13th International Symposium onf Toxicity Assessment

Toyama, Japan 2007

Ring-testing and Quality Control testing in Ecotoxicology :

State of the art with Toxkit microbiotests

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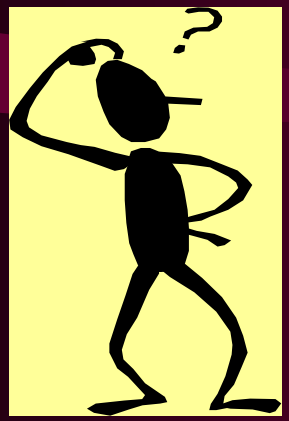
Various toxicity tests have been endorsed by national and international organisations (*DIN, AFNOR, DTA… OECD, ISO, EPA, ASTM…*)

Bacteria

Micro-algae

Daphnia

Fish



But have all "standard" tests been submitted to ring-testing to determine :

a) If they are really standardised from the **methodological** point of view ?

b) If they are practicable from the **technical** point of view ?

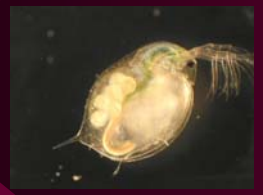
c) If the results of **reference tests** obtained by different labs are "sufficiently" similar ?

d) If their **costs** are "acceptable" for repeated applications ?

ISO norms for the 4 most commonly applied aquatic toxicity tests

ISO 11348 1,2,3 Bacterial luminescence inhibition test
Ring-tests in 1991-1993 (with 16-22 laboratories)

ISO 147 – Freshwater algal growth inhibition test
Ring-tests in 1980-1981 (with 9- 20 laboratories)

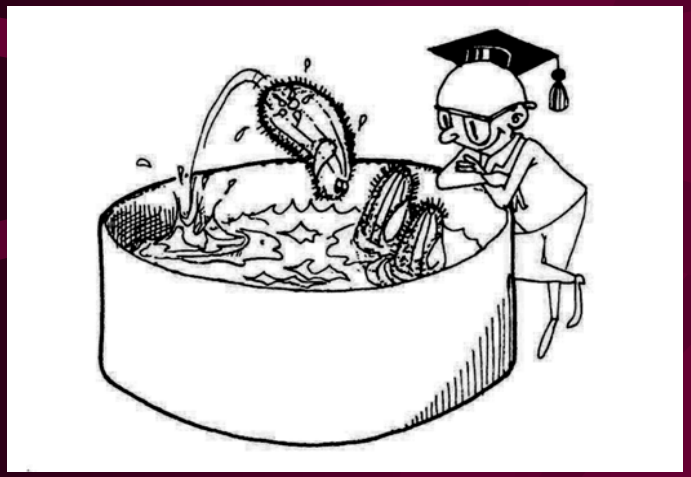


ISO 6341- Acute Daphnia test
Data collection in 1994 (from 36 laboratories)

ISO 7346 – Acute fish test
No ring-test

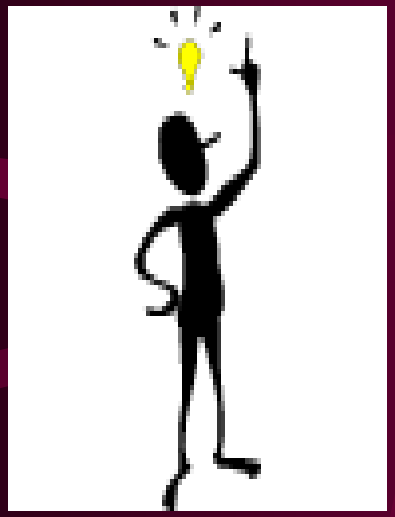
Burden of most toxicity tests :

