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Water quality – Determination of the acute lethal toxicity of substances to a freshwater fish [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)] – Part 3: Flow-through method (ISO 7346-3:1996)

The European Standard EN ISO 7346-3:1997 has the status of a Swedish Standard. This document contains the official English version of EN ISO 7346-3:1997.

The European Standard EN ISO 7346:1997 consists of the following parts:

- Part 1: Static method
- Part 2: Semi-static method
- Part 3: Flow-through method

In the static method, is the test solution not renewed during the test period. This makes the method limited to study of substances whose tested concentrations remain relative constant during the test period. In the semi-static method, is the test solution renewed every 24 h or 48 h. In the flow-through method is the test solution renewed continuously, which makes the method suitable for most types of substances.

Swedish Standards corresponding to documents referred to in this Standard are listed in "Catalogue of Swedish Standards", issued by SIS. The Catalogue lists, with reference number and year of Swedish approval, International and European Standards approved as Swedish Standards as well as other Swedish Standards.

Vattenundersökningar – Bestämning av olika ämnens akuta toxicitet för en sötvattensfisk [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)] – Part 3: Genomflödesmetod (ISO 7346-3:1996)

Europastandarden EN ISO 7346-3:1997 gäller som svensk standard. Detta dokument innehåller den officiella engelska versionen av EN ISO 7346-3:1997.

Europastandarden EN ISO 7346:1997 består av följande delar:

- Del 1: Statisk metod
- Del 2: Semistatisk metod
- Del 3: Genomflödesmetod

I den statistiska metoden förnyas inte testlösningen under försöksperioden. Detta gör att metoden är begränsad till ämnen vars testkoncentration är relativt konstant under försöksperioden. I den semistatiska metoden förnyas testlösningen var 24 h eller 48 h. I genomflödesmetoden förnyas testlösningen kontinuerligt, vilket gör metoden lämplig för de flesta typer av ämnen.

Motsvarigheten och aktualiteten i svensk standard till de publikationer som omnämns i denna standard framgår av "Katalog över svensk standard", som ges ut av SIS. I katalogen redovisas internationella och europeiska standarder som fastställts som svenska standarder och övriga gällande svenska standarder.

ICS 13.060.01

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English version

Water quality – Determination of the acute lethal toxicity of substances to a freshwater fish [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)] – Part 3: Flow-through method (ISO 7346-3:1996)

Qualité de l'eau – Détermination de la toxicité aiguë létale de substances vis-à-vis d'un poisson d'eau douce [*Brachydanio rerio* Hamilton-Buchanan (Téléostei, Cyprinidae)] – Partie 3: Méthode avec renouvellement continu (ISO 7346-3:1996)

Wasserbeschaffenheit – Bestimmung der akuten letalen Toxizität von Substanzen gegenüber einem Süßwasserfisch [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)] – Teil 3: Durchflußverfahren (ISO 7346-3:1996)

This European Standard was approved by CEN on 30 October 1997.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

The European Standards exist in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.

CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

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Foreword

The text of the International Standard from Technical Committee ISO/TC 147 "Water quality" of the International Organization for Standardization (ISO) has been taken over as an European Standard by 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 May 1998, and conflicting national standards shall be withdrawn at the latest by May 1998.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of the International Standard ISO 7346-3:1996 has been approved by CEN as a European Standard without any modification.

Introduction

The three parts of ISO 7346 describe methods of determining the acute lethal toxicity of substances to the zebra fish (*Brachydanio rerio* Hamilton-Buchanan) but it must be emphasized that the recommended use of the zebra fish does not preclude the use of other species. The methodologies presented here may also be used for other species of freshwater, marine or brackish water fish, with appropriate modifications of, for example, dilution water quality and the temperature conditions of the test.

Within the three parts of ISO 7346, a choice can be made between static, semi-static and flow-through methods. The static test, described in ISO 7346-1, in which the solution is not renewed, has the advantage of requiring simple apparatus, although the substances in the test vessel may become depleted during the course of the test and the general quality of the water may deteriorate. The flow-through method, described in this part of ISO 7346, in which the test solution is replenished continuously, overcomes such problems but requires the use of more complex apparatus. In the semi-static procedure, described in ISO 7346-2, the test solutions are renewed every 24 h or 48 h, this method being a compromise between the other two.

The flow-through method can be used for most types of substances, including those unstable in water, but the concentrations of the test substance are determined wherever possible. The static method is limited to the study of substances whose tested concentrations remain relatively constant during the test period. The semi-static method can be used for testing those substances whose concentrations can be maintained satisfactorily throughout the test by renewal of the solutions every 24 h or 48 h. Special arrangements may be necessary for substances which are highly volatile.

To assist in the preparation and maintenance of concentrations of substances which may be lethal at concentrations close to that of their aqueous solubility, a small volume of solvent may be used, as specified in the methods.

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Water quality — Determination of the acute lethal toxicity of substances to a freshwater fish [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)] —

Part 3: Flow-through method

1 Scope

This part of ISO 7346 specifies a flow-through method for the determination of the acute lethal toxicity of stable, non-volatile, single substances, soluble in water under specified conditions, to a freshwater fish [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae) — common name, zebra fish] in water of a specified quality.

