mm diameter 0.2 μm hydrophilic polyethersulphone membrane syringe filter (as an option, overlain by a 0.8 μm prefilter) will result in a solution essentially free of solids.

However, in the event that suspensions occur, stopping the agitation to allow the suspension to settle for about 5 minutes prior to taking a solution sample may be useful.

A10.5.2   *Prerequisites*

A10.5.2.1   *Analytical method*

A suitable validated analytical method for the total dissolved metal analysis is essential to the study. The analytical detection limit should be lower than the appropriate chronic or long term value from the exotoxicity tests.

The following analytical validation aspects are at a minimum to be reported:

(a)   detection and quantification limit of the analytical method;

(b)   analytical linearity range within the applicable analytical range;

(c)   a blank run consisting of transformation medium (this can be done during the tests);

(d)   matrix effect of the transformation medium on the measurement of the dissolved metal ion;

(e)   mass balance (%) after completion of the transformation test;

(f)   reproducibility of the analysis;

(g)   adsorptive properties of the soluble metal ions on the filters (if filtration is used for the separation of the soluble from the solid metal ion).

A10.5.2.2   *Determination of the appropriate pH of the dissolution medium*

If no relevant literature data exist, a preliminary screening test may need to be carried out in order to ensure that the test is performed at a pH maximizing transformation/dissolution within the pH range described in A10.2.3.2 and A10.5.1.6.

A10.5.2.3   *Reproducibility of transformation data*

A10.5.2.3.1   For a standard set-up of three replicate test vessels and two replicate samples per test vessel at each sampling time, it is reasonable to anticipate that for a constant loading of a substance, tested in a narrow particle size (e.g. 37 - 44 μm) and total surface area range, the within-vessel variation in transformation data should be < 10% and the between-vessel variation should be < 20 % (reference 5, this annex).

A10.5.2.3.2   To estimate the reproducibility of the transformation test, some Guidance is given in the following. The results can be used to eventually improve on reproducibility by adjusting the final test set-up through varying the number of replica test vessels and/or replica samples or further screening of the particles. The preliminary tests also allow for a first evaluation of the transformation rate of the tested substance and can be used to establish the sampling frequency.

A10.5.2.3.3   In preparing the transformation/dissolution medium, the pH of the medium should be adjusted to the desired pH (air buffering or CO_2 buffering) by agitation for about half an hour to bring the aqueous medium into equilibrium with the buffering atmosphere. At least three samples (e.g. 10 - 15 ml) are drawn from the test medium prior to addition of the substance, and the dissolved metal concentrations are measured as controls and background.

At least five test vessels, containing the metal or metal compound (e.g.100 mg solid/l medium), are agitated as described in A10.5.1.9 at a temperature ± 2 °C in the range 20 - 25 °C, and triplicate samples are taken by syringe from each test vessel after 24 hours. The solid and solution are separated by membrane filter as described in A10.5.1.10, the solution is acidified with 1% HNO_3 and analysed for total dissolved metal concentration.

A10.5.2.3.4   The within-test vessel and between-test vessel means and coefficients of variation of the measured dissolved metal concentrations are calculated.

A10.5.3   *Test performance*

A10.5.3.1   *Dissolution screening test – sparingly soluble metal compounds*

A10.5.3.1.1   After dissolution medium is prepared, add the medium into at least three test vessels (number of test vessels depend on the reproducibility obtained during the preliminary test). After a half-hour of agitation to bring the aqueous medium into equilibrium with the atmosphere or buffering system (paras. A10.5.1.6 to A10.5.1.8), the pH, temperature and dissolved O_2 concentrations of the medium are measured.

Then at least two 10 - 15 ml samples are taken from the test medium (prior to addition of the solids) and the dissolved metal concentration measured as controls and background.

A10.5.3.1.2   The metal compound is added to the test vessels at a loading of 100 mg/l and the test vessels are covered and agitated rapidly and vigorously. After the 24 hours agitation, the pH, temperature and dissolved O_2 concentrations are measured in each test vessel, and two to three solution samples are drawn by syringe from each test vessel and the solution is passed through a membrane filter as described in A10.5.1.10 above, acidified (e.g. 1 % HNO_3) and analysed for total dissolved metal concentration.

A10.5.3.2   *Full test - metals and metal compounds*

A10.5.3.2.1   Repeat A10.5.3.1.1

A10.5.3.2.2   For 7 day test, substance loadings of 1, 10 and 100 mg/l, respectively, are added to the test vessels (number of which depends on the reproducibility as established in sub-section A10.5.2.3), containing the aqueous medium. The test vessels are closed and agitated as described in A10.5.1.9. If a 28-day test is to be conducted, the test with 1 mg/l loading may be extended to 28 days, provided that the same pH value is to be chosen for both 7 day and 28-day tests. However, since 7-day tests are only conducted at pH ranges of 6 and higher, separate 28-day tests are needed to cover the pH range between 5.5 and 6. It may also be useful to include a concurrent control test with no substance loaded (i.e. a blank test solution). At established time intervals (e.g. 2 hours, 6 hours, 1, 4 and 7 days), the temperature, pH and dissolved O_2 concentrations are measured in each test vessel, and at least two samples (e.g. 10 - 15 ml) are drawn by syringe from each test vessel. The solid and dissolved fractions are separated as per A10.5.1.10 above. The solutions are acidified (e.g. 1 % HNO_3) and analysed for dissolved metal concentration. After the first 24 hours, the solution volumes should be replenished with a volume of fresh dissolution medium equal to that already drawn. Repeat after subsequent samplings. The maximum total volume taken from the test solutions should not exceed 20% of the initial test solution volume. The test can be stopped when three subsequent total dissolved metal concentration data points vary no more than 15%. The maximum duration for the loadings of 10 and 100 mg/l is seven days (the short term test) and 28 days for the loading of 1 mg/l test medium (long term test).

A10.5.4 *Test conditions*

A10.5.4.1   The transformation/dissolution tests should be done at a controlled ambient temperature ± 2 °C in the range 20 – 25 °C.

A10.5.4.2   The transformation/dissolution tests are to be carried out within the pH range described in A10.2.3.2 and A10.5.1.6. The test solution pH should be recorded at each solution sampling interval. The pH can be expected to remain constant (± 0.2 units) during most tests, although some short-term pH variations have been encountered at 100 mg/l loadings of reactive fine powders (reference 7, this annex), due to the inherent properties of the substance in the finely divided state.

A10.5.4.3   Above the aqueous medium, the head space provided by the reaction vessel should be adequate in most instances to maintain the dissolved oxygen concentration above 70% of its saturation in air, which is about 8.5 mg/l. However, in certain instances, reaction kinetics may be limited not by the availability of molecular oxygen in the head space above the solution but by the transfer of dissolved oxygen to, and removal of reaction product away from, the solid-solution interface. In this case, little can be done, other than await the restoration of equilibrium.