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



Infrastructure **Space** **Time** **Costs**

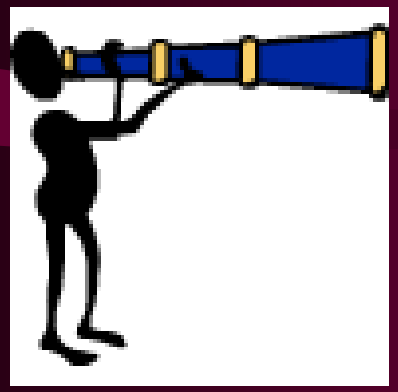
CULTURE FREE MICROBIOTESTS



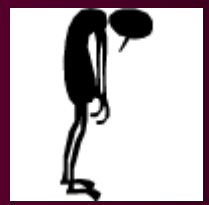
as alternatives to

“conventional”

toxicity tests



Development of cost-effective culture/maintenance free microbiotests

With bacteria	☺	**Microtox**
With micro-algae	☺	**Toxkits**
With invertebrates	☺	**Toxkits**
With fish	☹	

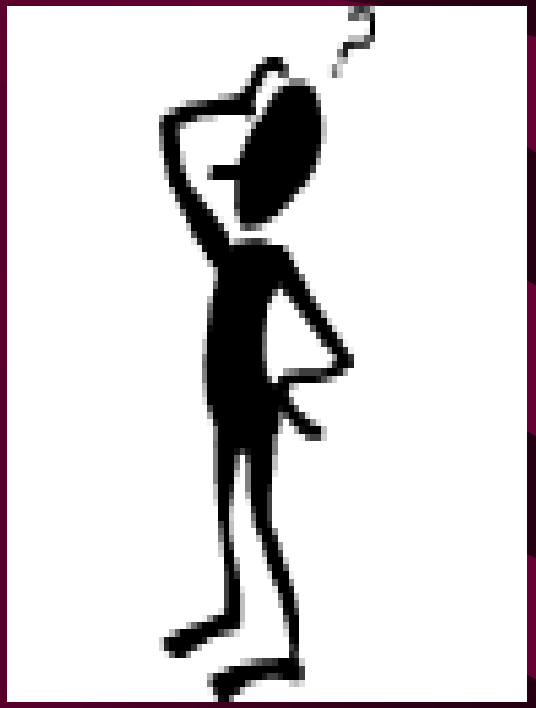
MICROBIOTESTS

Degree of standardisation ?

Sensitivity ?

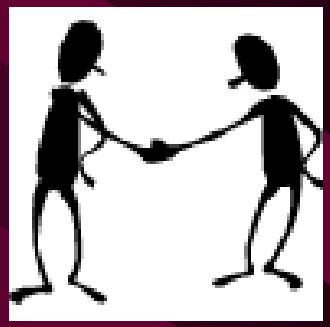
Precision ?

Ring-testing ?



The MICROTOX bacterial luminescence inhibition test with freeze-dried *Vibrio fischeri*

- 13 ring-tests have been organised yearly since 1993 by the Environmental Toxicology Laboratory of the Technical University of Catalonia in Spain



- 31 laboratories participated in the ring-test 2005
- Overall CV = 27 %

TOXKIT microbiotests

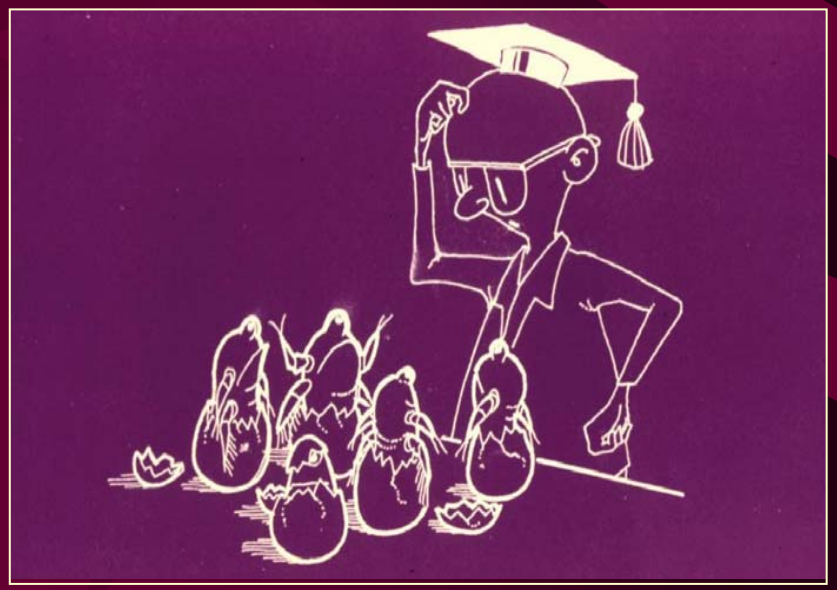
Basic approach :

