Irish Standard
I.S. EN ISO 6341:2012

# Water quality - Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) - Acute toxicity test (ISO 6341:2012)

# I.S. EN ISO 6341:2012

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EUROPEAN STANDARD

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English Version

# Water quality - Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) - Acute toxicity test (ISO 6341:2012)

Qualité de l'eau - Détermination de l'inhibition de la mobilité de Daphnia magna Straus (Cladocera, Crustacea) - Essai de toxicité aiguë (ISO 6341:2012)

Wasserbeschaffenheit - Bestimmung der Hemmung der Beweglichkeit von Daphnia magna Straus (Cladocera, Crustacea) - Akuter Toxizitäts-Test (ISO 6341:2012)

This European Standard was approved by CEN on 14 October 2012.



EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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# Foreword

This document (EN ISO 6341:2012) has been prepared by Technical Committee ISO/TC 147 “Water quality” in collaboration with Technical Committee CEN/TC 230 “Water analysis” the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by April 2013, and conflicting national standards shall be withdrawn at the latest by April 2013.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN ISO 6341:1996.

According to the CEN/CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

**Endorsement notice**

The text of ISO 6341:2012 has been approved by CEN as a EN ISO 6341:2012 without any modification.

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I.S. EN ISO 6341:2012

INTERNATIONAL STANDARD

**ISO 6341**

Fourth edition
2012-10-15

# Water quality — Determination of the inhibition of the mobility of *Daphnia magna* Straus (*Cladocera*, *Crustacea*) — Acute toxicity test

*Qualité de l'eau — Détermination de l'inhibition de la mobilité de* Daphnia magna *Straus (*Cladocera*,* Crustacea*) — Essai de toxicité aiguë*

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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 2.

The main task of technical committees is to prepare International Standards. 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 document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 6341 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

This fourth edition cancels and replaces the third edition (ISO 6341:1996), which has been technically revised. It also incorporates the Technical Corrigendum ISO 6341:1996/Cor. 1:1998.

# Introduction

This International Standard specifies a procedure for the determination of the acute toxicity of chemicals, waters and waste waters to the water flea *Daphnia magna* Straus.

The evaluation of harmful effects on water quality has for several years involved the performance of biological tests. Crustaceans are of interest from the ecotoxicological point of view because they are primary consumers and a major component of the zooplankton in aquatic ecosystems.

The test specified in this International Standard involves the determination of the immobilization of the water flea *Daphnia magna* Straus after 24 h or 48 h exposure (depending on the requirement of users or national authorities) to the test sample under the conditions specified in this International Standard.

# Water quality — Determination of the inhibition of the mobility of *Daphnia magna* Straus (*Cladocera*, *Crustacea*) — Acute toxicity test

**WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.**

**IMPORTANT — It is absolutely essential that tests conducted in accordance with this International Standard be carried out by suitably qualified staff.**

## 1 Scope

This International Standard specifies a method for the determination of the acute toxicity to *Daphnia magna* Straus (*Cladocera, Crustacea*).

This method is applicable to:

— chemical substances which are soluble under the conditions of the test, or can be maintained as a stable suspension or dispersion under the conditions of the test;

— industrial or sewage effluents;

— treated or untreated waste water;

— aqueous extracts and leachates;

— fresh water (surface and ground water);

— eluates of fresh water sediment;

— pore water of fresh water sediment.

## 2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 5667-16:1998, *Water quality — Sampling — Part 16: Guidance on biotesting of samples*

ISO 5814, *Water quality — Determination of dissolved oxygen — Electrochemical probe method*

ISO 10523, *Water quality — Determination of pH*

## 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

**3.1**
**control batch**
series of replicates containing control solution

[ISO 20665:2008,[3] 3.3]

**3.2**
**control solution**
test medium without sample under test

**3.3**
**immobilization**
inability of the organisms to swim during the 15 s which follow gentle agitation of the test and control solutions, even if they can still move their antennae

**3.4**
**$EC_{50}$**
concentration at which there is an effect on 50 % of the organisms in line with the test criterion

[ISO 15088:2007,[1] 3.3]

**3.5**
**neonate**
newly born or newly hatched individual

NOTE In this International Standard, a neonate is a first-instar daphnid, <24 h old.

[ISO 20665:2008,[3] 3.6]

**3.6**
**test batch**
series of replicates filled with the same test solution

[ISO 20665:2008,[3] 3.8]

## 4 Principle

Determination of the initial concentration (i.e. the concentration present at the beginning of the test) which, in 24 h or 48 h, immobilizes 50 % of exposed *D. magna*, under the conditions specified in this International Standard. This concentration, known as the effective initial inhibitory concentration, is designated 24 h $EC_{50}$ or 48 h $EC_{50}$.

