

REFERENCES



Client ID : TOROBALL
Description : Paintball•
Comment :

TORO Distribution
62 rue Armand Barbes
76300 SOTTEVILLE-LES-ROUEN
ØÜÆÒÒ

Rouen, February 19th, 2010

Report
RN10-01225.001 Page 1 / 13

Parameter :

**DETERMINATION OF MOBILITY INHIBITION WITH DAPHNIA
- ACUTE TOXICITY ESSAY -**

NF EN ISO 6341 - OCDE 202

Principle: Determination of the sample concentration, in which a substance is lethal for 50% of a trial population of *Daphnia magna*, after periods of exposure in this substance of 48 hours without renewal (EC50-48h).

Reference chemical for this test is potassium dichromate:

Test organism: *Daphnia magna* Straus (*Cladocera*, *Crustacea*) from acyclic parthenogenesis in laboratory farming condition and under 24 hours old.

Sample:

- TOROBALL
- Conservation: room temperature
- Preparation of tests solutions: shaking sample in test media during 24h, so filtration and dilution

Essay dates: from 10/02/09 to 10/02/11

Results :

TOROBALL respects the NF EN ISO 6341 - OCDE - 202 standards to non micro-organism toxicity. (see results page 2).

Mobility inhibition with *Daphnia magna*

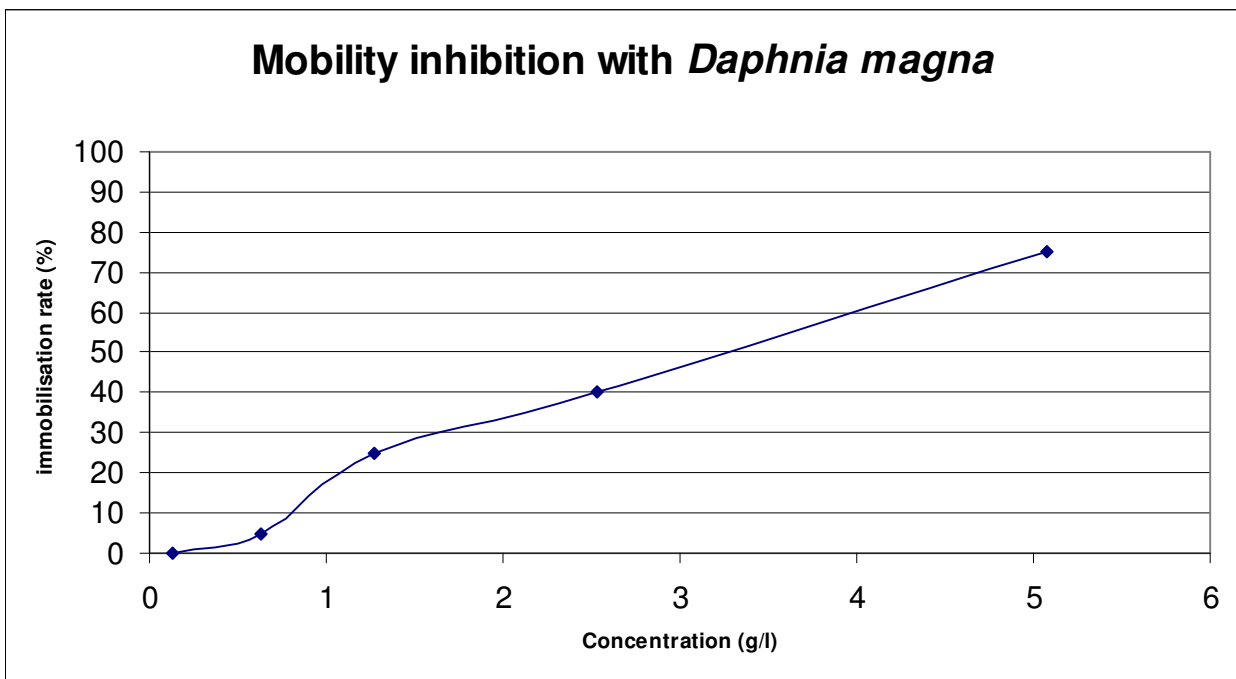
Sample :
RN10-01225.001

Essay date :
du 09 au 11/02/10

Concentrations g/l	total immobilisation per 5 in each replicate				Total immobilisatio	Rate immobilisatio	pH	O2 dissolved mg/l
	A	B	C	D				
control	0	0	0	0	0	0	7,8	8,7
0,1268	0	0	0	0	0	0	-	-
0,634	0	0	1	0	1	5	-	-
1,268	2	1	1	1	5	25	-	-
2,536	2	2	3	1	8	40	-	-
5,072	4	4	4	3	15	75	-	8

Confidence limits at 95%

EC _{50-48h} (g/l) :	2,80	2,39	3,21
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Parameter :**ALGAL GROWTH INHIBITION WITH
*Pseudokirchneriella subcapitata*****NF EN ISO 8692 - OCDE 201**

