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Water quality — Determination of toxicity to embryos and larvae of freshwater fish — Semi-static method

*Qualité de l'eau — Détermination de la toxicité vis-à-vis des embryons et
larves de poissons d'eau douce — Méthode semi-statique*



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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.

International Standard ISO 12890 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

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

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Introduction

Fish are particularly susceptible to the influence of substances, for example chemicals, during the reproductive stage (gametogenesis) and early developmental stages (embryo and larval stages). Determination of the toxicity to fish in early developmental stages is thus a more sensitive index of tolerance than that obtained by determination of acute toxicity to adult fish.

Only tests incorporating all stages of the life cycle of fish are expected to give an accurate estimate of the chronic toxicity of chemicals to fish. A reduced exposure with respect to life stages may reduce the sensitivity and, thus, underestimate the chronic toxicity. Therefore, the present method using embryos and larvae is expected to be less sensitive than a full life cycle test, and it may also be less sensitive than an early life-stage test incorporating the growth of the larvae for several weeks. The difference in sensitivity between these types of test will depend upon several factors, including the chemicals exerting toxicity. Therefore, it is not possible to generalize about the relationship between the sensitivity of full life cycle, embryo-larval (including growth) and embryo-larval (excluding growth) tests. However, experience has shown that for many chemicals the sensitivity obtained in embryo-larval tests correlates with that obtained in full life cycle tests.

Most experience with embryo-larval tests in Europe has been obtained with the freshwater fish *Danio rerio* (Hamilton-Buchanan) *Teleostei, Cyprinidae*, commonly called zebrafish. The systematic name of this species was recently changed from *Brachydanio rerio* to *Danio rerio*. More detailed guidance on the maintenance of stock fish and egg production for testing with this species is given in annex A. References to previous studies on these matters and embryo-larval testing are given in annex B. Reference to the zebrafish does not preclude the use of other species of freshwater fish for which experience is available.

Water quality — Determination of toxicity to embryos and larvae of freshwater fish — Semi-static method

1 Scope

This International Standard specifies a semi-static method for determination of toxicity of chemicals, waters and wastewaters to embryos and early larval developmental stages of a species of freshwater fish, *Danio rerio* (Hamilton-Buchanan), *Teleostei*, *Cyprinidae* (common name zebrafish). Where necessary this determination may include an acute toxicity test using *Danio rerio* to determine the 96-h LC₅₀ for zebrafish in accordance with ISO 7346-1, ISO 7346-2 or ISO 7346-3.

The method is also applicable to freshwater fish other than zebrafish if appropriate modifications of the test conditions are made, particularly with regard to the temperature and volume per fish biomass.

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

ISO 6341, *Water quality — Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) — Acute toxicity test*.

ISO 7346-1, *Water quality — Determination of the acute lethal toxicity of substances to a freshwater fish [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)] — Part 1: Static method*.

ISO 7346-2, *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, *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 Terms and definitions

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

3.1

effect concentration

EC_X

(for X % effect) the experimentally derived concentration that is calculated to affect X % of the test organisms

NOTE The EC₅₀ for survival is equivalent to the LC₅₀, the concentration lethal to 50 % of the test organisms.