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## Introduction

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|                  |                        |
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| 1  | 2                                          | (3)                          | 4               | 5                                                                                                                                  | (6)                                                                      | 7                                                  |
|----|--------------------------------------------|------------------------------|-----------------|------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|----------------------------------------------------|
| MB | Clause No./ Subclause No./Annex (e.g. 3.1) | Paragraph/Figure/ Table/Note | Type of comment | Commend (justification for change) by the MB                                                                                       | Proposed change by the MB                                                | Secretariat observations on each comment submitted |
|    | 3.1                                        | Definition 1                 | ed              | Definition is ambiguous and needs clarifying.                                                                                      | Amend to read '...so that the mains connector to which no connection...' |                                                    |
|    | 6.4                                        | Paragraph 2                  | te              | The use of the UV photometer as an alternative cannot be supported as serious problems have been encountered in its use in the UK. | Delete reference to UV photometer.                                       |                                                    |

# DRAFT INTERNATIONAL STANDARD

## ISO/DIS 4979

ISO/TC 147/SC 5

Secretariat: DIN

Voting begins on:  
2022-06-10

Voting terminates on:  
2022-09-02

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## Water quality — Aquatic toxicity test based on root re-growth in *Lemna minor*

ICS: 13.060.70

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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 documents 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](http://www.iso.org/directives)).

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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](http://www.iso.org/members.html).

## Introduction

*Lemna gibba* and *L. minor* have been most extensively used in phytotoxicity testing and there are several standard methods which have been adopted by major international standardization agencies e.g. ISO 20079:2005, U.S. Environmental Protection Agency,<sup>[4]</sup> Organization for Economic Cooperation and Development.<sup>[5]</sup> Tests with duckweed have typically favoured measurements of frond (e.g. their number, biomass, area, carbon uptake, chlorophyll content<sup>[9]</sup>) require standard exposure durations of at least 7 d to detect toxicity.

On the other hand, tests based on root elongation are some of the most widely used phytotoxicity methodologies for terrestrial angiosperms because of their simplicity and rapidity. Despite reports that roots of *L. minor* are highly sensitive to environmental stressors and that they play important ecological roles by providing stability<sup>[6],[7],[8]</sup> little attention has been paid to the roots in *Lemna* since it was generally considered that root fragility made their handling for measurements difficult and that it was impractical to obtain sufficient numbers of individual plants with identical root lengths to initiate tests. However, the ecotoxicological significance of the root endpoint has been re-evaluated and root length was shown to be a sensitive, precise and ecologically significant endpoint in comparison with more traditional frond growth or biomass endpoints.<sup>[9]</sup>

The proposed root re-growth bioassay differs in several key aspects from three internationally standardized methods (ISO, OECD and US EPA):

- a) the test can be completed within 72 h;
- b) the test vessel is a 24-well cell plate;
- c) the required volume of test water samples is 3 ml;
- d) roots were excised prior to exposure with subsequent measurements on newly developed roots. The technique of excising roots prior to exposure means that there is no requirement to pre-select roots of uniform length, which reduces the handling of these fragile roots.

Artificial severance of roots may never happen in natural settings since root abscission in *Lemna* has not been reported previously. However, according to recent studies the tiny globally distributed water ferns of the genus *Azolla* lost their roots under stress conditions<sup>[10]</sup> a phenomenon known as rapid root abscission. Such shedding sets its fronds free from root-entangled mats and facilitates their dispersion to a potentially better environment. Therefore, rapid root abscission is considered an important survival strategy of *Azolla*.<sup>[10]</sup> This may indicate that the endpoint root re-growth has its ecological relevance.

It is also well known that *Lemna* will thrive without any roots. Thus, *Lemna* roots appear nonessential organs, but are nonetheless important for plant anchorage, nutrient absorption and cytokinin biosynthesis. Therefore, the manipulation of roots by simple severance may not be a serious issue and does not justify the conclusion that removal of roots prior to ecotoxicological testing is inappropriate.

The 3 d root re-growth test is useful for the rapid screening of either wastewater effluents or hazardous contaminants in natural waters<sup>[11]</sup> as it is easy to perform, quick to run, and cost-effective to operate for wastewater toxicity screening and may have an operational benefit of testing time since management decision should be made in a timely manner in the case of unexpected pollution events.

The root re-growth endpoint from this 72-hour protocol is not a direct substitute for the 7-day growth rate/biomass endpoints.

The present protocol provides detailed information on how to set up and conduct the root re-growth test with *Lemna minor* as well as how to analyse toxicity data. This protocol is intended for use with *Lemna minor*, but it can also be applied to other *Lemna* species and *Spirodela* species with some modifications.

