

ANNEX 10

**GUIDANCE ON
TRANSFORMATION/DISSOLUTION OF
METALS AND METAL COMPOUNDS
IN AQUEOUS MEDIA**

Annex 10

GUIDANCE ON TRANSFORMATION/DISSOLUTION OF METALS AND METAL COMPOUNDS IN AQUEOUS MEDIA¹

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 oxidation 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:

¹ *OECD Environment, Health and Safety Publications, Series on Testing and Assessment, No. 29, Environment Directorate, Organisation for Economic Co-operation and Development, April 2001.*

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) Safety Data Sheets (SDS) 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) acrodisc filter should be flushed at least three times with fresh medium to avoid elevated trace metals in sample at time 0;
- (f) means to control the temperature of the reaction vessels to ± 1.5 °C in the range 20-23 °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 coupled axial plasma spectrometry) of acceptable accuracy, preferably with a limit of quantification (LOQ) five times lower than the lowest chronic ecotoxicity reference value;

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 used², as the standard transformation/dissolution medium. The medium should be sterilized by filtration (0.2 µm) before use in the 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 ₂ O	:	294 mg/l
MgSO ₄ ·7H ₂ O	:	123 mg/l

For tests carried out at lower or higher 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 ₂ O	:	20 mg/l
H ₃ BO ₃	:	30 mg/l
KBr	:	100 mg/l
KCl	:	700 mg/l
CaCl ₂ ·2H ₂ O	:	1.47g/l
Na ₂ SO ₄	:	4.0 g/l
MgCl ₂ ·6H ₂ O	:	10.78 g/l
NaCl	:	23.5 g/l
Na ₂ SiO ₃ ·9H ₂ O	:	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 CO₂ 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 and up to pH 8 and 8.5, Table A10.1 shows the recommended chemical compositions of the media, as well as the CO₂ concentrations in air to be passed through the headspace, and the calculated pH values under these conditions.

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 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	64.75 mg/l	194.25 mg/l
	KCl	0.58 mg/l	2.32 mg/l	5.75 mg/l	5.74 mg/l
	CaCl ₂ ·2H ₂ O	29.4 mg/l	117.6 mg/l	294 mg/l	29.4 mg/l
	MgSO ₄ ·7H ₂ O	12.3 mg/l	49.2 mg/l	123.25 mg/l	123.25 mg/l
CO ₂ concentration (balance is air) in test vessel		0.50%	0.10%	0.038% (air)	0.038% (air)
Calculated pH		6.09	7.07	7.98	8.5

NOTE 1: The pH values were calculated using the FACT (Facility for the Analysis of Chemical Thermodynamics) System (<http://www.crct.polymtl.ca/fact/fact.htm>).

NOTE 2: While the protocol was only validated for the pH range 6.0-8.0, this table does not prevent attaining pH 5.5. Composition for pH 8.5 has not been verified experimentally in presence of metal.

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. pH should not be adjusted during the test using an acid or alkali.

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/cm³ 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 CO₂ 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 ± 1.5 °C in the range 20 - 23 °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 one or two drops of trace metal grade HNO₃ with the target pH 1 and analyzed 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.

A10.5.2.3.5 To ensure reproducibility of transformation data, it is recommended that:

- (a) new laboratories use a training set;
- (b) one metal powder with specified surface conditions be used as standard control; and
- (c) one or two laboratories be responsible for reference chemicals.

It may be necessary to check specific surface areas of the powders.

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 O₂ 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 O₂ 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 % HNO₃) 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 O₂ 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 % HNO₃) 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 ± 1.5 °C in the range 20 – 23 °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 about 6.0 mg/l, which is 70% of the saturation level of 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:

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

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:

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

where:

A = limiting dissolved metal concentration (mg/l) at apparent equilibrium
= constant;

C_t = total dissolved metal concentration (mg/l) at time t ;

k = first order rate constant, 1/days.

(c) Second order model:

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

where:

- C_t = 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:

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

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 equation, 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.

Annex 10

APPENDIX

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