Principle: Determination of the sample concentration, in which a substance inhibit 50% of the cellular growth rate of *Pseudokirchneriella subcapitata*, after periods of exposure in this substance of 72 hours without renewal (ECi50-72h).

Reference chemical for this test is potassium dichromate.

Test organism: Exponential growing algae cultivated in our laboratory. Strain from S.A.G strain banck (Göttingen).

Sample :

- TOROBALL
- Conservation: room temperature
- Preparation of tests solutions: shaking sample in test media during 24h, so filtration and dilution

Incubation: Thermostatic incubator with continuous lighting (7000Lux)

Essay dates: from 10/02/12 to 10/02/15

Results :

TOROBALL respects the **NF EN ISO 8692 - OCDE 201** standards to non algal toxicity.
(see results pages 4-5 & 6).

Date : 12 au 15/02/2010

sample : RN10-01225.001

concentrations	Mesure D0	Mesure D3	Growth rate	Growth inhibition
Time (hours)	0	72		
Control	10000	5404000	0,0874	
	10000	5404000	0,0874	
	10000	5404000	0,0874	
	10000	5404000	0,0874	
	10000	5404000	0,0874	
	10000	5404000	0,0874	
Average	10000	5404000	0,0874	
C1	10000	3972000	0,0831	4,89
	10000	3972000	0,0831	4,89
	10000	3972000	0,0831	4,89
Average	10000	3972000	0,0831	4,89
C2	10000	1020000	0,0642	26,50
	10000	1020000	0,0642	26,50
	10000	1020000	0,0642	26,50
Average	10000	1020000	0,0642	26,50
C3	10000	868000	0,0620	29,06
	10000	868000	0,0620	29,06
	10000	868000	0,0620	29,06
Average	10000	868000	0,0620	29,06
C4	10000	56000	0,0239	72,62
	10000	56000	0,0239	72,62
	10000	56000	0,0239	72,62
Average	10000	56000	0,0239	72,62
C5	10000	10000	0,0000	100,00
	10000	10000	0,0000	100,00
	10000	10000	0,0000	100,00
Average	10000	10000	0,0000	100,00