A10.5.4.4   To reduce chemical and biological contamination as well as evaporation, the transformation/dissolution kinetics must be performed in closed vessels and in the dark, whenever possible.

A10.6   **Treatment of the results**

A10.6.1   *Screening test*

         The mean dissolved metal concentrations at 24 hours are calculated (with confidence intervals).

A10.6.2   *Full test: Determination of the extent of transformation/dissolution*

A10.6.2.1   *Short term test*

The dissolved metal concentrations, measured during the different short term (7 days) tests, are plotted versus time, and the transformation/dissolution kinetics may be determined, if possible. The following kinetic models could be used to describe the transformation/dissolution curves:

       (a)   Linear model:

$$C_t \quad = \quad C_0 + kt, \text{ mg/l}$$

            where:
                 C_0 = initial total dissolved metal concentration (mg/l) at time t = 0;
                 C_t = total dissolved metal concentration (mg/l) at time t;
                 k = linear rate constant, mg/l-days.

       (b)   First order model:

$$C_t \quad = \quad A (1 - e^{(-kt)}), \text{ mg/l}$$

            where:
                 A = limiting dissolved metal concentration (mg/l) at apparent equilibrium = constant;
                 Ct = total dissolved metal concentration (mg/l) at time t;
                 k = first order rate constant, 1/days.

       (c)   Second order model:

$$Ct \quad = \quad A (1 - e(-at)) + B (1 - e(-bt)), \text{ mg/l}$$

            where:
                 Ct = total dissolved metal concentration (mg/l), at time t;
                 a = first order rate constant, 1/days;
                 b = second order rate constant, 1/days;
                 C = A + B = limiting dissolved metal concentration (mg/l).

       (d)   Reaction kinetic equation:

$$C_t = a \left[1 - e^{-bt} - (c/n)\{1 + (b\, e^{-nt} - n\, e^{-bt})/(n - b)\}\right], \text{ mg/l}$$

            where:
                 C_t = total dissolved metal concentration (mg/l) at time t;
                 a = regression coefficient (mg/l);
                 b,c,d = regression coefficients (1/days);
                 n = c+d.

         Other reaction kinetic equations may also apply (reference 7 and 8, this annex).

For each replicate vessel in the transformation test, these model parameters are to be estimated by regression analyses. The approach avoids possible problems of correlation between successive measurements of the same replicate. The mean values of the coefficients can be compared using standard analysis of variance if at least three replicate test vessel were used. The coefficient of determination, r^2, is estimated as a measure of the "goodness of fit" of the model.

A10.6.2.1   *Long term test*

The dissolved metal concentrations, measured from the 1 mg/l loading during the 28 day test, are plotted versus time and the transformation/dissolution kinetics determined, if possible, as described in A10.6.1 and A10.6.2.

A10.7   Test report

The test report should include (but is not limited to) the following information (see also A10.4 and A10.5.2.1):

(a)   Identification of the sponsor and testing facility;

(b)   Description of the tested substance;

(c)   Description of the reconstituted test medium and metal loadings;

(d)   Test medium buffering system used and validation of the pH used (as per paras. A10.2.3.2 and A10.5.1.6 to A10.5.1.8) description of the analytical method;

(e)   Detailed descriptions of the test apparatus and procedure;

(f)   Preparation of the standard metal solution;

(g)   Results of the method validation;

(h)   Results from the analyses of metal concentrations, pH, temperature, oxygen;

(i)   Dates of tests and analyses at the various time intervals;

(j)   Mean dissolved metal concentration at different time intervals (with confidence intervals);

(k)   Transformation curves (total dissolved metal as a function of time);

(l)   Results from transformation/dissolution kinetics, if determined;

(m)   Estimated reaction kinetic quation, if determined;

(n)   Deviations from the study plan if any and reasons;

(o)   Any circumstances that may have affected the results; and

(p)   Reference to the records and raw data.

APPENDIX

References

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2. OECD Metals Working Group Meeting, Paris, June 18-19, 1996

3. European Chemicals Bureau. Meeting on Testing Methods for Metals and Metal Compounds, Ispra, February 17-18, 1997

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2. OECD Guideline for testing of chemicals, Paris (1984). Guideline 202:Daphnia sp. Acute im-mobilisation test and Reproduction Test

3. OECD Guideline for testing of chemicals, Paris (1992). Guideline 203: Fish, Acute Toxicity Test

4. OECD Guideline for testing of chemicals, Paris (1992). Guideline 204: Fish, Prolonged Toxicity Test: 14- Day study

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[1]   *LISEC, Craenevenne 140, 3600 Genk, Belgium.*

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5.   OECD Guideline for testing of chemicals, Paris (1992). Guideline 210: Fish, Early-Life Stage Toxicity Test

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**ANNEX 2: EXTRACT FROM THE PROCEDURE FOR THE TRANSFORMATION/
DISSOLUTION OF METALS AND SPARINGLY SOLUBLE METAL COMPOUNDS (SOP),
FEBRUARY 2004**

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NOTE FOR READERS:

As the T/DP is a general guidance document only, CANMET and LISEC jointly developed a procedure (called SOP) to provide a detailed methodology for the ring test for the purpose of the Validation of the T/DP.

CONTENTS

CONTENTS (cont.)

2 APPARATUS

2.1 test vessels:

- for 1, 10 and 100 mg/L loadings of metal and metal compounds in powder form:
    - 2.8 L Pyrex© Fernbach flask;
    - 1 L Pyrex© reaction kettle, covered with a Pyrex© four-neck lid comprising three outer 24/40 standard taper joints and one centre 34/45 standard taper joint for the radial impeller;
    - Schott Duran bottles in volumes of 1, 2, 5 or 10 L; and

- for 1 mg/L loadings of massives (normally applied to metals and alloys, but may also apply to metal compounds):
    - 20 L fluorinated high-density polyethylene carboys (see Sections 2.2 and 4.3); or
    - Schott Duran bottles in volumes of 5 or 10 L.

2.2 agitation methods:

- orbital shaker:
    - top-loading LabLine Model 3530-1 refrigerated environmental shaker with a 1" (2.5 cm) or 2" (5 cm) orbit and temperature range of 5 to 60°C; note that these are no longer manufactured and the industry standard is now a 1" (2.5 cm) orbit;
    - test vessel:
        - 2.8 L Fernbach flasks, capacity of eight; or
        - 1 L Schott Duran bottles, capacity of 24.

- horizontal laboratory shaker:
    - Invicon type IVC-60;
    - 50 kg capacity;
    - amplitude: 2" (5 cm).

