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**Water quality — Biochemical and  
physiological measurements on fish —  
Part 2:  
Determination of  
ethoxyresorufin-O-deethylase (EROD)**

*Qualité de l'eau — Mesurages biochimiques et physiologiques sur  
poisson —*

*Partie 2: Dosage de l'éthoxyrésorufine-O-dééthylase (EROD)*



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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.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of normative document:

- an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;
- an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

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/TS 23893-2 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

ISO 23893 consists of the following parts, under the general title *Water quality — Biochemical and physiological measurements on fish*:

- *Part 1: Sampling of fish, handling and preservation of samples*
- *Part 2: Determination of ethoxyresorufin-O-deethylase (EROD) [Technical Specification]*

## Introduction

The measurement of pollution biomarkers in fish, such as the measurement of biotransformation enzyme activities, is likely to provide information about exposure levels, bioavailability and the early biological effects of substances present in aquatic ecosystems. The measurement of the EROD enzyme activity allows the diagnosis of the exposure of fish to inducers of the P450 1A cytochrome, such as certain polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and dioxins. A large amount of research work bears witness to the extent of the studies conducted (see Bibliography).

An induction of EROD activity reflects the presence of inducers such as those mentioned above. On the other hand, the absence of induction does not necessarily reflect the absence of exposure of the fish to organic contaminants, account being taken of the inhibition phenomena of the EROD induction of possible modification of the bioavailability of the inducers or of low exposure concentrations.

The application of a standardised reference method is strongly advised within a monitoring programme. The intercalibration exercises on the measurement of the EROD enzyme activity undertaken since 1991 have revealed multiple sources of errors, which are very easy to avoid (dilution of resorufin, determination of proteins, calculation of the enzyme activity, etc.) once laboratories have become familiar with the analysis of enzyme activities and the possible error factors.

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# Water quality — Biochemical and physiological measurements on fish —

## Part 2:

## Determination of ethoxyresorufin-O-deethylase (EROD)

### 1 Scope

This part of ISO 23893 specifies a method for measuring the ethoxyresorufin-O-deethylase (EROD) enzyme activity on a post-mitochondrial fraction of fish liver homogenate (subcellular fraction in which the EROD activity is located) employing a cell or microplate fluorimetric method.

It applies to fish that are sampled in their natural environment (fresh water or salt water) or exposed to substances or effluents in a laboratory.

This method is applicable for EROD values greater than or equal to 1 pmol/(min·mg) of proteins. A higher sensitivity may be achieved by using a cell (test tube) procedure.

### 2 Normative references

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

ISO 23893-1, *Water quality — Biochemical and physiological measurements on fish — Part 1: Sampling of fish, handling and preservation of samples*

### 3 Principle

Samples of fish are collected and dissected as described in ISO 23893-1 to obtain pieces of liver. Homogenates of fish liver are prepared by crushing (homogenisation) and the supernatant S9 fraction recovered by centrifugation. The EROD enzyme activity in the S9 fractions is determined by measurement of the increase in fluorescence due to the transformation of the 7-ethoxyresorufin into resorufin. The fluorescence is reported in quantities of resorufin by means of a calibration range (external calibration of resorufin or of rhodamine B). The EROD activity is related to the quantity of proteins in the S9 fraction.

### 4 Test environment

All of the tests and handling operations with the S9 fraction shall be carried out at a temperature close to 4 °C (e.g. handling in crushed ice), except the enzyme reaction which shall be performed at 20 °C ± 2 °C.