Use of dormant or immobilized stages

of selected aquatic organisms

from which the test biota

can be obtained "on demand"



DAPHTOXKIT F *magna*

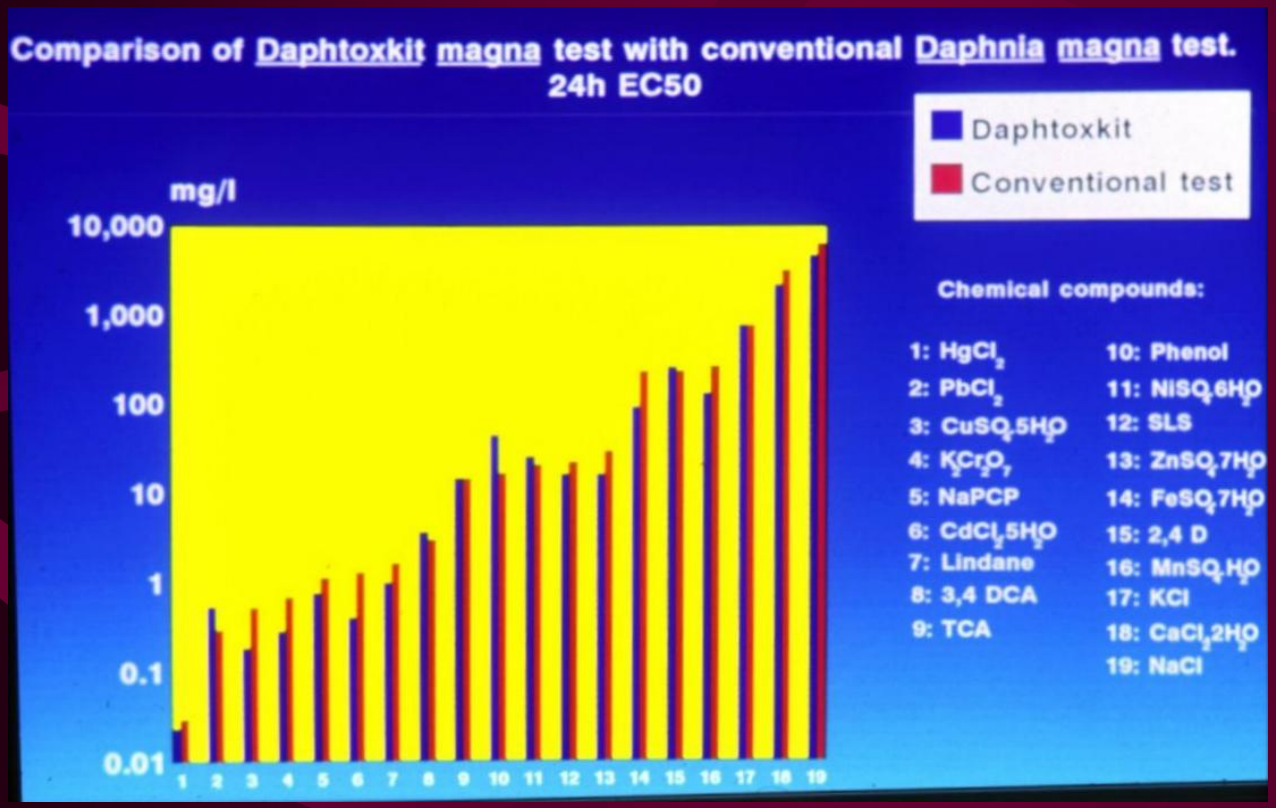
Hatched neonates

Dormant egg

Sensitivity comparison

Conventional *Daphnia magna* test / Daphtoxkit F *magna*

Persoone 1998 **Pure chemicals**



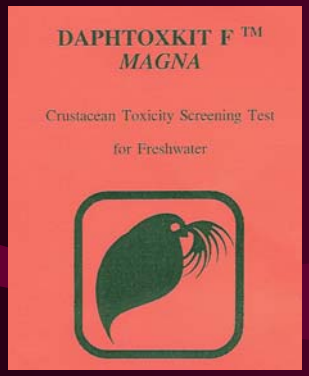
Correlation coefficient

R = 0.98

Intra- and inter-laboratory sensitivity comparison studies Daphtoxkit F magna/conventional *Daphnia magna* test (1998-2005)

- 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)

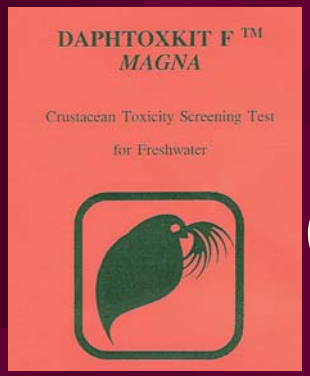
2003 and 2005



76

43

105 labs !



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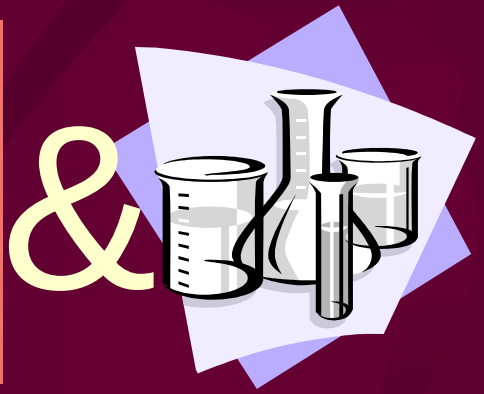
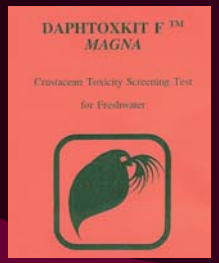


14

2005 Results

for potassium dichromate



n = 40, 24h EC50 = **1.20 mg/l**

within-lab variability 9.9 % ; between-lab variability **23.9 %**

n = 33, 24h EC50 = **1.01 mg/l**

within-lab variability 8.5 % ; between-lab variability **34.3 %**

No stastistical difference !

ISO International Organization for Standardization

6341/98

n = 36, 24h EC50 = **1.12 mg/l**

within-lab variability 5 % ; between-lab variability **50 %**

QUALITY CONTROL TESTS

with the Dapthoxkit F magna

on potassium dichromate ($K_2Cr_2O_7$)

1. Performed by MicroBioTests Inc – Belgium (from 2002 to 2007)

164 tests with Daphnias from 21 batches of dormant eggs

Mean 24h EC50 : 1.15 mg/l (CV = 18.31 %)

2. Performed by Grupo Interlab – Spain (from 1997 to 2007)

63 tests with Daphnias from 34 batches of dormant eggs

Mean 24h EC50 : 1.05 mg/l (CV = 28.59 %)

No stastistical difference !

