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Water quality — Determination of long term toxicity of substances to *Daphnia magna* Straus (*Cladocera*, *Crustacea*)

Qualité de l'eau — Détermination de la toxicité à long terme de substances vis-à-vis de Daphnia magna *Straus (*Cladocera, Crustacea*)*

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 10706 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

Annexes A, B, C and D of this International Standard are for information only.

Introduction

This International Standard defines a procedure for the determination of the long term sublethal toxicity of chemicals, waters and waste waters to the water-flea *Daphnia magna* Straus. The methodology is adapted from a guideline produced by the Organisation for Economic Co-operation and Development (OECD Guideline 211, see reference [1] in the Bibliography).

Water quality — Determination of long term toxicity of substances to *Daphnia magna* Straus (*Cladocera, Crustacea*)

WARNING — Activated sludge and sewage contain potentially pathogenic organisms. Therefore, appropriate precautions should be taken when handling them. Toxic test compounds and those whose properties are unknown should be handled with care.

1 Scope

This International Standard describes a method for the determination of the long term sublethal toxicity to *Daphnia magna* Straus (*Cladocera, Crustacea*) of:

a) chemical substances, which are soluble under the conditions of the test, or can be maintained as stable suspensions or dispersions under the conditions of the test,

b) industrial or sewage effluents, treated or untreated, after decanting, filtration, or centrifugation,

c) surface or ground waters.

NOTE This International Standard is adapted from OECD Guideline 211.[1]

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 5667-2, *Water quality — Sampling — Part 2: Guidance on sampling techniques.*

ISO 5667-16, *Water quality — Sampling — Part 16: Guidance on biotesting of samples.*

3 Principle

Daphnia magna females, less than 24 h old, are exposed for a period of 21 days to a test substance, industrial or sewage effluent or surface/ground water added to dilution water in a range of concentrations in a semi-static or flow-through system. The survival of parents is recorded together with the number of live offspring produced per live parent at the end of the test.

The survival is reported and the reproductive output of exposed parents living at the end of the test (expressed in a number of terms) is compared to those of the control parents.

4 Test environment

The test atmosphere shall be free from vapours or dusts which may be toxic to *Daphnia magna* Straus.

The exposure solutions shall not be aerated.

The dissolved oxygen concentration in the exposure solutions shall be above 3 mg/l and the pH shall be within the range of pH 6 to pH 9 and not change by more than 1,5 pH units for the duration of the test. Hardness shall be above 140 mg/l (as $CaCO_3$), which has been demonstrated to be necessary to promote reproductive performance that shall meet the validity criteria (see 8.7).

The photoperiod of the test shall be 16 h of light and 8 h of darkness. The intensity shall be in the range 600 lux to 800 lux but not exceeding 1 200 lux.

The temperature of the test exposures shall be maintained within the range of 18 °C to 22 °C and the temperature for the test shall not vary by more than 2 °C (for instance 18 °C to 20 °C, 19 °C to 21 °C or 20 °C to 22 °C) throughout the test.

5 Reagents and materials

5.1 Test organism: *Daphnia magna* Straus (*Cladocera, Crustacea*), hereafter referred to as *D. magna,* obtained by acyclical parthenogenesis for at least three generations under specified culture conditions.

The age and the source (including clone, if possible) of the *D. magna* culture shall be indicated in the test report, since the sensitivity of *D. magna* to toxicants can be affected by the source of the culture.

The animals used for the test shall be less than 24 h old and be from the second to fifth brood. The *D. magna* shall be from a healthy stock showing no signs of stress such as mortality > 20 %, presence of males, ephippia, or discoloured animals and there shall be no delay in the production of the first brood.

The stock animals shall be maintained in culture conditions (light, temperature, medium, feeding and animals per unit volume) similar to those in the test. If the culture conditions differ from test conditions, it is recommended that one generation be acclimated under the test conditions for about three weeks so as to avoid stressing the parent animals.

5.2 Dilution water, synthetic as described in 5.3 or uncontaminated natural waters with comparable pH and hardness characteristics to these dilution waters for culturing and testing.

5.3 Synthetic dilution water, prepared using one of the following methods:

a) OECD M4 and M7 media (see informative annex A);

b) ASTM reconstituted hard freshwater (see informative annex B).

The dilution water shall be aerated until the dissolved oxygen concentration has reached 95 % saturation and the pH has stabilized. If necessary, adjust the pH to $8{,}0 \pm 0{,}5$ by adding sodium hydroxide (NaOH) solution or hydrochloric acid (HCl) solution. The dilution water prepared in this way shall not be further aerated before use.

