

ISO 20227:2017-06 (E)

Water quality - Determination of the growth inhibition effects of waste waters, natural waters and chemicals on the duckweed *Spirodela polyrhiza* - Method using a stock culture independent microbiotest

| Contents | | Page |
|--------------------|---|-------------|
| Foreword | | iv |
| Introduction | | v |
| 1 | Scope | 1 |
| 2 | Normative references | 1 |
| 3 | Terms and definitions | 1 |
| 4 | Principle | 2 |
| 5 | Test organisms | 3 |
| 6 | Growth medium | 3 |
| 6.1 | Preparation of stock solutions | 4 |
| 6.2 | Preparation of the final concentration of modified Steinberg medium | 4 |
| 7 | Apparatus | 4 |
| 8 | Reference chemicals | 5 |
| 9 | Procedure | 5 |
| 9.1 | Germination of the <i>Spirodela polyrhiza</i> turions | 5 |
| 9.2 | Tests on effluents (and waste waters) | 5 |
| 9.2.1 | Addition of concentrated growth medium to the effluent sample | 5 |
| 9.2.2 | Preparation of the effluent dilutions | 6 |
| 9.2.3 | Procedure | 6 |
| 9.3 | Tests on chemical compounds | 7 |
| 9.3.1 | Range finding test | 7 |
| 9.3.2 | Definitive test | 8 |
| 9.4 | Filling of the test plate with the toxicant dilutions | 9 |
| 9.4.1 | General | 9 |
| 9.4.2 | Procedure | 9 |
| 9.5 | Transfer of the germinated turions in the test cups | 10 |
| 9.6 | Photo of the multiwell at the start of the toxicity test | 10 |
| 9.7 | Incubation of the multiwell | 10 |
| 9.8 | Photo of the multiwell at the end of the toxicity test | 11 |
| 9.9 | Measurement of the area of the first fronds | 11 |
| 10 | Data treatment -- Calculation of the growth inhibition | 11 |
| 11 | Validity criterion | 12 |
| 12 | Test sensitivity | 12 |
| 13 | Test with reference chemicals | 12 |
| 14 | Test report | 15 |

| | |
|--|-----------|
| Annex A (informative) Spirodela polyrhiza stock culturing for turion production | 16 |
| Annex B (informative) Sensitivity of the Spirodela polyrhiza microbiotest | 17 |
| Annex C (informative) Performance data | 19 |
| Bibliography | 20 |