- radial impellers:
    - a radial impeller may be:
        - two blades of polypropylene, dimensions 40 mm width x 15 mm height affixed to a PVC-coated steel rod 8 mm diameter and 350 mm long for use with the 1 L reaction kettle; or
        - two blades of glass, dimensions 58 mm width x 12 mm height affixed to a glass rod 10 mm diameter and 500 mm long for use with the 20 L carboy;
    - blades deployed 5 cm from the bottom of a the test vessel maintained at constant temperature;
    - driven by a Caframo Stirrer Type motor, 70-700 r.p.m.;
    - test vessels, for powders and massives, respectively:
        - 1 L reaction kettle (see Section 2.1 above); or
        - 20 L carboy (see Sections 2.1 above and 4.3 below).

- note that there is no need to conduct the T/D tests in darkness since the aqueous media do not contain micronutrients (see Section 3.3 below).

2.3 pH meter: range: -2.00 to 19.99; resolution: 0.01; accuracy: ± 0.02; (e.g., Orion pH Meter Model 250A, battery operated) with temperature measurement capability and automatic temperature adjustment.

2.4 centrifuge: Clay-Adams Dynac Model no. 420101 or equivalent.

2.5 balance: readability: 0.01 mg. (e.g., Sartorius Research RC 210D), calibrated prior to each use.

2.6 dissolved oxygen (D.O.) meter: range: 0.0 to 20.0 mg/L; % saturation: 0 to 200%; resolution: 0.01/0.1 mg/L; relative accuracy ±1% full scale; temperature range: -5 to 45°C; temperature resolution: 0.1; temperature accuracy ± 1 (e.g., Orion Dissolved Oxygen Meter Model 820) with temperature measurement capability and automatic temperature adjustment; or equivalent.

2.7 particle size analyser: range of particle size displayed: 0.1 to 600 μm (e.g., Horiba Laser Scattering Particle Size Distribution Analyzer LA-300).

2.8 specific surface area instrumentation: applicability: specific surface area - approximately 0.01 m^2/g (minimum); accuracy/reproducibility: low specific surfaces - typically better than ±3% (single point method), ±2% (multipoint method) reproducibility within ±0.5%. moderate-to-high specific surfaces - typically better than ±2% (single point method); ±1.5% (multipoint method) reproducibility within ±0.5% (e.g., Micromeritics Flowsorb II 2300).

2.9 sample tubes: Sarstedt 105 mm x 16.8 mm polypropylene tubes, 14 mL capacity; high density polyethylene tubes with caps; or equivalent.

2.10 gas handling and control equipment (Figure 1):

- the following is an example of a gas handling and control system for the T/DP; other designs should follow the same general setup;

- Matheson model no. 8H or equivalent CO_2 regulator;

- Gelman Acro 50 vent filter, 0.2 μm, 50 mm diameter;

- regulator to vent filter: Nalgene 5/8" O.D. x 1/16" wall thickness (15.9 mm O.D. x 1.5 mm wall) PVC tubing;

- Cole Parmer gas flow control valve 6939-1;

- vent filter to control valve: Nalgene 5/8" O.D. x 1/16" wall thickness (15.9 mm O.D. x 1.5 mm wall) PVC tubing;

- Matheson 601 flowmeter;

- control valve to flowmeter: Nalgene 5/8" O.D. x 1/16" wall thickness (15.9 mm O.D. x 1.5 mm wall) PVC tubing;

- manifold:
    - Nalgene 5/8" O.D. x 1/16" wall thickness (15.9 mm O.D. x 1.5 mm wall) PVC tubing;
    - Becton Dickson 21G1½ Precision Glide sterile needles inserted into Nalgene 5/8" O.D. x 1/16" wall thickness (15.9 mm O.D. x 1.5 mm wall);

- Intramedic polyethylene 0.023" O.D. x 0.0075" wall thickness (0.965 mm O.D. x 0.19 mm wall) tubing;
- flowmeter to manifold: Nalgene 5/8" O.D. x 1/16" wall thickness (15.9 mm O.D. x 1.5 mm wall) PVC tubing.

2.11 volumetric labware:

- 20 L carboys: Nalgene polypropylene with spigot and handles, or equivalent;
- pipettes:
  - 1.000 mL: ± 0.006 mL @ 20°C (e.g., Pyrex no. 7100);
  - 2.000 mL: ± 0.006 mL @ 20°C (e.g,. Sibata);
  - 10.00 mL: ± 0.02 mL @ 20°C (e.g., Fisherbrand);
  - 10 - 100 μl ±5% @ 20°C (Biohit);
  - 100 - 1000 μl ±2% @ 20°C (Biohit);
  - 1.00 - 5.00 ml ±2% @ 20°C (Finnpipette).
- volumetric flasks:
  - 200.00 mL ± 0.10 mL (e.g., Pyrex TD20°C no. 5640);
  - 1000.00 mL ± 0.30 mL (e.g., Kimax TC(IN)20°C no. 28014);
- graduated cylinders:
  - 1000 mL (e.g., Pyrex 20°C no. 3042);
  - 2000 mL (e.g., Pyrex 20°C no. 3022).

2.12 sampling:

- for each test vessel:
  - a Latex- and oil-free syringe (e.g, Henke Sass Wolfe DIN/EN/ISO 7886-1) of at least 10 mL volume; and
  - optional if sampling directly by syringe: an 8" (20 cm) length of Nalgene tubing 3/16" O.D. (outside diameter) x 1/32" wall thickness (0.48 mm O.D. x 0.08 mm wall).

2.13 filtration:

- for aqueous media:
  - 0.2 μm (e.g., Pall Life Science Supor©-200, or equivalent) in a water aspirator or high pressure configuration;
- for filtration of samples:
  - 25 mm diameter membrane filter, 0.2 μm, (e.g., Pall Corp. Acrodisc© Gelman Syringe Filter (hydrophilic polyethersulfone) or equivalent); a 0.8/0.2 μm membrane filter in a serial configuration is also acceptable;
  - a Latex- and oil-free syringe of at least 10 mL volume (unnecessary if centrifugation of samples is not required);
- for filtration of final T/D solutions:
  - 25 mm or 45 mm diameter (e.g., Pall Life Sciences©,, Supor Millipore© 0.45 μm, or equivalent) in a water aspirator or high pressure configuration.

2.14 stroboscope: General Radio Company Strobotac Type 1531-A, range 110-25,000 r.p.m., or equivalent, for monitoring rotation rate of radial impeller motors.

3 REAGENTS

3.1 deionized (d.i.) water: 5-18 MΩ resistance water produced, for example, by a Biolab Water Purification system comprising the following or its equivalent:

- a Biolab pre-filter;

- a Bruner water softener;

- a Biolab organic bed carbon unit;

- a Biolab reverse osmosis system;

- a Biopure 710 ion exchanger resin system;

- an Atlantic Ultraviolet Corp. 3284 ultraviolet lamp; and

- a Millipore CWSCOIS polishing filter.

