TOXKIT MICROBIOTESTS
as practical and low cost tools
for toxicity screening
and biomonitoring

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- Ghent University, Laboratory of Environmental Toxicology and Aquatic Ecology
- MicroBioTests Inc.
Conventional bioassays

or

Microbiotests?
Short-term « conventional » toxicity tests:

- 72 h Algal growth inhibition test
- 24-48 h Daphnia immobilisation test
- 96 h Fish mortality test
Culturing of micro-algae

Infrastructure
Space
Labour
Inoculation of test vessels with micro-algae

Incubation of test vessels

Daily counting of micro-algae

ALGAL TEST PROCEDURE
The waterflea *Daphnia magna*
Culturing of Daphnias

Daily maintenance

Daily feeding with micro-algae

Infrastructure
Space
Labour
Fish toxicity test
Fish culturing/maintenance facilities

Infrastructure

Space

Labour
Sediment contact tests

Stock culturing of the amphipod crustacean

*Hyalella azteca*
Burden of most toxicity tests:

Dependence of the (continuous) culturing/maintenance of live stocks of test species

Infrastructure  Space  Time  Costs
MICROBIO TESTS

as alternatives to

“conventional”

toxicity tests
Major characteristics of microbiotests  
(Blaise, 1991)

- Inexpensive or cost-effective
- Generally not labour intensive
- High sample throughput potential
- Cultures that are easily maintained or maintenance free
- Modest laboratory and incubation space requirement
- Low cost of consumables (e.g. test containers)
- Low sample volume requirements
at the Laboratory for Biological Research in Aquatic Pollution
(Ghent University – Belgium)

Basic approach:
Use of dormant or immobilized stages of selected aquatic organisms from which the test biota can be obtained “on demand”
TOXKIT microbiotests

Development of technology

» User friendliness
» Cost-effectiveness
» Miniaturization
DAPHTOXKIT F *magna*

- **Dormant egg**
- **Hatched neonates**
Sensitivity comparison

Daphtoxkit F magna / Conventional *Daphnia magna* test

Persoone 1998 Pure chemicals

Correlation coefficient

\[ R = 0.98 \]
Intra- and inter-laboratory sensitivity comparison studies
Daphtoxkit F magna/conventional *Daphnia magna* test
(1998-2007)

- Pesticides (Poland)
- Household products (Croatia)
- Waste leachates (Austria)
- Reference chemical and fly ash leachate (Slovak Republic)
- Chemical mixtures (Slovenia)
- Industrial effluents (UK)
- Industrial effluents (Flanders, Belgium)
- Reference chemical (Italy)
- Waste ringtest (EU)
QUALITY CONTROL TESTS
with the Dapthoxkit F magna on potassium dichromate (K₂Cr₂O₇)
performed by MicroBioTests Inc. from 2002 to 2006

Number of batches of dormant eggs : 21
Total number of tests : 164
Mean 24h EC50 : 1.15 mg/l (CV = 18.31 %)
Mean 48h EC50 : 0.79 mg/l (CV = 16.50 %)

Mean 24h EC50 : 1.12 mg/l (CV = 50 %)
CONCLUSIONS FROM ALL THE INTRA- AND INTER-LABORATORY COMPARISON STUDIES ON THE DAPHTOXKIT F MAGNA

1. The sensitivity of the young Daphnias obtained from dormant eggs is the same as that of Daphnias from laboratory cultures.

2. The Daphtoxkit F magna microbiotest is a well-validated low cost alternative to the conventional Daphnia magna test.
ALGALTOXXKIT F

Micro-algae immobilised in algal beads

Algal beads stored in tube

De-immobilised micro-algae

Long cells as test containers
Sensitivity comparison

Conventional algal test / Algaltoxkit F

Persoone 1998  Pure chemicals

Correlation coefficient

$R = 0.98$
- Waste leachates (Austria)
- Sediment pore waters (Flanders, Belgium)
- Reference chemical (Wallonia, Belgium)
- Reference chemical and fly ash leachate (Slovak Republic)
- Waste water treatment plant effluents (Denmark)
- Industrial effluents (UK)
- Industrial effluents (Flanders, Belgium)
- Waste ringtest (EU)
Objectives:

1. Determination of the “precision” (degree of standardisation) of the Algaltoxkit

2. Sensitivity comparison of the Algaltoxkit F microbiotest with the “conventional” algal toxicity test
Ring-test micro-algae 2006