An indication of the lowest concentration tested which immobilizes all the *D. magna* and the highest concentration tested which does not immobilize any of the *D. magna* is desirable and provides useful information in cases where the $EC_{50}$ cannot be determined.

The test is carried out in one or two stages:

— a preliminary test which determines the range of concentrations to be tested in the definitive toxicity test and gives an approximate value of the 24 h $EC_{50}$ or 48 h $EC_{50}$;

— a definitive test, conducted when the approximate value given by the preliminary test is not sufficient, which permits calculation of the 24 h $EC_{50}$ or 48 h $EC_{50}$, and determines concentrations corresponding to 0 % and 100 % immobilization.

If the method specified in this International Standard is used for biotesting of chemical substances, a limit test can be performed at 100 mg/l or at a lower concentration, at which the substance is soluble or is in stable dispersion under the conditions of the test (see 9.5). If it provides useful information, a limit test may also be performed at concentrations above 100 mg/l as long as the substance is soluble or in stable dispersion.

## 5 Test environment

The exposure of organisms as specified in this International Standard shall be carried out either in the dark or under a 16 h + 8 h light + dark photoperiod, in a temperature-controlled room or incubator at (20 ± 2) °C in the test containers.

The testing atmosphere shall be free from vapours or dusts toxic to *D. magna*. Photodegradable chemicals shall be tested in the dark, or using minimal lighting with the specified photoperiod, or minimal red lighting, as appropriate.

The use of controls (3.1) also allows checking that the test is performed in an atmosphere free from toxic dusts and vapours.

## 6 Reagents, test organisms and media

Use only reagents of recognized analytical grade, unless otherwise specified.

**6.1 Test organisms**. The test organisms are neonates of *D. magna* Straus (*Cladocera*, *Crustacea*), obtained by acyclical parthenogenesis under specified breeding conditions (see Annex C).

The animals used for the test shall be less than 24 h old and should not be first brood progeny. The *D. magna* shall be from a healthy stock, showing no signs of stress such as mortality >20 % in 2 d, presence of males, ephippia, or discoloured animals, and there shall be no delay in the production of the first brood. Isolate gravid females and collect newly released neonates within 24 h.

If the culture conditions differ significantly from test conditions, it is recommended that one generation be acclimated under the test conditions for about one week to avoid stressing the parent animals and the offspring.

The age of the stock culture 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 *D. magna* may also derive from the hatching of ephippia obtained from laboratory cultures of the crustacean as described in Annex D or can be purchased from a specialized company.[1)] The neonates hatched from the ephippia may be used directly as test organisms if they comply with all validity criteria given in this International Standard.

**6.2 Pure water**, conductivity below 10 µS/cm.

**6.3 Dilution and culturing water**.

**6.3.1 General**. Natural water (surface or ground water), reconstituted water or dechlorinated tap water are acceptable as culturing and dilution water if *D. magna* survives in it for the duration of the culturing, acclimation and testing without showing signs of stress. These waters may be used if they comply with all criteria and conditions specified in this International Standard. Waters in the range pH 6 to pH 9, with hardness between 140 mg/l and 275 mg/l (as $CaCO_3$) are recommended.

For stock culture of *D. magna* in the laboratory, the M4 medium (see Annex A) may also be used.

M4 medium (Annex A) should not be used as dilution water for samples containing bivalent metal ions. The EDTA in this medium can reduce the bioavailability of such ions, resulting in a decrease in apparent toxicity. In addition, for the same reason, M4 medium should not be used as the dilution water for samples of unknown composition.

NOTE If the test is performed for purposes necessitating the use of a dilution water with characteristics differing from those described in the preceding three paragraphs, state the main characteristics of the synthetic dilution water used in the test report.

As an example, the preparation of dilution water meeting the requirements is described below.

Dissolve known quantities of reagents in pure water (6.2) The dilution water prepared shall have a pH of 7,8 ± 0,5, a hardness of (225 ± 50) mg/l (expressed as $CaCO_3$), a molar Ca + Mg ratio close to 4 + 1 and a dissolved oxygen concentration above 7 mg/l.

Prepare the solutions specified in 6.3.2 to 6.3.5.

1) MicroBioTests Inc., Mariakerke, Belgium, is an example of a suitable supplier. This information is given for the convenience of the users of this document and does not constitute an endorsement by ISO of this supplier.