3.2 gases: 0.1% CO_2-balance air and 0.5% CO_2-balance air, certified, in size 1A or 1H cylinders, internal volume 1.55 ft^3 (43.8 L), or equivalent.

3.3 aqueous media and pH:

- for aqueous media: the aqueous test media used in the T/D tests are based on the aquatic toxicity testing medium OECD 203 (ISO 6341), the composition of which is presented in Table 1;

- a list of reagents and their analyses used to make the OECD 203 medium should be presented as per Table 2;

- it is possible to work with i) stock OECD203 solutions, or ii) concentrated OECD 203 stock solutions;

    i) to make up 20 L of the stock OECD 203 medium, perform the following:
    - weigh 20 times the mg/L quantities of the reagents listed in Table 1, dissolve in d.i. water, transfer to a 1,000 mL volumetric flask, dilute to the mark, cap and mix thoroughly;
    - filter through the 0.2 μm filter and transfer to 20 L carboy;
    - repeat filtration with 19 L of d.i. water and transfer to 20 L carboy;

    ii) to make up 1,000 mL of concentrated OECD 203 stock solution:
    - weigh 1,000 times the mg/L quantities of the reagents listed in Table 1 for the pure OECD 203, dissolve in d.i. water, transfer to a 1,000 mL volumetric flask, dilute to the mark, cap and mix thoroughly;

- the compositions of the aqueous test media are derived from the requirement in the T/DP [2, p. 434] calling for the seven-day tests to be conducted at a pH in the range 6-8.5 that maximizes the concentration of the dissolved metal ions. The pH range 6-8.5 is also to be used in the 28-day tests;

- the pH of an aqueous solution is established by i) its composition, ii) the composition of the atmosphere with which it is in equilibrium and iii) any solids that may react with the solution;

- accordingly, the pHs of the media in the range 6 to 8.5 are established by equilibrating CO_2 with the bicarbonate in the media. Thermochemical calculations [15] show that the pH of the OECD 203 medium in equilibrium with air, which contains 0.033% CO_2, is 8.09. Similar calculations show that pH values of 6 and 7 can be attained by using greater concentrations of CO_2 and more dilute aqueous media.

- the dilutions of the OECD 203 medium, the corresponding required concentrations of CO_2 and the calculated resulting values of pH [15] are presented in Table 3;

- to make up the aqueous test media from the stock OECD 203 medium i) above, perform the following:
    - 10X dilute OECD 203: transfer 2,000 mL of stock OECD 203 to a separate carboy and add 18 L of d.i. water filtered through a 0.2 μm filter.

- to make up the aqueous test media from the concentrated stock OECD 203 medium ii) above, perform the following:
    - pure OECD 203: transfer 20 mL of concentrated stock OECD 203 to a separate carboy and make up to 20 L with d.i. water; filter through a 0.2 μm filter;
    - 10X dilute OECD 203: transfer 2 mL of concentrated stock OECD 203 to a separate carboy and make up to 20 L with d.i. water; filter through a 0.2 μm filter.

3.4 standard solutions: Buck Scientific Puro-Graphic Calibration Atomic Absorption Certified Standards of appropriate element @ 1,000 μg dissolved metal/mL in 2% HNO_3, or equivalent.

3.5 quality assurance (QA) standards:

- prepare the following matrix and aqueous standards for the target metal, Me, for use in analytical measurement quality assurance, as required;

- standard stock A:
    - pipette 10.00 mL (± 0.02 mL) of certified 1,000 μg/mL atomic absorption standard solution into a 100 mL volumetric flask, dilute to the mark and mix thoroughly;

- other QA standards: pipette aliquots of standard stock A into volumetric flasks as per Table 4, dilute to the mark with the appropriate diluent, cap and mix thoroughly.

4 PROCEDURES

4.1 general:

- **cleaning of reaction kettles and Fernbach flasks**:
    - new kettles and flasks:
        - fill with tap water, and allow to soak for at least 24 h;
        - repeat with d.i. water;
    - previously used kettles and flasks:
        - rinse (~15 seconds) with concentrated HCl or HNO_3 or both to dissolve any remaining and adhering metal-bearing solids;
        - fill with tap water, and allow to soak for at least 24 h; and
        - fill with d.i. water and allow to soak for at least 24 h;
    - prior to a test series, rinse kettles and flasks three times with the medium to be used.

- **cleaning of Schott Duran bottles**:

    - one cycle of laboratory dishwasher;

    - rinse with 5% HCl;

    - rinse three times with d.i. water; and

    - air dry.

- **cleaning of carboys**: thoroughly rinse with tap water, d.i. water and aqueous medium to be stored.

- **cleaning of syringes and Sarstedt sample tube tops**: soak in 10% HCl or HNO_3 overnight and thoroughly rinse with d.i. water; however, Latex- and oil-free syringes need not be acid-washed prior to use.

4.2 24-hour screening test:

- apply to metal compounds;

- aqueous medium: select to deliver the pH that achieves the maximum total dissolved metal concentration, usually pH 6 with 10X dilute OECD 203 under 0.5% CO_2;

- volume of aqueous medium per test: 1,000 mL;

- temperature: controlled to ±1.5C° in the range 20-23°C;

- agitation:
    - orbital shaker: set at 200 r.p.m. using the rotation speed control and check by counting;
    - radial impeller: set at 400 r.p.m. and check using the stroboscope; or
    - horizontal laboratory shaker: set at 200 v.p.m. (vibrations per minute);

- loadings of test substance: 100 mg/L;

- sample times: 0 (before addition of substance) and 24 h;

- replicates:
  - test vessels:
    - per loading: 5;
    - blanks containing only test medium: 3;
  - samples drawn per test vessel:
    - per sample time: at least 2;

- T/D test operation: as per below;

- the total dissolved metal concentration that the test substance delivers to the aqueous medium after 24 h is compared to the L(E)C50:
  - if the 24-h concentration exceeds the L(E)C50, then the substance fails the 24-h test, and no further testing is required;
  - if the 24-h concentration is less than the L(E)C50, the substance passes the 24-h test, so proceed to the seven-day full and 28-day tests [2, p. 395].

4.3 seven-day full test:

- apply to:
  - metal compounds that pass the 24-hour screening test;
  - metals; and
  - alloys.

