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Third edition
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Soil quality — Inhibition of reproduction of Collembola (*Folsomia candida*) by soil contaminants

*Qualité du sol — Inhibition de la reproduction de Collembola (*Folsomia candida*) par des contaminants du sol*

Reference number
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Contents

Page

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Foreword

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This document was prepared by Technical Committee ISO/TC 190, *Soil quality,* Subcommittee SC 4, *Biological characterization*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 444, *Environmental characterization of solid matrices,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This third edition cancels and replaces the second edition (ISO 11267:2014), which has been technically revised.

The main change is as follows:

— addition of an annex to provide specific information when using alternative Collembola species for reproduction test.

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Introduction

Ecotoxicological test systems are applied to obtain information about the effects of contaminants in soil and are proposed to complement conventional chemical analysis (see References [2] and [4]). Reference [2] includes a list and short characterization of recommended and standardized test systems and Reference [4] gives guidance on the choice and evaluation of the bioassays. Aquatic test systems with soil eluate are applied to obtain information about the fraction of contaminants potentially reaching the groundwater by the water path (retention function of soils), whereas terrestrial test systems are used to assess the habitat function of soils.

Soil-dwelling Collembola are ecologically relevant species for ecotoxicological testing. Springtails are prey animals for a variety of endogeic and epigeic invertebrates and they contribute to decomposition processes in soils. In acidic soils they are probably the most important soil invertebrates besides enchytraeids with respect to that function, since earthworms are typically absent.[19] Additionally, Collembola represent arthropod species with a different route and a different rate of exposure compared to earthworms[1] and enchytraeids.[3] Various species were used in bioassays of which four species were used most commonly, *Folsomia candida* Willem, *Folsomia fimetaria* L., *Onychiurus armatus*, and *Orchesella cincta*.[20] Numerous soil toxicity tests supported by Environment Canada (EC) resulted in the development and standardization of a biological test method for determining the lethal and sublethal toxicity of samples of contaminated soil to Collembola.[10] The method prepared by EC includes four species, *Orthonychiurus folsomi*, *Proisotoma minuta*, *F. candida*, and *F. fimetaria*. As standardized test systems using Collembola as indicator organisms for the habitat function of soil, another two methods exist. One is designed for assessing the effects of substances on the reproductive output of the Collembola, *F. fimetaria* and *F. candida* in soil[19],[21], and the other method described here, focuses on testing contaminated soil. Optionally the method can be used for testing substances added to standard soils (e.g. artificial soil) for their sublethal hazard potential to Collembola.

This document describes a method that is based on the determination of sublethal effects of contaminated soils to adult Collembola of the species *Folsomia candida* Willem. The species is distributed worldwide. It plays a similar ecological role to *F. fimetari*[10],[19]. *F. candida* reproduces parthenogenetically and is an easily accessible species as it is commercially available and easy to culture. *F. candida* is considered to be a representative of soil arthropods and Collembola in particular. Background information on the ecology of springtails and their use in ecotoxicological testing is available in Reference [22].

Distinct Collembolan species inhabit various ecological niches at different soil depths and in different soil types across the globe. Although considered a surrogate species and therefore frequently used in ecotoxicological reproduction tests, *F. candida* is not common in most natural soils.[28] Furthermore, species specific morphological adaptations can influence exposure and toxic effects of chemicals on organisms.[102] Thus, the use of a variety of Collembolan species representing different morphological adaptations can be advantageous to obtain a broad spectrum of sensitivities for this group. Therefore, other species like *F. fimetaria* (euedaphic, distributed worldwide and found in agricultural soils[28]), *Onychirus yodai* (an euedaphic Asian species,[31] *Proisotoma minuta* (hemiedaphic, distributed worldwide and inhabiting agricultural soils [31],[36]), *Protaphorura fimata* (euedaphic, occuring through mild temperate to cold zones [31],[37]), and *Sinella curviseta* (epedaphic, distributed from North America to Europe, Southeast Asia and Japan [42]) were added as potential alternative test species (Annex E). These species have been used as ecotoxicological test species before, but available testing experience is limited.

Effects of substances are assessed using a standard soil, preferably a defined artificial soil substrate. For contaminated soils, the effects are determined in the soil to be tested and in a control soil. According to the objective of the study, the control and dilution substrate (dilution series of contaminated soil) are either an uncontaminated soil comparable to the soil to be tested (reference soil) or a standard soil (e.g. artificial soil).

NOTE The stability of the test substance cannot be ensured over the test period. No provision is made in the test method for monitoring the persistence of the substance under test.

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