	g/l
C1	0,1268
C2	0,634
C3	1,268
C4	2,536
C5	5,072

ALGAL GROWTH INHIBITION

Sample :	RN10-01225.001	Date :	from 10/02/12 to 10/02/15
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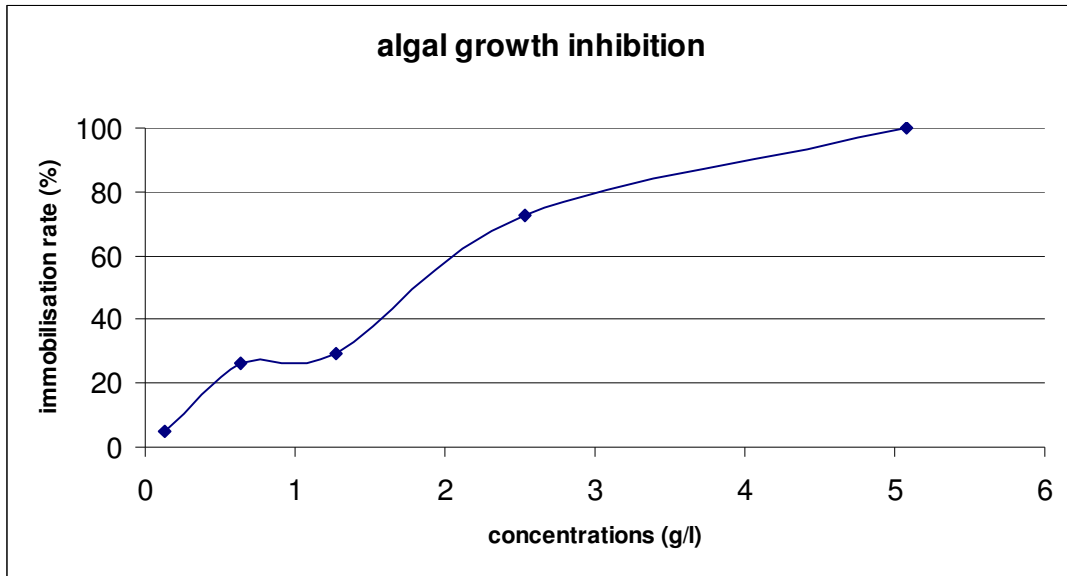
	Control	C1	C2	C3	C4	C5
pH D0	8.1	8.12	8.02	7.43	7.42	7.48
pH D3	8.27	9.27	8.01	7.85	7.61	7.62

	observed	reference		Conformity
Growth factor in control vessel	540.40	>	67	C
Relative Standard Deviation :	0,00	<	5,00%	C
pH Variation in control vessel :	0,17	<	1,5	C
Exposure luminosity (lux) :	7000,00	6000	10000	C
Temperature in incubator (°C) :	24	21	25	C
Growth factor of inoculum :	160,00	>	67	C
Sensibility (K2Cr2O7 mg/l) :	1,90	0,92	1,46	C
Visual check for algae purity :	ok			C
Date of preparation of the media :	10/02/12			C
Sample preparation :	filtration 0,45 μ			C

Calculation for EC 50 and EC 10 in mg/l

		Confidence limits (95%)	
EC 50 g :	1.61	0.81	2.41

g : growth



Parameter :**FISH, ACUTE TOXICITY TEST WITH
*Brachydanio rerio*****NF EN ISO 7346-1 - OCDE 203**

Principle : Determination in the conditions of the essay of the concentrations in which a substance is lethal for 50 % of a trial population of *Brachydanio rerio* (Zebra fish), after periods of exposure in this substance of 24 hours, 48 hours, 72 hours, 96 hours without renewal.

Test Organism: Zebra fish (*Brachydanio rerio*) resulting from the same origin, from the size included between 2.5 and 3 cms and maintained in breeding in the laboratory more than fortnight without any loss.

Sample :

- TOROBALL
- Conservation: room temperature
- Preparation of tests solutions: shaking sample in test media during 24h, so filtration and dilution

Essay dates: from 10/02/05 to 10/02/09

Results :

TOROBALL respects the **NF EN ISO 7346-1 - OCDE 203** standards to non fish toxicity.
(see results page 8).

Essays date	hour of beginning
from 10/02/05 to 10/02/09	10h00

total of dead fish per 7 during exposure

	C(g/l)	24h	48h	72h	96h
control (dilution water)	0	0	0	0	0
dilution 1	1,268	0	0	0	0

EC50 (g/l)	>1,268
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Parameter:

**Inherent aerobic biodegradability :
Zahn-Wellens Test
NF EN ISO 9888 - OCDE 302 B**

Principle: A mixture containing the test substance, mineral nutrients and a relatively large amount of activated sludge in aqueous medium is agitated and ventilated at 20-25°C in the dark or under diffuse light for up to 28 days. The biodegradation process is monitored by determination of DOC (or COD) in filtered samples taken at daily or other time intervals.