- aqueous medium: select to deliver the pH that achieves the maximum total dissolved metal concentration, usually 10X dilute OECD 203 under 0.5% CO_2;

- temperature: controlled to ± 1.5C° in the range 20-23°C;

- agitation:
  - orbital shaker set at 100 r.p.m. using the rotation speed control and check by counting;
  - horizontal laboratory shaker: set at 100 v.p.m.; or
  - radial impeller set at 200 r.p.m. and check using the stroboscope;

- loadings of test substance: 1, 10 and 100 mg/L;

- volume of aqueous medium per test:
  - for powders, usually 1,000 mL;
  - for solid metals, metal compounds or alloys of density greater than about 3 g/cm^3, the smallest obtainable particle size may well be approximately 1 mm^3 (e.g., samples available only as shot, or as cuttings or machinings from wire, rod or tube); then it will be necessary to use a solution volume that will achieve the loading of 1 mg/L; for instance, for an alloy of 8 g/cm^3 density, a single sample of 1 mm length cut from a 1 mm diameter wire would weigh about 6.3 mg and would therefore require a solution volume of 6.3 L for a 1 mg/L loading; however, to avoid the possibility of unrepresentative data caused by the use of only one cutting, it may be advisable to use a minimum of two and preferably three cuttings, in which case solution volumes of 12.6 and 19 L, respectively, would be required; cuttings weighing more than 6.3 mg would obviously require greater solution volumes to achieve the 1 mg/L loadings; besides the 20 L carboy-radial impeller method noted in Section 2.2 above, alternative equivalent methods for achieving the 1 mg/L loadings may be applied.
- set pH: to desired value as per Section 3.3;

- sample times:
  - depending on the degree of certainty required for the test, options are:

- – 0, 1, 2, 4, 8, 24, 48, 96 and 168 h; or
  - – 0, 2, 6, 24, 96 and 168 h;
- replicates:
  - – test vessels:
    - – per loading: 3;
    - – blanks containing only test medium: 1;
  - – samples drawn per test vessel, depending on the degree of certainty required for the test:
    - – at 1, 2, 4 and 8 h: 1;
    - – at 2 and 6 h: at least two;
    - – at 0, 24, 48, 96 and 168 h: at least 2;
- T/D test operation: as per below.

4.4 28-day full test:

- apply to:
  - – metal compounds that pass the screening test;
  - – metals; and
  - – alloys.

- aqueous medium: select to deliver the pH that achieves the maximum total dissolved metal concentration, usually 10X dilute OECD 203 under 0.5% CO_2;

- temperature: controlled to ± 1.5C° in the range 20-23°C;

- agitation:
  - – orbital shaker set at 100 r.p.m.;
  - – horizontal laboratory shaker set at 100 v.p.m.;
  - – radial impeller set at 200 r.p.m.; or

- loadings of test substance: 1 mg/L;

- volume of aqueous medium per test: see Section 4.3 above;

- sample times, depending on the degree of certainty required for the test, options are:
  - – 0, 1, 2, 4, 8, 24, 48, 96, 168, 240, 336, 408, 504, 576 and 672 h; or
  - – 0, 2, 6, 24, 96, 168, 336, 504 and 672 h;

- replicates:
  - – test vessels:
    - – per loading: 3;
    - – blanks containing only test medium: 1;
  - – samples drawn per test vessel:
    - – at 1, 2, 4 and 8 h: 1;
    - – at 0, 24, 48, 96, 168, 240, 336, 408, 504, 576 and 672 h: at least 2;
- T/D test operation: as per below.

4.5 T/D test operation:

- if the test is to be at pH 7, or 6:
  - – set up the gas train as per Figure 1; read and apply all relevant instructions concerning gas regulation and flow control equipment;

- ensure that the gas is flowing through the line at a rate of at least one bubble per second by briefly immersing one of the capillary tubes in a beaker of d.i. water;

- introduce the aqueous medium into the test vessel;

- if the test is to be at pH 7 or 6:
  - ensure that all capillary tubes are introduced into the headspace of the vessel to a depth of about 2" (5 cm);

- start the agitation and allow to run for at least ½ h to equilibrate the aqueous medium with the atmosphere, after which time draw three blank samples using the syringe and Nalgene sample tubing;

- quantitatively transfer the weighed test substance into the aqueous medium, washing the weighing container with a small aliquot of aqueous medium, if necessary, to remove the last traces of the test substance; record the initial exposure time;

- at the prescribed sample times:
  - with the syringe (and Nalgene sample tubing if applicable), e.g. draw 15 mL solution samples, remove Nalgene tubing from syringe (if applicable), apply 0.8/0.2 μm syringe filters to syringes, filter into the labelled Sarstedt tubes, acidify with 1-2 drops of Fisher Trace Metal grade HNO_3 (68-71%) and submit for analysis of total dissolved metal;
  - read and note the pH, D.O. (see Appendix A) and temperature;
    - if the pH does not lie within the anticipated range, carefully note any factors that may have contributed to this situation, such as gas flow anomalies or any obvious reaction of the test substance with the aqueous test medium;
  - if, due to agitation, the solution obviously contains a suspension, it may be advisable to centrifuge the sample prior to filtration;
  - guidance on the pH includes:
    - "There should be no pH adjustment during the test using an acid or alkaline" [16];
    - "The pH can be expected to remain constant (± 0.2 units) during most tests, although some short-term pH variations have been encountered at 100 mg/L loadings of reactive fine powders [8], due to the inherent properties of the substance in the finely divided state" [2, p. 439].

- after the 6/8 h samples have been taken, replenish the test solution with a volume of fresh medium equal to the total volume already drawn; replenishment is continued for the duration of the test;

- if the T/D solutions are to be subjected to ecotoxicity testing upon completion of the test, or if a mass balance is to be calculated on the test data, then remove the test vessels from agitation, filter the solutions through previously weighed 0.45 μm filters, measure and note the volumes of the filtrate, and recover and note the weight of the solids after they have been air-dried; the T/D solutions can be retained and then subjected to ecotoxicity testing, if required;

- if no ecotoxicity tests are to be performed or if a mass balance need not be calculated, then the solutions may simply be discarded upon completion of the test;

- with every 25-40 test solutions submitted for analysis, identify and prepare appropriate QA samples, as per Table 4, to submit with the test samples based on estimated solution concentrations of the metal in the test series, including matrix and d.i. water samples B through F;

- submit all samples for analysis.

4.6 chemical analyses:

- analytical instrumentation:
    - ICP-MS (inductively coupled plasma-mass spectrometer);
    - ICP-DADS (inductively coupled plasma-diode array detector spectrometer);
    - GF-AAS (graphite furnace-atomic absorption spectrophotometer); and
    - or equivalent to provide required sensitivity.

4.7 matrix effect:

- draw, acidify and submit for analysis samples of solutions:
    - B, C, D and E; and
    - F, G, H and J.;
- to assess the matrix effect, compare the analyses from the matrix and d.i. water solutions.