It is recommended that the TOC levels be < 5 mg/l before the addition of algae. The use of dilution water containing EDTA (for instance M4 and M7) is not recommended when testing compounds containing metals because chelation may reduce the toxicity of the compound.

If the test has to be performed for purposes necessitating the use of a dilution water with characteristics differing from those described above, mention is necessary in the test report of the main characteristics (for instance pH, hardness, TOC, COD) of the synthetic dilution water used.

6 Apparatus

Ordinary laboratory apparatus and in particular the following.

6.1 Environmental controls, for controlling temperature, photoperiod and light intensity.

6.2 Measuring apparatus and/or instruments, for measuring dissolved oxygen, pH, hardness, total organic carbon, chemical oxygen demand, light intensity and temperature.

6.3 Test containers, of chemically inert material and of sufficient capacity for the tests (for example 50 ml or 100 ml glass test tubes or beakers) and which are clean and uncontaminated.

7 Treatment and preparation of samples

7.1 Special precautions for sampling and transportation of water or effluent samples

Sampling of water or effluent shall be carried out in accordance with the general procedure specified in ISO 5667-2. Bottles shall be completely filled to exclude air.

The preservation and storage of water or effluent samples shall be carried out in accordance with ISO 5667-16; the following is only a summary. Carry out the toxicity test as soon as possible, ideally within 12 h of collection. If this time interval cannot be observed, cool the sample (0 °C to 4 °C) and test the sample within 24 h. If testing cannot be carried out within 48 h, the sample may be frozen (below –18 °C) for testing within 2 months of collection.

All portions (subsamples) shall be pretreated identically (i.e. if freezing of unstable water is necessary all portions, including those needed for the first day, shall be frozen prior to testing, see ISO 5667-16).

Because of the duration of this test (21 days) and the periodic renewal of solutions, a sufficient number of portions of the sample must be frozen to renew test solutions and to repeat the test, if needed (reserve samples). The minimum volume of the frozen portions is dependent upon the toxicity of the sample to be tested. The volume of sample needed for a semi-static test with ten daphnids exposed individually in 100 ml of undiluted sample is 9 l; additional sample will be required for diluted exposures.

If the test is conducted on site or close to the sampling site, fresh samples may be used to replace the test solutions. In this case the variations at the sampling site are incorporated into the test design.

7.2 Preparation of solutions of substances to be tested

7.2.1 Preparation of stock solutions

A stock solution of the substance(s) to be tested shall be prepared by dissolving a known quantity of the substance(s) in a specified volume of dilution water, deionized water or distilled water in a glass container. The stock solution shall be prepared immediately before preparing the exposure solutions unless the substance(s) is/are known to be stable in defined storage conditions, in which case the stock solution may be prepared in advance of testing and stored in these conditions.

Stock solutions or suspensions of substance(s) which are poorly soluble in water can be solubilized or dispersed directly in the medium with ultrasonic dispersion, and/or stirring or with solvents or dispersants of low toxicity to *D. magna* as discussed in ISO 5667-16. If a solvent is used, the concentration of the solvent in the stock solution shall be such that the concentration in the highest exposure solution does not exceed 0,1 ml/l.

The use of organic solvents should be avoided. If they are required organic solvents such as acetone, ethanol, methanol, dimethylformamide, triethylene glycol or dispersants such as Cremophor RH40, methylcellulose 0,1 %, and HCO-40 may be used to produce a suitably concentrated stock solution. They are not toxic to *D. magna* at 0,1 ml/l concentrations. No single procedure for the preparation of stock solutions of poorly soluble substances can be recommended due to the differing nature of chemicals.

7.2.2 Preparation of exposure solutions

The exposure solutions shall be prepared (8.2) by adding the stock solutions (7.2.1) or effluent samples (7.1) to the dilution water (5.2) in specified quantities (see 8.2).

If the stock solutions are prepared in deionized or distilled water, no more than 100 ml of stock solution shall be added to each litre of dilution water.

The chosen concentrations can also be prepared separately by the direct addition of the test substance to dilution water where the amounts to be added can be accurately weighed out or pipetted for liquids.

If the sample pH is not between 6 and 9, the test shall be carried out after adjusting the pH to the value of the nearest limit (6 or 9) using solutions of hydrochloric acid or sodium hydroxide.

NOTE Testing water samples at concentrations above 100 ml/l may reduce the reproduction and survival of *D. magna* because of deficiency in the medium (for instance hardness). Identifying effects of such deficiencies may require the addition of the same salts to the sample as in the dilution water.