Inoculums: Activated sludge from Rouen wastewater treatment plant. Microbial material recovered after centrifugation and re-suspension in the test media. The rate of inoculums (suspended materials) with test substance (DOC) is between 2.5/1 and 4.0/1 in final test vessel.

Sample:

- TOROBALL
- Conservation: room temperature

Essays date :

D=0 : 10/02/03
 D=1 : 10/02/04
 D=2 : 10/02/05
 D=5 : 10/02/08
 D=6 : 10/02/09
 D=7 : 10/02/10
 D=9 : 10/02/12
 D=15 : 10/02/18

TOROBALL respects the **NF EN ISO 9888 - OCDE 302 B** standards to inherent biodegradability.
 TOROBALL is 100% biodegradable after 9 days.
 (see results pages 10-11 & 12).

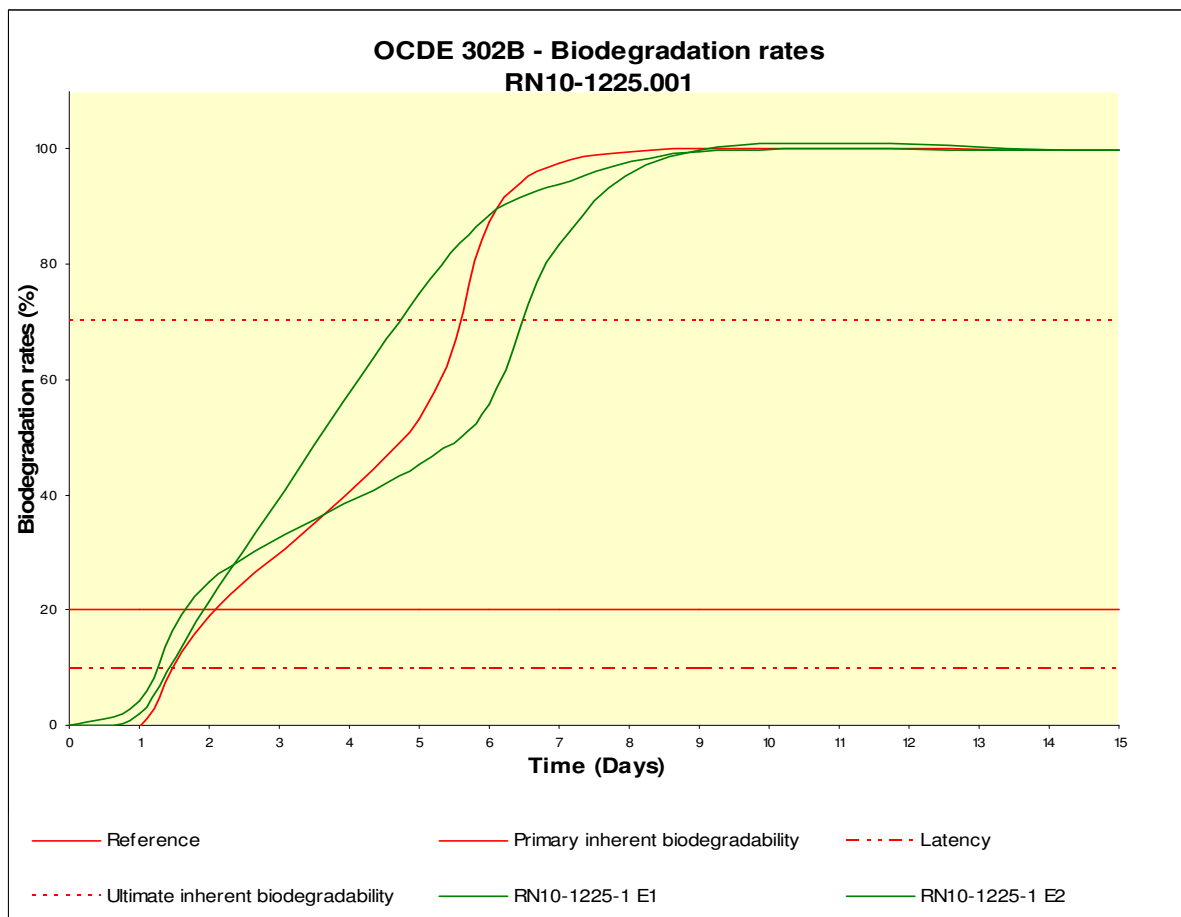
Results :

