

Water quality —

Part 5: Biological methods —

Section 5.17 Determination of the prolonged toxicity of substances to freshwater fish — Method for evaluating the effects of substances on the growth rate of rainbow trout [*Oncorhynchus mykiss* Walbaum (Teleostei, Salmonidae)]

Committees responsible for this British Standard

The preparation of this British Standard was entrusted by Technical Committee EPC/44, Water quality, to Subcommittee EPC/44/5, Biological methods, upon which the following bodies were represented:

- British Laboratory Ware Association Ltd.
- Department of the Environment (Water Directorate)
- Department of the Environment for Northern Ireland
- Department of Health
- Freshwater Biological Association
- Institution of Water and Environmental Management
- Ministry of Agriculture, Fisheries and Food
- National Rivers Authority
- Royal Society of Chemistry
- Scottish Natural Heritage
- Soap and Detergent Industry Association
- Water Companies Association
- Water Research Centre
- Water Services Association of England and Wales

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National foreword

This Section of BS 6068 has been prepared by Subcommittee EPC/44/5 and is identical with ISO 10229:1994 *Water quality — Determination of the prolonged toxicity of substances to freshwater fish — Method for evaluating the effects of substances on the growth rate of rainbow trout [Oncorhynchus mykiss Walbaum (Teleostei, Salmonidae)]*.

The international standard was prepared by Subcommittee 5, Biological methods, of Technical Committee 147, Water quality, of the International Organization for Standardization (ISO) with the active participation and approval of the UK.

BS 6068 is being published in a series of Parts subdivided into Sections that will generally correspond to particular international standards. Sections are being, or will be, published in Parts 1 to 7, which, together with Part 0, are listed below.

- Part 0: Introduction;
- Part 1: Glossary;
- Part 2: Physical, chemical and biochemical methods;
- Part 3: Radiological methods;
- Part 4: Microbiological methods;
- Part 5: Biological methods;
- Part 6: Sampling;
- Part 7: Precision and accuracy.

NOTE The tests described in this Section of BS 6068 should only be carried out by suitably qualified persons with an appropriate level of biological expertise. Usual biological procedures should be followed throughout.

Cross-references

International standard	Corresponding British Standard
ISO 7346-2:1984 ^a	BS 6068-5.3:1985 <i>Determination of the acute lethal toxicity of substances to a freshwater fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)]: Semi-static method</i> (Identical)
ISO 7346-3:1984 ^a	Section 5.4:1985 <i>Determination of the acute lethal toxicity of substances to a freshwater fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)]: flow-through method</i> (Identical)

^a Revision to be published shortly.

A British Standard does not purport to include all the necessary provisions of a contract. Users of British Standards are responsible for their correct application.

Compliance with a British Standard does not of itself confer immunity from legal obligations.

Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, pages 1 to 12, an inside back cover and a back cover.

This standard has been updated (see copyright date) and may have had amendments incorporated. This will be indicated in the amendment table on the inside front cover.

1 Scope

This International Standard specifies a method for the determination of the long-term sublethal toxicity of substances soluble in water under specified conditions to a species of anadromous fish [*Oncorhynchus mykiss* Walbaum (Teleostei, Salmonidae) — common name, rainbow trout] in fresh water.

NOTE 1 “Substances” is considered to include pure chemicals tested singly or in mixtures, waste waters, process waters or other waters whose sublethal toxicity to fish it is necessary to determine. A range of concentrations of waste and process waters should be tested (see 8.2).

The response measured is the change in rate of growth of fish exposed to the test substance over periods of 14 d and 28 d (see [1] in Annex B).

NOTE 2 The mass of each fish at the start of the test, the period of exposure, the feeding regime and temperature range have been chosen to ensure that the control fish will be growing exponentially throughout the test.

The method is applicable for assigning, for each test substance, a category of prolonged sublethal toxicity to *Oncorhynchus mykiss* under the test conditions.

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

It is possible for the method to 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 temperature, the quantity and quality of the dilution water, the food and the fish-marking technique.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 7346-2:1984, *Water quality — Determination of the acute lethal toxicity of substances to a freshwater fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)] — Part 2: Semi-static method*.

ISO 7346-3:1984, *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*.

3 Principle

Determination, under specified conditions, of the concentrations at which a substance statistically and significantly reduces the rate of growth of a test population of *Oncorhynchus mykiss* after exposure periods of 14 d and 28 d to that substance in the ambient water.

