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Standard Guide for Conducting Static, Axenic, 14-Day Phytotoxicity Tests in Test Tubes with the Submersed Aquatic Macrophyte, *Myriophyllum sibiricum* Komarov[1]

This standard is issued under the fixed designation E 1913; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ϵ) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 Submersed rooted aquatic macrophytes are important components of aquatic systems. They contribute to primary productivity, improve water quality, cycle nutrients, generate oxygen, affect flow patterns, provide habitat and food for other organisms, and stabilize the sediment. These plants can be adversely affected when pesticides are sprayed to control aquatic weeds and algal blooms or when phytotoxic chemicals enter the waterway through atmospheric fallout, soil erosion, industrial effluent, sewage discharge, spills or drift from aerial or ground applications.

1.2 This guide is designed to give guidance for assessing the potential phytotoxicity of a test material added to a sterile liquid growth medium on a species of freshwater submersed macrophyte (*Myriophyllum sibiricum* Komarov) during a 14-day static exposure. A sterile system is recommended to determine the direct effect of the test chemical upon individual parameters of the submersed macrophyte because there is no degradation of the test item by micro-organisms. For similar reasons, other aquatic plant testing, such as those of, *Lemna* and algae, is commonly conducted in an axenic fashion. Overall environmental impact can not be directly determined. The main other disadvantage of the axenic test system is the difficulty in preventing accidental contamination. These procedures could possibly be useful for conducting toxicity tests with other species of submersed macrophytes, although modifications might be necessary **(1-8)**[2].

1.3 The procedures in this guide are applicable to most chemicals, either individually or in formulations, commercial products, or known mixtures. These procedures might be used to conduct tests for dependency on temperature, light, nutrients and pH. With appropriate modification, these procedures might be used to conduct tests for contaminated surface waters and aqueous effluents (see Guide E 1192). This static, axenic toxicity test might not be applicable to materials that contain microorganisms unless the sample can be filter sterilized without removing the toxicant. If the test materials are highly volatile, care should be taken to ensure that the test chambers are isolated. It might be necessary to replace the test material on a regular basis if the test material is rapidly biologically or chemically transformed in aqueous solution, or is removed from the test solutions in substantial quantities by the test chambers or organisms during the test. This toxicity test is not suitable for testing interactions between aquatic plants and other organisms, such as plant pathogens.

1.4 Results from the toxicity test outlined in this guide can be reported in terms of a 14-day IC25, IC50, or NOEC. This parameter may be based on several endpoints including inhibition of plant growth during the 14-day period, inhibition of shoot length, inhibition of root number and length, inhibition of fresh or dry weight (see Guide E 1415), inhibition of oxygen production, change in membrane permeability, and change in chlorophyll *a*, chlorophyll *b* and carotenoid content extracted from sections of the plants (see Practice D 3731 and Guide E 1218) **(9-18)**. All or some of these endpoint parameters may be examined depending upon the mode of phytotoxic action or researcher preference. It might be necessary to conduct the toxicity test at only one concentration to determine whether or not that specific concentration is inhibitory to plant growth and development.

1.5 This guide is arranged as follows:

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[2] The boldface numbers given in parentheses refer to a list of references at the end of the text.

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