4.8 filter effect:

- repeat procedure for matrix effect, except filter solutions with 0.8/0.2μm filters before acidifying;
- to assess the filter effect, compare the analyses from the filtered and unfiltered solutions.

5 REPORTING AND RECORD-KEEPING

5.1 test substances: characterize and record:

- chemical formula;
- supplier;
- lot or batch no.;
- chemical analysis, including impurities;
- physico-chemical method of production if available;
- particle size distribution and particle size range if available;
- specific surface area measured by the BET technique [17] if available; and
- other information from client.

5.2 recording of test parameters:

- for each test vessel, record the following on a separate data sheet, as per Figure 2:
    - test number;
    - starting substance and particle size;
    - agitation method;
    - r.p.m.;
    - atmosphere (Table 3);
    - aqueous medium (Table 3) and date of preparation;
    - sample volumes;
    - sample times;
    - nominal loading;
    - initial weighed amount of test substance;
    - name or identification number of test vessel;
    - initial solution volume;
    - target pH;
    - final weighed amount of substance;
    - final solution volume; and
    - readings of pH, D.O. and temperature, with calibrations initialled by the technologist.

5.3 report for client:

- write a report based on the following outline, providing all details of the study:
    - Executive Summary;
    - Abstract;
    - Introduction;
    - Experimental;
    - Results and Discussion;

- Conclusions;
- Acknowledgements; and
- References.

5.4 report contents:

- in the client report, a typical results table and data plot for which are presented in Figure 3, present all data concerning:
  - methodology, including specifications of instrumentation such as balances and pH meters;
  - estimates of weighing and, as appropriate, other errors;
  - total dissolved metal concentration as a function of time;
  - temperature;
  - dissolved oxygen;
  - pH;
  - the detection and quantification limit of the analytical method;
  - the range of linearity within the applicable range of analyses;
  - a consideration of the matrix effect of the aqueous medium on the analysis of the dissolved metal ions;
  - mass balances, if possible;
  - limits of quantification in chemical analyses;
  - a discussion of the reproducibility of the analyses;
  - an examination of the adsorptive properties of the filters with respect to the soluble metal ions, if applicable; and
  - other pertinent aspects of the study as agreed upon between the client and CANMET-MMSL, as well as factors that may have caused the study conditions to vary from those prescribed in the T/DP, and related recommendations.

5.5 record-keeping:

- for each project, maintain a dedicated binder for the following:
  - data sheets;
  - requests for analysis;
  - analytical results; and
  - other relevant data and information.

6 QUALITY SYSTEMS

6.1 general:

- laboratories conducting T/D studies should have procedures in place that ensure the quality, integrity, traceability and validity of the data thereby generated; and

- these goals can be achieved through the implementation of such quality management systems as ISO (International Standards Organization) 9002 [18] and GLP (Good Laboratory Practice) [19].

6.2 examples of quality systems:

- certifications under ISO 9002 and excerpts from the quality management system in place at CANMET-MMSL are presented in the Appendix B; and

- the principles of Good Laboratory Practices as specified by Directive 1999/11/EEC of the commission of March 08, 1999, relating to the adjustment of the principles of GLP to technical progress according to the Directive 87/18/EEC of the council for the adaptation of legal and administrative regulations for the application at test with chemical test items. (official journal of the European Union N° L77 p-8-21 of 23 March 1999).

7 ACKNOWLEDGEMENTS

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LIST OF TABLES

Table 1: Composition of modified OECD 203 (ISO 6341) aqueous medium.

reagent	molecular weight	mg/L	$[Mg^{2+}]$	$[Ca^{2+}]$	$[Cl^-]$ (mg/L)	$[HCO_3^-]$	$[SO_4^{2-}]$	mmol/L
$CaCl_2 \cdot 2H_2O$	147	293.8		79.95	141.9			2
$MgSO_4 \cdot 7H_2O$	246	123.3	12.03				48.12	0.5
$NaHCO_3$	84	64.8				47.06		0.77
KCl	74.5	5.8			2.76			0.08
total			12.03	79.95	144.67	47.06	48.12	
mmol/L			0.5	2	4.08	0.77	0.5	
hardness as $CaCO_3$		250						

The concentration of total organic carbon in the medium should not exceed 2.0 mg/L [1].

Table 2: Analyses of reagents used for the OECD 203 (ISO 6341) aqueous medium

reagent	supplier	assay	insol.[2]	oxidisers as NO_3	SO_4	NH_4	Ba	heavy metals as Pb	Fe	Mg
$CaCl_2 \cdot 2H_2O$	Anachemia	74-78*	<0.01	<0.01	<0.01	<0.005	<0.005	< 5 ppm	<0.001	<0.005
$MgSO_4 \cdot 7H_2O$	BDH	98-102	0.005			0.002		5 ppm	5 ppm	
$NaHCO_3$ [1]	BDH	99.7-100.3	0.015		0.003	5 ppm		5 ppm	0.001	
$MgCl_2 \cdot 6H2O$	BDH	99-102	0.005	0.001	0.002	0.002	0.005	5 ppm	5 ppm	
NH_4Cl	BDH		0.005			0.002		5 ppm	2 ppm	
KCl	Baker	99.9	0.002	<0.003	0.001		<0.001	< 2 ppm	1 ppm	

* as $CaCL_2$

	K	Na	Sr	Cl	Br	I	NO_3	As	Ca	Mn	PO_4
$CaCl_2 \cdot 2H_2O$	<0.01	<0.02	<0.1						0.02		
$MgSO_4 \cdot 7H_2O$	0.005	0.005	0.005	5 ppm			0.002	2 ppm		5 ppm	0.001
$NaHCO_3$ [1]	0.005			0.003							
$MgCl_2 \cdot 6H2O$	0.005	0.005	0.005				0.001		0.01	5 ppm	5 ppm
NH_4Cl									0.002		2 ppm
KCl		0.002			<0.01	<0.001			0.002		< 2 ppm

Note: assays and analyses are in % unless otherwise indicated

[1] dried basis

[2] insoluble matter

Table 3: Recommended chemical compositions of T/D media [1, p. 241], CO_2 concentrations in air to be passed through the headspace of reaction vessel, and calculated pH values under these conditions.

	50X	20X	10X	5.14X	OECD 203 1X
dilution of OECD 203 (ISO 6341) with respect to $NaHCO_3$	50X	20X	10X	5.14X	1X
mL OECD 203/20 L of d.i. water	400	1,000	2,000	*	-
calculated pH [15]	5.47	5.80	6.09	7.07	8.09
component			concentration, mg/L		
$NaHCO_3$	1.296	3.25	6.5	12.6	64.8
KCl	0.116	0.29	0.58	2.32	5.8
$CaCl_2{\cong}2H_2O$	5.876	14.7	29.4	117.6	293.8
$MgSO_4{\cong}7H_2O$	2.466	6.15	12.3	49.2	123.3
per cent CO_2 (balance is air) in test vessel	0.50	0.50	0.50	0.10	0.033

* The pH 7 medium cannot be readily made from stock OECD 203, but instead should be made up with the appropriate weighed amounts of the components (see text).