8 Procedure

8.1 Controls

Every test shall include a control containing no test substance.

Two controls are required when a solvent or dispersant is used. One control shall contain no solvent or dispersant. The second control shall contain a concentration of solvent or dispersant equal to that in the highest exposure concentration.

8.2 Selection of exposure concentrations

There shall be at least five exposure concentrations arranged in a geometric series with a separation factor not exceeding 3,2.

In setting the range of concentrations, the following shall be borne in mind:

a) if the objective is to obtain the NOEC (no observed effect concentration), the range of concentrations shall include at least one concentration producing a significant effect compared to the control (LOEC: lowest observed effect concentration), preceded by a NOEC.

   If this is not the case, the test shall be repeated with a reduced lowest concentration. See footnote 1 to clause 9.

b) If the objective is to obtain the EC_p (effect concentration producing a percentage response; typically this is 20 % and/or 50 %) for the effect on reproduction and survival, it is advisable that two exposure concentrations be higher than this EC_p concentration (p is the percentage response selected). Otherwise, although it will still be possible to estimate the EC_p, the confidence interval for the EC_{50} will be very wide and it may not be possible to assess satisfactorily the adequacy of the fitted model.

NOTE Prior knowledge of the toxicity of the test substance from an acute test and/or from range-finding studies (ISO 6341) is helpful in selecting appropriate exposure concentrations.

Where a solvent or dispersant is used to aid preparation of stock solutions, the concentration of solvent or dispersant in the highest concentration shall not be greater than 0,1 ml/l. The concentration of solvent or dispersant shall, as far as possible, be the same in all vessels.

8.3 Renewal of exposure solutions

The exposure solutions shall be renewed at least three times per week. If preliminary stability tests indicate that the test substance concentration falls below 80 % of the initial measured concentration before day three, consideration shall be given to either increasing the frequency of exposure solution renewal or using a flow-through test. Changes in the frequency of solution replacement or the rate of continuous flow shall be reported.

When semi-static tests are conducted, minimize the volume of medium transferred with the *D. magna.*

8.4 Introduction of animals into the test system

Transfer young animals (< 24 h old) from the culture system to the test system at the beginning of the test. Place each individual in a single vessel containing 50 ml to 100 ml of exposure solution.

Larger volumes of exposure solution may be required for chemical analysis of exposure concentrations. Alternatively, replicates can be pooled for chemical analysis.

Semi-static testing shall require at least ten animals exposed individually to each exposure concentration and control. Furthermore, a second series of test vessels shall be prepared at the time of solution renewal and the parent animals are transferred to them by means of a glass pipette of suitable bore diameter.

Organisms shall be exposed for a total of 21 days.

If a continuous flow test is being conducted, this International Standard should be used for guidance.

8.5 Feeding of organisms

The diet of the parent animals shall be living algal cells of one of the following species: *Chlorella* spp, *Pseudokirchneriella subcapitata* (formally known as *Selenastrum capricornutum*) or *Scenedesmus subspicatus*. It is preferable to feed the animals daily but as a minimum when solutions are being replaced. The organisms shall be fed at a rate of 0,1 mg to 0,2 mg of carbon per animal per day. The ration shall be supplied either at a constant rate throughout the test period or at a gradually increasing rate consistent with the growth of the parent animals. When volumes greater than 100 ml are used in the test, the ration given shall be increased proportionately.

NOTE Supplementing the algal food with other sources of particulate carbon has proved valuable, to avoid nutritional deficiencies in pure, monospecific, cultured algae provided that the total carbon does not exceed the limits above.

Where a surrogate measure of carbon is used, such as algal cell number or light absorbance, the testing laboratory shall generate a nomograph relating the surrogate measure to carbon content. Nomographs shall be confirmed at least annually or when algal culture conditions are changed.

A concentrated algal suspension produced by centrifugation or decanting, followed by re-suspension in culture medium shall be transferred to the animals to minimize the volume of algal culture medium transferred to the test vessels.

8.6 Observations and measurements

Record all observations on a data sheet. An example of a suitable format is presented for information in annex C; other formats are acceptable.

The light intensity at the surface of the test solution shall be reported.

Measure and record the dissolved oxygen, temperature, water hardness, and the pH of the control(s) and the highest exposure concentration weekly, at the beginning and end of one of the renewal periods.

Live offspring produced by each parent shall be counted and removed at least three times per week when changing solutions. Record the presence of dead parents, offspring, males or ephippia eggs.