Test method: ISO 147 – OECD 201
Test species: *Pseudokirchneriella subcapitata*
Test compound: reference chemical $K_2Cr_2O_7$
Number of participating laboratories: 33
Number of countries: 14
Number of tests performed:  
  Algaltoxkit: 42  
  Erlenmeyers: 6  
  Microplates: 8
QUALITY CONTROL TESTS
with the Algaltoxkit on potassium dichromate (K₂Cr₂O₇)
performed by MicroBioTests Inc. from 2002 to 2006

Number of batches of algal beads : 21
Total number of tests : 76
Mean 72h EbC50 : 0.46 mg/l (CV = 21.53 %)
CONCLUSIONS FROM ALL THE INTRA- AND INTER-LABORATORY COMPARISON STUDIES ON THE ALGALTOXKIT

1. The sensitivity of micro-algae de-immobilized from algal beads is similar to that of micro-algae from laboratory cultures

2. The Algaltoxkit microbiotest is a well-validated low cost alternative to the conventional algal test
Toxkit for sediment toxicity testing

The culture/maintenance free

OSTRACODTOXKIT

with the ostracod crustacean

*Heterocypris incongruens*

hatched from dormant eggs (cysts)

Developed specifically for *direct contact* tests
OSTRACODDTOXKIT F

6 days mortality and growth inhibition test
Battery of acute and short-chronic Toxkit microbiotests with various test species
WATER CONTAMINATION EMERGENCIES

Very rapid detection of toxic hazard
Bacterial luminescence inhibition tests
(Microtox – Lumistox – Checklight, etc…)

- very rapid (15-30 minutes)
- sensitive to many chemicals
- but not sensitive to all compounds
  (e.g. metals, biological toxins, etc)

Need for a « complementary »
(non-bacterial) rapid assay
The RAPIDTOXKIT microbiotest with larvae of the crustacean *Thamnocephalus platyurus* for detection of toxic hazard in 30-60 minutes.
The RAPIDTOXXKIT microbiotest in a nutshell

**Suspected water**

15-60 minutes exposure

Addition of red microspheres (15-30 minutes)

No particle uptake

**Control water**

15-60 minutes exposure

Addition of red microspheres (15-30 minutes)

Uptake of red particles

**Toxic hazard**

YES: Toxic hazard

NO: Not toxic
Each kit contains all the materials to perform 3 independent series of tests on 21 up to 45 water samples.

The Rapidtoxkit is presently already in use in various countries and has recently been evaluated by the USEPA in the framework of Anti-Terrorism Water Monitoring Technologies.
SOIL TOXICITY TESTING

Tests with invertebrates

Tests with plants
Soil toxicity tests with invertebrates

- Toxicity test with earthworms (14 days)
- Toxicity test with collemboles (28 days)

but no specific Toxkit microbiotest …
Application of the 6 days ostracod microbiotest for soil toxicity testing

1. Application of the ostracod solid phase microbiotest for toxicity monitoring of contaminated soils – Comparison with the springtail (Folsomia candida) test (published in 2003)

2. PAH degradation and ecotoxicity during a phytoremediation experiment (published in 2004)

3. Effects of PAH spiking on soil ecotoxicity during a bioremediation process (published in 2006)

4. Bioaugmentation and biostimulation effects on PAH dissipation and soil ecotoxicity under controlled conditions (published in 2007)

The Ostracodtoxkit is as sensitive as “conventional” soil bioassays
Soil toxicity tests with higher plants

- Several standard methods
- Various test species
- Exposure time up to 28 days
- Tests in pots (direct soil contact)
- Various endpoints

- Space
- Labor
- Time
Development of a practical and cost-effective phytotoxicity test

1. Flat test containers only allowing one-dimensional growth of the germinated seeds

2. Incubation of the test containers in vertical position

3. Automation of length measurements and data treatment by image analysis
Test procedure

1. Flat transparent test plate with cover (18 x 12 x 0.8 cm)
2. Filling of lower half of test plate with soil, and hydration
3. Placing of filter paper and seeds and closing of test plate with lid
4. Vertical incubation of test plates in holders
5. Shooting of picture of test plates with germinated seeds, with a webcam or a digital camera
6. Length measurements by image analysis
Test plate with germinated seeds after 3 days incubation ready for direct length measurements by image analysis
Each Phytotoxkit contains all the materials to perform a complete test on one soil in comparison to a reference soil, with 3 plant species and in 3 replicates.
Domains of application of the PHYTOTOXKIT microbiotest