Table 4: Preparation of QA standard solutions. Standard stock A is made from 10.00 mL of certified 1,000 µg/mL atomic absorption standard solution diluted to 100 mL to yield a solution containing 100.0 mg Me/L (see text). The example is for 10X dilute OECD 203 as the matrix and should be repeated for the other dilute OECD 203 media, as required.

QA standard	type	diluent	stock	stock vol., mL	dilution vol., mL	[Me], mg/L
B	matrix	10X OECD 203	A	2.000	200.00	1.000
C	matrix	10X OECD 203	A	1.000	200.00	0.500
D	matrix	10X OECD 203	A	1.000	1,000.00	0.100
E	matrix	10X OECD 203	B	2.000	200.00	0.010
F	water	d.i. water	A	2.000	200.00	1.000
G	water	d.i. water	A	1.000	200.00	0.500
H	water	d.i. water	A	1.000	1,000.00	0.100
I	water	d.i. water	F	2.000	200.00	0.010

LIST OF FIGURES

Figure 1: Schematic diagram of gas train.

0.2 μm vent filter

capillary tubing

CO₂ regulator

sterile needle

manifold

flowmeter

Parafilm cover

gas flow control valve

Fernbach flasks

cylinder of 0.5% CO₂-balance air
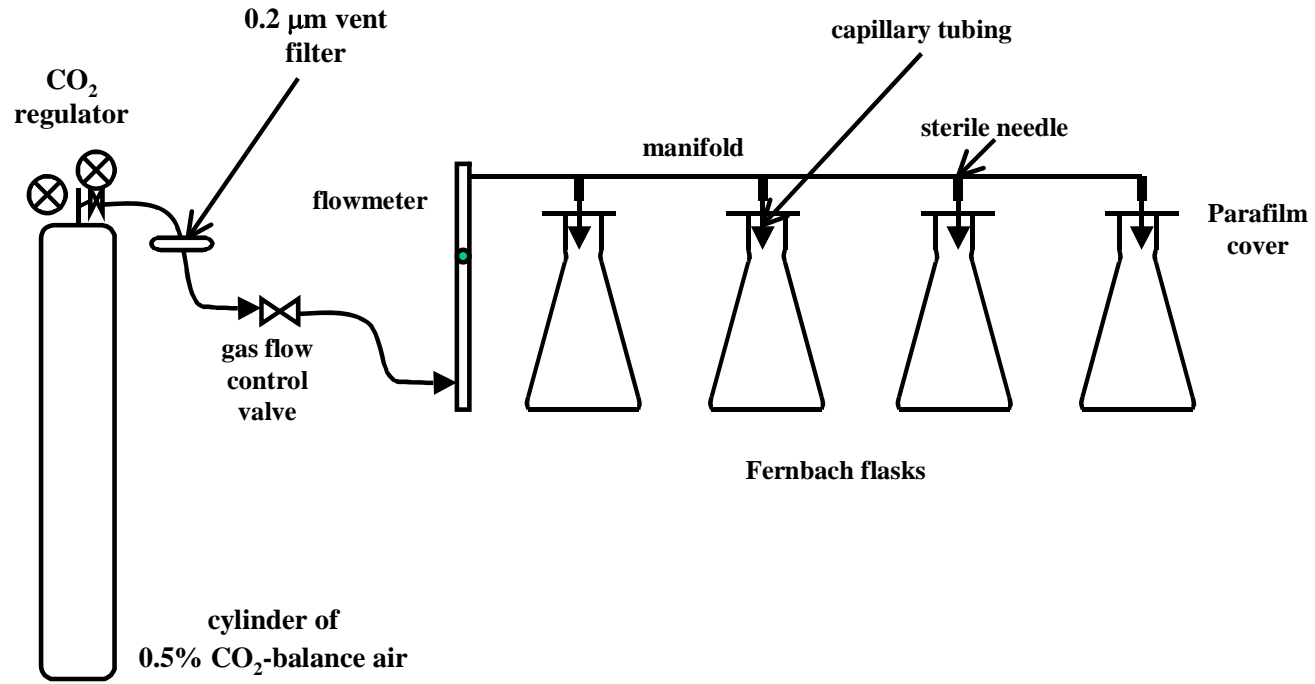
Figure 2: Typical T/D test sheet

OECD 203 10X DILUTION

Vessel #9   prepared Sept. 26/02   **Vessel #27**

Test # **Me25-5**		Element: **Me**
Loading: **100** mg/L	Initial leachate volume: **1.00** L	
Particle Size: **Me powder Type C**	Feed solution: **203 10X DILUTION**	
Contact Method: **Orbital Shaker**	Final solid weight: **97.6** g	
Actual Weight: **100.5** mg	Final volume: **990** mL	
Atmosphere: **0.5% CO₂**	Target pH = **6**	
RPM: **100**		

sample number		time, hr	date, observations	sample volume, mL	T, °C	D.O. mg/L	pH		initials
	Me25-5	0	**07-Oct-02**	**45**	**21.6**	**9.0**	**6.24**		*D.H.*
	Me25-5	1	**07-Oct-02**	**15**	**21.0**	**9.1**	**6.22**		*D.H.*
	Me25-5	2	**07-Oct-02**	**15**	**21.4**	**8.9**	**6.09**		*D.H.*
	Me25-5	4	**07-Oct-02**	**15**	**21.0**	**9.0**	**6.11**		*D.H.*
	Me25-5	8	**07-Oct-02**	**15**	**21.7**	**9.0**	**6.24**		*D.H.*
	Me25-5	24	**08-Oct-02**	**45**	**21.5**	**8.4**	**6.36**		*D.H.*
	Me25-5	48	**09-Oct-02**	**45**	**20.6**	**8.6**	**6.26**		*D.H.*
	Me25-5	96	**11-Oct-02**	**45**	**21.1**	**8.5**	**6.21**		*D.H.*
	Me25-5	168	**14-Oct-02**	**45**	**20.5**	**9.1**	**6.10**		*D.H.*