The test is carried out in two stages:

a) a preliminary test, carried out using rainbow trout as the test species but in accordance with ISO 7346-2 or ISO 7346-3 (although the rainbow trout is not specifically named in these parts of ISO 7346), which determines the concentrations at which the substance is lethal to 50 % of a test population after exposure to the substance in the ambient water for 96 h: this median lethal concentration is designated as 96 h LC₅₀ and serves to determine the range of concentrations for the final test (see note 11 to 8.1).

b) a final test, using 16 fish per concentration, in which the results are recorded as both the lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC). They are prefixed by an indication of the exposure period concerned, for example “28 d LOEC”. The data may also be fitted to a regression model in order to calculate the IC₁₀ (inhibition concentration for a 10 % effect), or a similar statistic.

Where analytical data show that median test concentrations remain relatively constant (i.e. within 20 % of the nominal values) throughout the test, the method allows the use of either measured or nominal concentrations in the statements on LOEC and NOEC. Where such analyses show that the concentrations present remain relatively constant (i.e. ± 20 % of the median) but are less than 80 % or greater than 120 % of the nominal values, then the analytical values derived from this method are used in presenting the LOEC and NOEC. Where no evidence is available to show that the test concentrations remained at an acceptable level throughout the test period or where it is known that the concentrations of the test chemical have changed significantly (i.e. by greater than 20 % of the median) at any stage during the test, then irrespective of whether or not chemical analytical data are available the LOEC and NOEC cannot be defined using this test method. In these cases, the test is not necessarily invalidated but it can only be stated that the LOEC of the substance is $\leq x$ mg/l, and that the NOEC of the substance is $\leq y$ mg/l, the values x and y given being estimated from the nominal concentrations used.

NOTE 3 Wherever possible, it is very important to determine the concentrations of the test substance to which the fish are exposed.

Fish can be individually recognized throughout the test if the freeze-branding technique or an equivalent method (e.g. tagging) is used for marking, but this is not mandatory.

4 Test organism

The recommended test species is *Oncorhynchus mykiss* Walbaum (Teleostei, Salmonidae), commonly known as the rainbow trout. Test fish shall be selected from a population of a single stock. This stock shall have been acclimatized and, in any case, maintained for at least 2 weeks prior to the test in dilution water, continuously aerated (using bubbled air), under conditions of water quality, feeding regime and illumination similar to those used in the test.

Except where otherwise stated (see clause 8), the fish shall be fed at a minimum rate of 1 % of their wet body mass per day during any pre-acclimation and acclimation period and 4 % of their mass per day during the final test (see 8.2).

NOTE 4 This latitude in feeding rate during the pretest period enables the fish to be grown to, or held at, a suitable size (see below) for the start of the test.

The food shall be a dry proprietary salmonid fry food and shall be divided into two equal portions and given to the fish in two feeds per day separated by at least 5 h. After exposure of 14 d to the test substance, when the fish are weighed again, the rations shall be recalculated for each test vessel.

At the start of the test, each fish shall have a mass of not less than 3 g and not more than 5 g (but see 8.2). For the whole batch of fish used in the test, the range in individual masses at the start of the test shall lie within ± 10 % of the arithmetic mean of the masses.

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.

Maintain the fish in environmental conditions suitable for rainbow trout.

NOTE 5 Fish cultivation manuals should be consulted, for example [2] in Annex B.

5 Reagents

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

5.1 Dilution water

The dilution water shall be suitable for the long-term survival and growth of the test fish. The average pH of the dilution water shall be within the range 6,7 to 8,5, but during a given test the pH of the dilution water shall not vary by more than $\pm 0,2$ pH units from the mean value.

NOTE 6 Such a pH range can be expected to be associated with a bicarbonate alkalinity of 1 mg/l to 100 mg/l CaCO_3 and total hardness values of 20 mg/l to 300 mg/l CaCO_3 , but these ranges will depend on the precise constitution of the dilution water and the time available for equilibration of the water. A suitable artificial water can be made with distilled water, as follows:

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

5.2 Stock solutions of test substances

Prepare a stock solution of the test substance by dissolving a known amount of test substance in a defined volume of dilution water, deionized water or glass-distilled water.

NOTE 7 In the case where the test "substance" is a waste water, process water or other water (see clause 1, note 1) and not one or more pure chemicals, these waters are to be taken as the stock solution.

Prepare or obtain the stock solution as frequently as necessary to maintain stable solutions. Although the use of solvents is not desirable, to enable stock solutions to be prepared and to assist in their transfer to the test vessels, it shall be permissible to dissolve or disperse substances of low aqueous solubility by suitable means, for example ultrasonic devices or the use of 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 (and preferably be below 0,01 ml/l), and 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.

NOTE 8 It is preferable that the same concentration of solvent be used in all test vessels.

5.3 Test solutions

Prepare test solutions by adding appropriate amounts of the stock solution of the test substance (5.2) to the dilution water to give the required concentrations (8.2). 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. Wherever possible, it is very important to determine the concentrations of the test substance in each test vessel, with the exception of waste waters where this may not be possible.