Mean 24h EC50 : 1.12 mg/l (CV = 50 %)

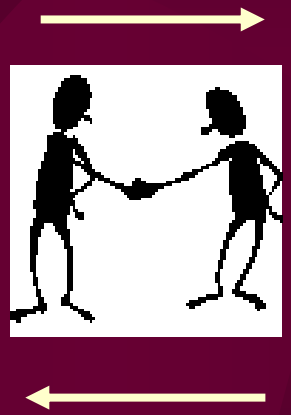
ISO International Organization for Standardization

6341/98

CONCLUSIONS FROM ALL THE INTRA- AND INTER-LABORATORY COMPARISON STUDIES ON THE DAPHTOXKIT F MAGNA

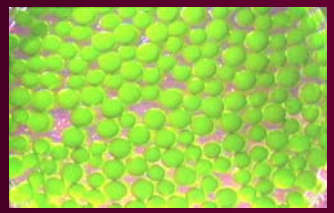
The sensitivity of the young Daphnias obtained from dormant eggs is the same as that of Daphnias from laboratory cultures

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



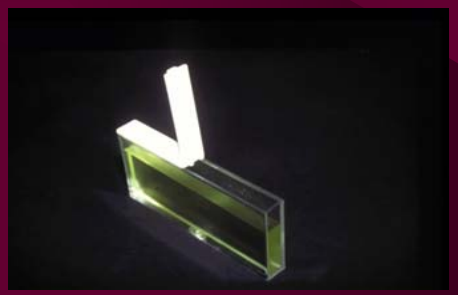
ALGALTOXKIT F

Micro-algae immobilised in algal beads



Algal beads stored in tube

De-immobilised micro-algae

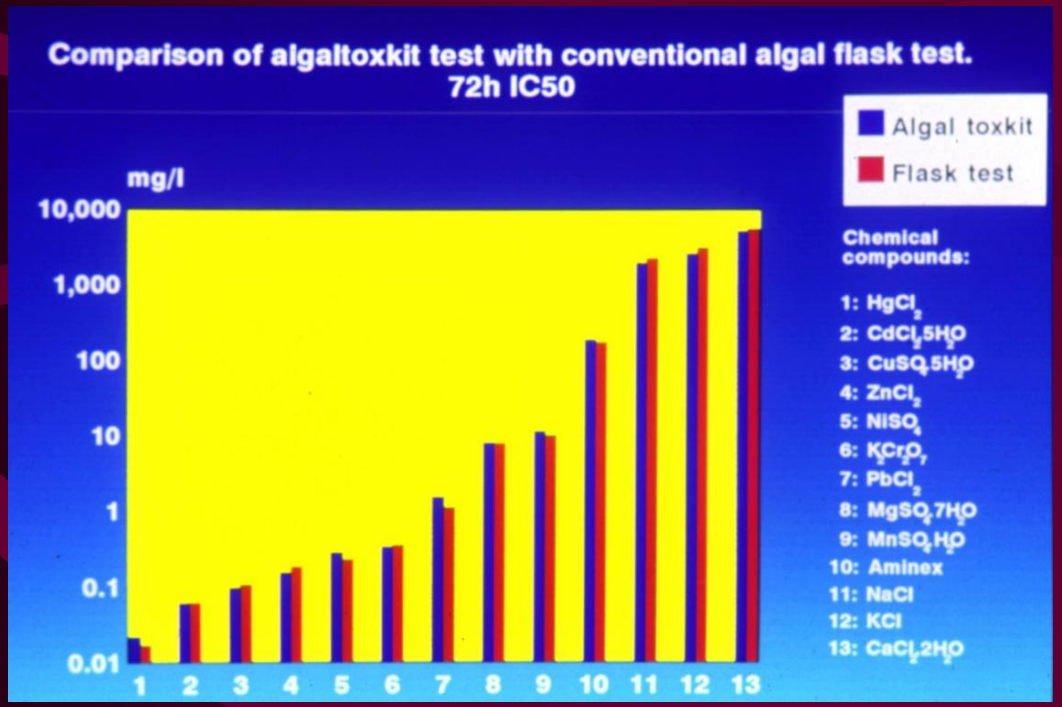


Long cells as test containers

Sensitivity comparison

Conventional algal test / Algaltoxkit F

Persoone 1998 **Pure chemicals**



Correlation coefficient

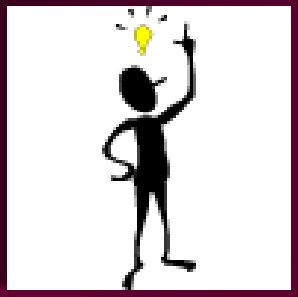
R = 0.98

Intra-laboratory sensitivity comparison studies Algaltoxkit F/conventional algal assay (1998-2005)