Biodegradation rate in percent according to time:

	J = 0	J = 1	J = 2	J = 5	J = 6	J = 7	J = 9	J = 15
Reference control	0	0	19	63	87	97	100	99
RN10-01225.001 E1	0	4	25	46	67	83	99	99
RN10-01225.001 E2	0	2	22	75	88	94	99	99

E1 and E2 are two different concentrations at D=0.

Tests are realized in two replicates with a difference between results lower than 20% according to validity criteria of OECD guidelines.



Remark: Not adsorption on activated sludge

Latency time (a) is less than 2 day
Biodegradation time (b) is 7 days
Maximum biodegradation level (c) is above 99 %

- a) Duration from the beginning of inoculation to the moment when the rate of degradation has reached 10%
- b) Interval between the end of latency time and the moment when approximately 90% of the maximum rate of biodegradation is reached.
- c) Approximate value from which there are more biodegradation during the test

Comments:

The reference is a substance known for its ability to be biodegraded and validate the whole experience (it gets 97% of degradation in 7 days, the experience was realised within favourable conditions).

The OECD guidelines have set a threshold of 20% biodegradation beyond which the substance demonstrates a primary inherent biodegradability and a 70% threshold beyond which the substance demonstrates an ultimate inherent biodegradability. For this analysis, there is a biodegradation of the test substance above 99% in 9 days.

Sample **TOROBALL** is « **inherent biodegradable without pre-adaptation** » according to OECD criteria extrapolated to a finish product.

OECD guidelines extract (March, 23rd 2006) (pure chemicals):

When the results indicate that inherent, ultimate biodegradability does occur, it indicates that the substance has a potential for degradation under favourable conditions, e.g. in well-operated STPs.

Inherent biodegradability tests are used to assess whether a chemical has any potential for biodegradation. The European Commission Technical Guidance Document (5) proposes that results of the Zahn-Wellens/EMPA Test (TG 302 B) and the Modified MITI Test (II) (TG 302 C) may be used for extrapolation to a rate constant in models for estimation of the elimination of chemicals in STPs (5). However, this extrapolation is only allowed, if the inherent biodegradability tests fulfil specific criteria.

-The pass level of 70% degradation in the Zahn-Wellens/EMPA Test must be reached within 7 days, including the lag-phase and the log-phase.

-The lag-phase should be no longer than 3 days.

-The percentage removal in the test before biodegradation occurs should be below 15%.

When a negative result is obtained in a test of inherent biodegradability, it may lead to a preliminary conclusion of environmental persistence and to an evaluation of potential adverse effects of transformation products.

RESULTS

Test	Biological material	Method	Data	Results
<i>Daphnia</i> sp., Mobility inhibition	<i>Daphnia magna</i>	OECD 202	EC50-48h	2,8 g/L
Algal growth inhibition	<i>Pseudokirchneriella subcapitata</i>	OECD 201	EC50-72h	1,61mg/L
Fish mortality test	<i>Brachydanio rerio</i>	OECD 203	LC50-96h	>1,268 g/L
Ultimate inherent biodegradability	Micro-organisms	OECD 302B	Biodegradation rate	>99% in 9 days inherent biodegradable without pre-adaptation

According to 67/548/CEE directive (EC50 above 100mg/l for daphnia, algae and fish), this product can be regarded as **non dangerous for aquatic environment**.

EC50-48h: Concentration with 50% effect in 48 hours (daphnia immobilisation)

EC50-72h: Concentration with 50% effect in 72 hours (algal growth)

LC50-96h: Concentration with 50% mortality in 96 hours (fishes)

Results validated electronically by **Aymeric BELLEMAIN**
 Project Manager

This validation is an electronic signature realised in conformity with requirements of ISO 17025.