Development and validation of a stock culture independent duckweed microbiotest with *Spirodela polyrhiza*

Guido Persoone[1,2], Manousos Foudoulakis[3], Gerassimos Arapis[4], Renato Baudo[5]

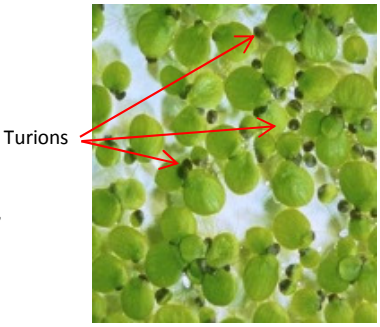
[1] Ghent University, Laboratory for Environmental Toxicology and Aquatic Ecology, Ghent, Belgium; [2] MicroBioTests Inc., Mariakerke(Gent), Belgium; [3] Dow AgroSciences, Lavrion, Greece ; [4] Agricultural University of Athens, Laboratory of Ecology and Environmental Sciences, Athens, Greece; [5] C.N.R. Istituto per lo Studio degli Ecosistemi (I.S.E), Pallanza, Italy

INTRODUCTORY STATEMENTS

1. For applications in routine, toxicity tests must be robust, practical and low cost
2. There are presently but very few test methods which abide by these 3 basic principles
3. One of the reasons is that most test methods are dependent on the (continuous) culturing/maintenance of live stocks of the test species
4. There is hence a need to develop additional practical and cost-effective methods for routine toxicity testing

DEVELOPMENT OF A *SPIRODELA POLYRHIZA* DUCKWEED MICROBIOTEST

1. The toxicity test with the duckweeds *Lemna minor* or *Lemna gibba* is presently the only standard method with a floating aquatic plant to assess the hazard of toxicants.
2. This assay is in regular use in the EU for the authorization of Plant protection products (herbicides and plant regulators – Reg EC N°1107/2009).
2. *Lemna* bioassays, besides their dependence on the culturing/maintenance of stocks, require substantial bench and incubation space and work, and are hence quite costly.
3. Extensive joint research by the authors of this poster with their associates has eventually led to the selection of a duckweed species which produces "dormant vegetative buds (turions)", and to develop a methodology for a practical and low cost "stock culture free" microbiotest with *Spirodela polyrhiza*, departing from stored and germinated turions.
4. The test procedure consists of 3 days germination of the turions in a Petri dish, followed by transfer of the germinated turions in the cups of a multiwell containing the toxicant concentrations. The areas of the first fronds are then measured by Image Analysis after 3 days incubation, followed by calculation of the percentage inhibition and the 72h EC50.



Spirodela polyrhiza

SENSITIVITY COMPARISON OF THE *LEMNA* ASSAY AND THE *SPIRODELA POLYRHIZA* MICROBIOTEST

A first sensitivity comparison was performed with 2 reference chemicals (KCl and 3,5 DCP; Tabl. 1), and subsequently with 18 inorganic and organic compounds (Fig. 1).
These comparisons revealed that the duckweed microbiotest was as sensitive as the "conventional" *Lemna* assay.

EC50	3,5 DCP	KCl
ISO *Lemna* test (acceptability range for the total number of fronds)	2,2 -3,8	5.000 – 10.000
Spirodela microbiotest (area of first frond)	3,3	5.200

Table 1

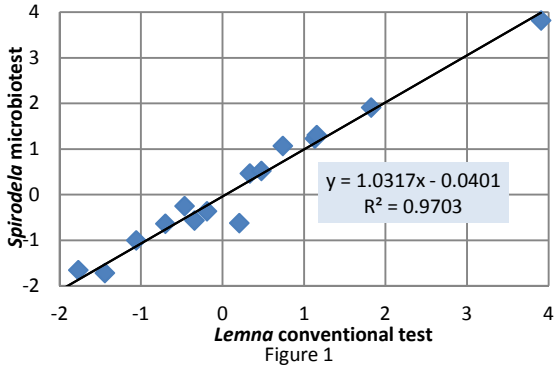


Figure 1

ORGANIZATION OF RINGTESTS

A preliminary ringtest with 6 laboratories from 6 countries has been performed on the reference chemical KCl and revealed a mean 72h EC50 of 6.593 mg KCl/l with a CV of only 6%. The test method was subsequently refined by also measuring the area of the first fronds at the start of the test (t0h), and calculation of the t72h-t0h growth, which was found to increase the sensitivity of the assay.
A second **International Interlaboratory Comparison** with the same reference chemical was launched in early 2014.
57 laboratories from 22 countries have participated in this ringtest and have submitted their data .
The data treatment is in progress and **the results confirm the simplicity of the "stock culture free" microbiotest, the rapidity of the measurements and the reliability of the test procedure.**

TEST PROCEDURE OF THE DUCKWEED *SPIRODELA* MICROBIOTEST