- 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)
- **No ring-test**

ISO ring-test on micro-algae :

25 years ago (1980-1981)



INTERNATIONAL RING-TEST ON THE ALGALTOXKIT F MICROBIOTEST (2006)

Organizer : *Laboratory of Environmental Toxicology and Aquatic Ecology – Ghent University, Belgium*

Objectives :

1. Determination of the "precision" (degree of standardisation) of the Algaltoxkit
2. Sensitivity comparison of the Algaltoxkit F with the "conventional" algal toxicity test

Ring-test micro-algae 2006

Test method : **ISO 147**

Test species : *Pseudokirchneriella subcapitata*

Test compound : reference chemical $K_2Cr_2O_7$

Number of participating laboratories : **33**

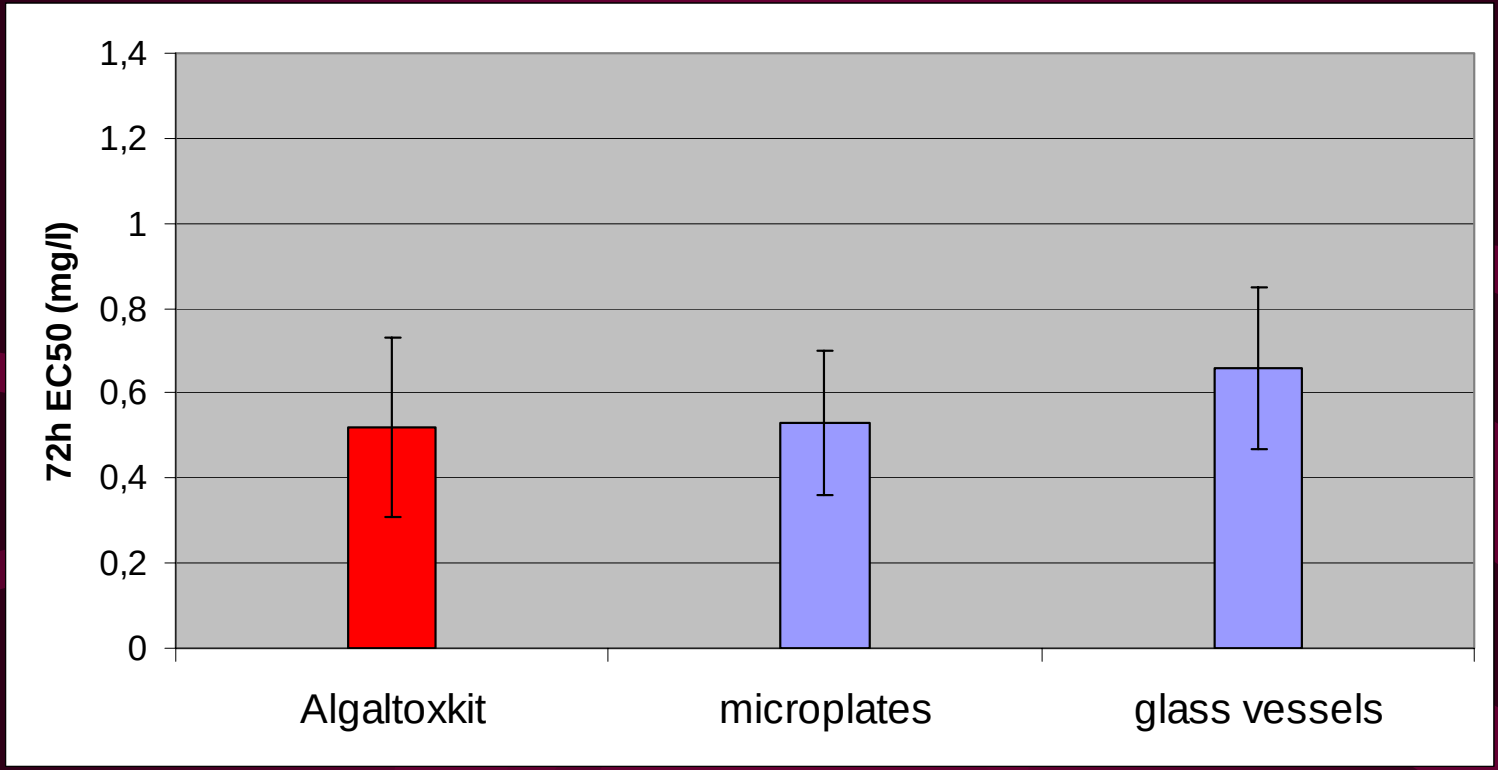
Number of countries : **14**

Number of tests performed : Algaltoxkit : 42

Erlenmeyers : 5

Microplates : 8

Comparison of the results of the Algaltoxkit with those obtained in micoplates and in Erlenmeyers



Mean 72h EbC50 Algaltoxkit : 0.52 mg/l (**CV : 40 %**)

Mean 72h EbC50 Microplates : 0.53 mg/l (**CV : 33 %**)

Mean 72h EbC50 Erlenmeyers : 0.66 mg/l (**CV : 29 %**)

QUALITY CONTROL TESTS

with the Algaltoxkit on potassium dichromate ($K_2Cr_2O_7$) performed by MicroBioTests Inc. (from 2002 to 2007)

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

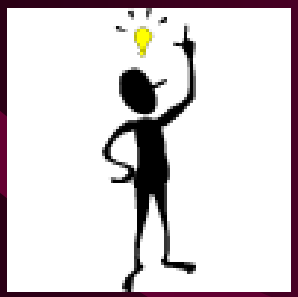
The sensitivity of micro-algae de-immobilized from algal beads is similar to that of micro-algae from laboratory cultures