Phytotoxicity analysis of:

- Soils
- Sludges
- Sediments
- Composts
- Effluents for irrigation
- Chemicals
- Biocides
- Phytosanitary products
PHYTOTOXKIT  ➔  For ecotoxicology

PHYTOTESTKIT  ➔  For agriculture

- Quality control of seeds
- Impact of soil fertilisers
- Dynamics of early growth of plants
Germination and early growth of *Sinapis alba*

Daily shooting of pictures of the test plates

Day 2  Day 3  Day 4
Application of Toxkit microbiotests

Chemical compounds  Surface waters
Groundwaters  Sediments
Biotoxins  Solid wastes
Waste waters  Composts
Soils  Sludges
Marine environments  Water contamination emergencies
Detoxification/ Bioremediation

Over 250 scientific papers on various applications listed on website www.microbiotests.be

More than 150,000 Toxkit tests performed worldwide to date
TOXKIT microbiotests

- Culture/maintenance free
- Miniaturized
- User-friendly
- Standardized
- Validated
- Highly reproducible
- Cost-effective

A practical and reliable tool, particularly suited for

Routine toxicity monitoring

Research
Field (in situ) analyses

- Toxicity of water
- Contamination of water by bacteria and biological residues

URGENT NEED FOR FIELD TESTS
Development of two low cost and user-friendly kits for ultra-rapid field analyses

Toxi-Screening Kit

Bacterial Contamination Screening Kit
Basic principle of the new field kits

*Luminescence measurement in a portable low cost luminometer*

Toxi-Screening Kit: *bacterial bioluminescence*

Bacterial Contamination Screening Kit: *ATP-chemical luminescence*
Development of a handy lightweight

“Luminescence Measurement Case
containing all the equipment and materials
1. **Test preparation**
Transfer 1 ml rehydration medium into vial with lyophilised bacteria

2. **Sampling**
Take a 1 ml water sample and transfer it in the sample tube
3. **Transfer of luminescent bacteria in control and sample tube:**

- **200 µl**
- Tube with rehydrated bacteria
- Control tube
- Sample tube

4. **Exposure**: 30 minutes
5. **Scoring** (at start and after 30 minutes exposure)

- Fix the tube in the holder
- Insert the tube + holder in the luminometer and close
- Score the luminescence (in Relative Light Units = \( RLU \))

6. **Data evaluation** : % toxicity = \( \frac{\Delta RLU \ (T0-T30) \ _{sample} \ - \ \Delta RLU \ (T0-T30) \ _{control}}{\Delta RLU \ (T0-T30) \ _{control}} \times 100 \)

**TOTAL TIME NEEDED FOR THE TEST** : 1 hour
1. Test preparation

Dipstick unit

Holder

Dipstick with cotton tip

Tube with 2 compartments with reagents

Remove the dipstick from the holder

Hydrate the dipstick with 50 µl water sample

Screening of Bacterial Contamination
2. Test performance

- Put the dipstick back in the holder
- Push the wet dipstick in the compartment containing ATP extractant
- Push the dipstick further down into the compartment containing ATP reagent
3. **Test scoring**

Insert the dipstick unit in the luminometer and close.

Score the luminescence (in Relative Light Units = RLU).

4. **Evaluation of bacterial contamination**

- $<100 \text{ RLU} = \text{low bacterial contamination}$
- $>1000 \text{ RLU} = \text{substantial bacterial contamination}$

**TOTAL TIME NEEDED FOR THE TEST:** <5 minutes
Verification of bacterial contamination in larger water samples with prior elimination of extracellular ATP

Take a 10 ml water sample with the syringe

Put the syringe on the mini-holder containing a 0.45 µ membrane filter

Flush the water through the filter
Separate the tube with reagents from the dipstick unit

Remove the sealed cover of the tube with the pincette

Take the membrane filter out of the holder with the pincette

Reassemble the dipstick unit

Submerge the membrane filter in the ATP extracting medium for 1 minute
Push the dipstick into the bottom compartment with ATP reagent

Insert the dipstick unit in the luminometer and close

Score the luminescence
(in Relative Light Units = RLU)
A breakthrough for field testing of suspected waters on their toxicity and/or bacterial contamination

Ultra-rapid
User-friendly
Low cost
Time out

Thank you for your attention!