Test # **Me25-6**		Element: **Me**
Loading: **1** mg/L	Initial leachate volume: **1.00** L	
Particle Size: **Me powder Type C**	Feed solution: **203 10X DILUTION**	
Contact Method: **Orbital Shaker**	Final solid weight: **1.1** g	
Actual Weight: **0.9** mg	Final volume: **980** mL	
Atmosphere: **0.5% CO₂**	Target pH = **6**	
RPM: **100**		

sample number		time, hr	date observations	sample volume mL	T, °C	D.O. mg/L	pH		initials
	Me25-6	0	**07-Oct-02**	**45**	**21.8**	**9.0**	**6.27**		*D.H.*
	Me25-6	1	**07-Oct-02**	**15**	**21.0**	**9.1**	**6.10**		*D.H.*
	Me25-6	2	**07-Oct-02**	**15**	**21.5**	**9.0**	**6.29**		*D.H.*
	Me25-6	4	**07-Oct-02**	**15**	**21.0**	**9.1**	**6.11**		*D.H.*
	Me25-6	8	**07-Oct-02**	**15**	**21.4**	**9.0**	**6.25**		*D.H.*
	Me25-6	24	**08-Oct-02**	**45**	**20.9**	**8.6**	**6.25**		*D.H.*
	Me25-6	48	**09-Oct-02**	**45**	**20.8**	**8.2**	**6.16**		*D.H.*
	Me25-6	96	**11-Oct-02**	**45**	**21.1**	**8.4**	**6.35**		*D.H.*
	Me25-6	168	**14-Oct-02**	**45**	**20.9**	**9.0**	**6.16**		*D.H.*

Figure 3: Typical T/D data table and graph

Table 1: Results of test Me25-7; transformations of Me metal (Alpha Me-5191) in 10X dilute OECD 203.

substance: Me metal powder, 50% - 40 µm; measured specific surface area = 0.20 m^2/g.

initial surface area loadings of Me metal to the aqueous medium are

       calculated as the product of the measured specific surface area and the mass loading

agitation: 1 L of solution, 100 rpm, orbital shaker, 0.5% CO_2 - balance air

test #	Me25-7						
mass loading:		100.5	mg/L				
surface area loading:		20,100	mm^2/L		Me(aq), mg/L		
time, hr	T, °C	[O], mg/L	pH	avg	a	b	c
0	21.6	9.00	6.24	0.3	0.1	0.3	0.4
1	21.0	9.10	6.22	137			
2	21.4	8.90	6.09	161			
4	21.0	9.00	6.11	187			
8	21.7	9.00	6.24	198			
24	21.5	8.40	6.36	293	343	229	307
48	20.6	8.60	6.26	365	354	351	389
96	21.1	8.50	6.21	443	467	417	446
168	20.5	9.10	6.10	578	578	581	576
avg	21.2	8.84	6.20				
std dev	0.43	0.27	0.09				
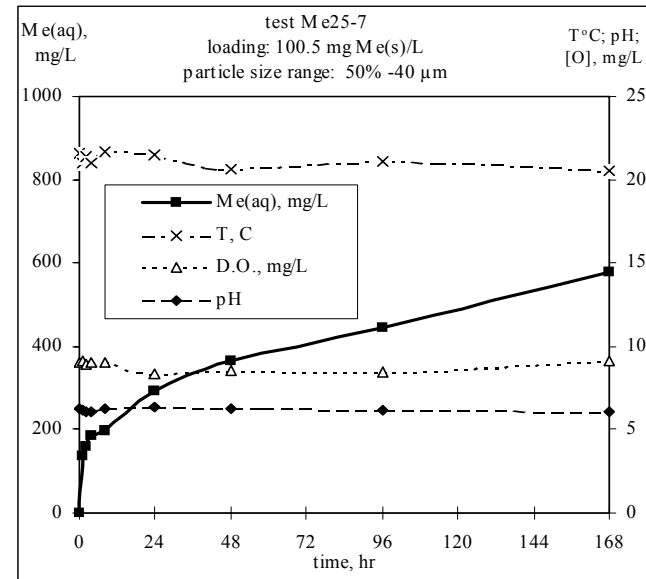
APPENDIX A: MEASUREMENTS

1. pH measurement

calibrate pH meter daily prior to sample readings according to meter's manual using buffers of pH = 4 and pH = 7. Use fresh buffer daily;

- use a small amount of buffer to avoid waste but enough to cover frit (liquid junction) in electrode;

- stir buffer or gently move electrode in buffer during calibration;

- a slope close to 100∀3% should be achieved;

- thoroughly rinse electrode with d.i. water, ensuring that the final rinse is the bulb of the electrode;

- place electrode into vessel such that the frit is submerged in the test solution;

- start rotation of flask ensuring that the electrode continually monitors the solution and the electrode is secured to prevent damage;

- allow the reading to stabilize; record value; if value appears to be abnormal, continue with the readings for some of the other vessels and return to check the abnormal reading;

- remove electrode and rinse electrode with distilled water into a waste container;

- continue with next reading.

2. Dissolved oxygen measurement

- calibrate d.o. meter daily prior to sample readings according to the meter's manual;

- thoroughly rinse electrode with d.i. water, ensuring that the final rinse is the tip of the electrode where the membrane is located;

- immerse the tip of the electrode in the test solution in the flask;

- start rotation of flask ensuring that the electrode continually monitors the solution and the electrode is stationary to prevent damage;

- allow the reading to stabilize; and

- record value.

APPENDIX B: QUALITY SYSTEMS

ISO 9002 at CANMET-MMSL (excerpts)

B.1.1 certification:

- CANMET-MMSL operates in compliance with ISO 9002 Certificate No. CC1535-010777 with the exception of:
    - the Analytical Services Group (ASG), which is in compliance with ISO 9002 Certificate No. 004638; and
    - the Canadian Centre for Reference Materials Project (CCRMP), ISO 9002 Certificate no. 004645.

B.1.2 responsibilities[1]:

- Project Leader for:
    - ensuring that MMSL has the necessary capability (e.g., resources, expertise, time frame, etc.) to undertake the project before the proposal for a project is submitted to the client;
    - all communications with the client regarding the technical and operational aspects, preparation of the project proposal, and, where applicable, preparation of the report on the project and its management;
    - ensuring that the relationship between the MMSL Project Number and all work performed for the project is maintained to establish traceability;
    - initiating and closing out a project file in the Project Management Information System (PMIS); and
    - for defining the handling, storage and preservation of chemical reagents, laboratory gases, supplies or components when the instructions from the supplier are inadequate and when a Material Safety Data Sheet (MSDS) is not available.

- Employees for:
    - the quality of work in their area.

- Program Manager for:
    - ensuring that personnel are qualified to carry out CANMET-MMSL's mandate in conformance with its quality system; and
    - ensuring sufficient financial resources are available for all necessary supplies and external services, and all necessary instruments and equipment.

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[1] CANMET-Mining and Mineral Sciences Laboratories. 2002. ISO 9000 Quality System Documents WI-CR-1.DOC., WI-CR-7, PM-MR and PM-HS.DOC.