The Algaltoxkit microbiotest is a well-validated low cost alternative to the conventional algal test

EU Ecotox Waste Ringtest 2006-2007

Tests on waste eluates : Bacterial luminescence inhibition test
Algal growth inhibition test
Daphnia acute test

Tests on solid wastes : Earthworms acute test
Plant test



Additional or alternative test methodologies also welcome

The following Toxkit microbiotests have been performed on the wastes in the framework of the EU ringtest :

Algaltoxkit F

Daphtoxkit F

Thamnotoxkit F

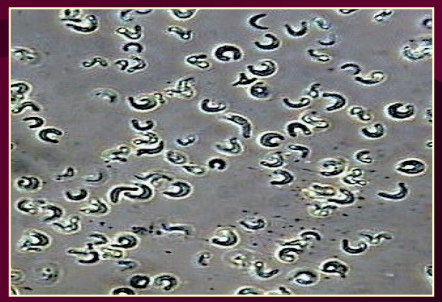
Chronic Rotoxkit F

Phytotoxkit

Toxkit data were received from 14 laboratories in 8 European countries

Name of laboratory	*Abbreviation*	*Number of different Toxkit tests performed*
MicroBioTests Inc. (Belgium)	MBT	5
Institut Provincial d'Hygiene et de Bactériologie (Belgium)	IPHB	2
EPAS (Belgium)	EPAS	2
AlControl Laboratories (United Kingdom)	ALC	4
Agenzia per la Protezione dell'Ambiente Tuscany (Italy)	ARPAT	1
Agenzia per la Protezione dell'Ambiente Grosseto (Italy)	ARPAG	1
Instituto do Ambiente (Portugal)	IDA	2
Mälardalen University (Sweden)	MALU	3
Technische Universität Braunschweig (Germany)	TUB	2
Insavalor-Polden (France)	POLD	1
INERIS (France)	INER	1
IRH Environnement (France)	IRH	1
Laboratoire Santé Environ. Hygiene de Lyon (France)	LSEH	1
Grupo Interlab (Spain)	GRINT	1