The method is applicable for assigning, for each test substance, broad categories of acute lethal toxicity to *Brachydanio rerio* under the test conditions.

The results are insufficient by themselves to define water quality standards for environmental protection.

The method is also applicable when using certain other species of freshwater fish as the test organism¹⁾.

The method may be adapted for use with other freshwater fish and marine and brackish water fish

with appropriate modification of the test conditions, particularly with respect to the quantity and quality of the dilution water and the temperature.

2 Principle

Determination, under specified conditions, of the concentrations at which a substance is lethal to 50 % of a test population of *Brachydanio rerio* after exposure periods of 24 h, 48 h, 72 h and 96 h to that substance in the ambient water. These median lethal concentrations are designated the 24 h - LC50, 48 h - LC50, 72 h - LC50 and 96 h - LC50.

The test is carried out in two stages:

- a) a preliminary test which gives an approximate indication of the acute median lethal concentrations and serves to determine the range of concentrations for the final test;
- b) a final test, the results of which alone are reported.

1) The following species of freshwater fish can be used, in addition to *Brachydanio rerio*, without modification to this part of ISO 7346.

— *Lepomis macrochirus* (Teleostei, Centrarchidae)

— *Oryzias latipes* (Teleostei, Poeciliidae)

— *Pimephales promelas* (Teleostei, Cyprinidae)

— *Poecilia reticulata* (Teleostei, Poeciliidae)

Where evidence is available to show that test concentrations remain relatively constant (i.e. within about 20 % of the nominal values) throughout the test, then either measured or nominal concentrations are used in the estimation of the LC50. Where such analyses show that the concentrations present remain relatively constant but are less than about 80 %, or greater than 120 %, of the nominal values, then the analytical values are used in estimating the LC50. Where evidence is not available to show that the test concentrations remained at an acceptable level throughout the test period, or where it is known (or suspected) that the concentrations of the test chemical have declined significantly at any stage during the test, then, irrespective of whether or not chemical analytical data are available, the LC50 cannot be defined using this test method. In these cases, the test is not necessarily invalidated but it can only be stated that the LC50 of the substance is $\leq x$ mg/l, the value, x , being estimated from the nominal concentrations used.

3 Test organism and reagents

The reagents shall be of recognized analytical grade. The water used for the preparation of solutions shall be glass-distilled water or deionized water of at least equivalent purity.

3.1 Test organism

The test species shall be *Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae), commonly known as the zebra fish. Each test fish shall have a total length of $30 \text{ mm} \pm 5 \text{ mm}$, which, in principle, corresponds to a mass of $0,3 \text{ g} \pm 0,1 \text{ g}$. They shall be selected from a population of a single stock. This stock should have been acclimatized and, in any case, maintained for at least 7 d prior to the test in dilution water, continuously aerated using bubbled air (see 3.2), under conditions of water quality and illumination similar to those used in the test. They shall be fed as normal up to the 24 h period immediately preceding the test.

Test fish shall be free of overt disease or visible malformation. They shall not receive treatment for disease during the test or in the 2 weeks preceding the test. Subsequent to the test, fish remaining alive should be suitably disposed of.

Environmental conditions for the maintenance and breeding of zebra fish are given in annex A.

3.2 Standard dilution water

The freshly prepared standard dilution water shall have a pH of $7,8 \pm 0,2$, and a calcium hardness of approximately 250 mg/l, expressed as calcium carbonate, and shall contain the following concentrations of salts dissolved in distilled or deionized water:

294,0 mg/l $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$

123,3 mg/l $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

63,0 mg/l NaHCO_3

5,5 mg/l KCl

Aerate the dilution water until the concentration of dissolved oxygen reaches at least 90 % of its air saturation value (ASV) and the pH is constant at $7,8 \pm 0,2$. If necessary, adjust the pH of the solution by adding sodium hydroxide solution or hydrochloric acid. The dilution water thus prepared shall receive no further forced aeration before use in the tests.

3.3 Stock solutions of test substances

A stock solution of the test substance should be prepared by dissolving a known amount of test substance in a defined volume of dilution water, deionized water or glass-distilled water. The stock solution should be prepared at a frequency appropriate to the stability of the test substance. To enable stock solutions to be prepared and to assist in their transfer to the test vessels, substances of low aqueous solubility may be dissolved or dispersed by suitable means, including ultrasonic devices and organic solvents of low toxicity to fish. If any such organic solvent is used, its concentration in the test solution shall not exceed 0,1 ml/l, or the volume containing 0,1 g/l, whichever is the greater. Where a solvent is used, two sets of controls, one containing solvent at the maximum concentration used in any test vessel and one without solvent or test substance, shall be included.

3.4 Test solutions

Test solutions are prepared by adding appropriate amounts of the stock solution of the test substance to the dilution water to give the required concentrations. It is recommended that, when a stock solution is prepared in distilled or deionized water, no more than 100 ml of stock solution should be added per 10 litres of dilution water.