



**Technical
Specification**

ISO/TS 21738

**Water quality — Active
biomonitoring method with in situ
caged benthic amphipods**

*Qualité de l'eau — Méthode de biosurveillance active avec des
amphipodes benthiques en cage in situ*

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

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

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Some chemical substances (metals or hydrophobic organic contaminants) accumulate in organisms. The measurement of bioaccumulation using living organisms is found to be a valid approach for monitoring chemical contamination.

Two biomonitoring strategies can be applied to measure chemical substances in the biota: either passive approaches based on sampling or collecting indigenous organisms at the monitoring station,^[1] or active approaches based on transplanting, by caging, organisms from a reference source population.^{[4],[7]}

Active biomonitoring makes it possible to:

- overcome the lack of indigenous organisms at certain monitoring stations;
- minimize the biological variability of the responses measured, associated with the impact of confounding factors such as the exposure history and time, reproductive status, physiological state, age, or sex of the organisms sampled.

This enables a reliable comparison of results between stations and between sampling dates.^[6]

This method limits the risk of organisms escaping during caging:

- enclosure chambers present holes with diameter inferior to organisms' size and are securely closed with screwed cap;
- enclosure chambers are fixed and protected from breakage by being installed inside an exposure system.¹⁾

This document describes a method to expose test organisms (amphipods), directly on the field by a caging methodology, with the aim to measure bioaccumulation of chemical substances on a monitoring station, i.e. either the concentrations of metals or organic compounds, or both, accumulated in the organisms. Unlike tests carried out on water samples, the major advantage of the caging method is to be able to integrate the temporal variability of exposure via continuous caging for several days or weeks.

This method can be used for a comparative study at large scale (including stations in several watersheds) and for upstream/downstream studies to assess discharge impacts.

The use of invasive species should be avoided at areas where they are not already present. The introduction of several species and the possibility to use local populations as source organisms in this document has the advantage of being able to circumvent this issue.

A summary table is proposed in [Annex A](#) to reference for each species its indigenous area. Further species could be added in this annex. Freshwater or marine species could be used, while paying attention to the cannibalistic behaviour of certain amphipod species.

This document also describes the specifications for test organism selection and conditioning, in situ exposure, and finally sorting and conditioning of the surviving organisms after exposure.

The organism preparation methods (freeze-drying, extraction, mineralization) and quantification of the chemical substances do not fall within the scope of this document.

Amphipods are relevant organisms for the analysis of the bioaccumulation of chemical substances.^{[2],[18]} Moreover, amphipods represent important keystone species in aquatic ecosystems, they play a key role in biogeochemical cycle (e.g., litter breakdown processes) and constitute an important element in food webs by providing prey for secondary consumers.

The application domain of method depends on the characteristics of the used species. A summary table is proposed in [Annex B](#) to reference for each species the main indications about exposure time, matrix,

1) Around 7,000 gammarid caging experiments were carried out in rivers mainly in France, but also in Belgium and Luxembourg. No broken or capless chamber was found during these experiments.

physicochemical parameters for optimal exposure, range of average weight per individual, organisms' density in cage. Further species can be added to this table.

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1 Scope

This document describes a method to expose test organisms (amphipods), directly on the field by a caging methodology, with the aim to measure bioaccumulation of chemical substances on a monitoring station, i.e. either the concentrations of metals or organic compounds, or both, accumulated in the organisms.

This document also describes the specifications for test organism selection and conditioning, in situ exposure, and finally sorting and conditioning of the surviving organisms after exposure.

This document does not apply to organism preparation methods (freeze-drying, extraction, mineralization) and quantification of the chemical substances.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1 acclimatization period

period prior to in situ exposure and during which the organisms are kept under controlled laboratory conditions

Note 1 to entry: Controlled laboratory conditions can include temperature, electric conductivity, oxygen, diet, organism density, medium replacement frequency, and photoperiod.

3.2 organism calibration

operation consisting of selecting specimens in order to obtain test organisms of homogeneous weight and of the same sex

Note 1 to entry: A size to weight ratio of the organisms used is measured at each experiment (detail for each species is given in [Annex A](#)).

3.3 monitoring station

location in the water-body characteristic of the impacted or reference location being studied

3.4 caging point

sub-area of the monitoring station