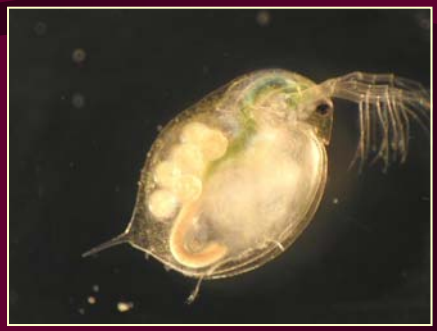
72h ErC50's of the tests on micro-algae



Incineration waste	Convent.tests	Algaltoxkit
	(n = 15)	*(n = 5)*
Mean	6.9 %	2.5%
St.dev.	9.7	1.9
CV%	141 %	77 %

Waste wood	Convent.tests	Algaltoxkit
	(n = 12)	*(n = 5)*
Mean	0.27 %	0.24 %
St.dev.	0.16	0.10
CV%	58 %	42 %

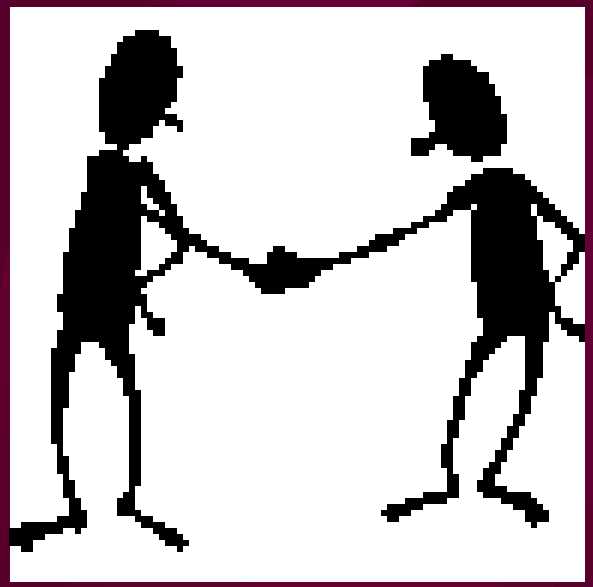
24h EC50's of the tests on *Daphnia magna*



Incineration waste		
	Convent.tests	Daphtoxkit
	(n = 37)	*(n = 7)*
Mean	2.85 %	2.51 %
St.dev.	1.10	0.92
CV%	39 %	37 %

Waste wood		
	Convent.tests	Daphtoxkit
	(n = 47)	*(n = 10)*
Mean	0.51 %	0.62 %
St.dev.	0.36	0.19
CV%	69 %	31 %

Overall, the results of the Toxkit assays in the EU waste ringtest also clearly revealed that the microbiotests are reliable and cost-effective tools in a test battery, for the determination of the hazard of solid wastes.

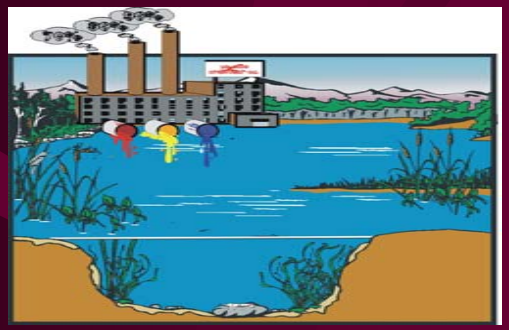


Microbiotests

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

A practical and reliable tool, particularly suited for

Routine toxicity monitoring



Research

Time out

Thank you
for your attention !