Test for Ready Biodegradability of Products

1	**Sample designation:**	**„Dryflex 1“**
1.1	Sample received on:	25-Apr-2008
1.2	Storage conditions:	room temperature
2	**Sponsor:**	Drytech Italia s.r.l.
2.1	Address:	Via Ravona 1H, I-20220 San Fermo, Italy
3	**Test facility:**	SGS INSTITUT FRESENIUS GmbH
3.1	Address:	Im Maisel 14, D-65232 Taunusstein-Neuhof; Germany
3.2	Study director:	Dr. H. Lebertz
4	**Test method:**	OECD-Guideline 301 B (CO_2 Evolution Test)
4.1	Inoculum:	Filtrate of homogenised activated sludge from the waste-water treatment plant at Taunusstein-Bleidenstadt; Lot-No.: 27-May-2008
4.2	Application:	28 days
4.3	Detection method:	CO_2-measurement
5	**Control:**	Sodiumbenzoate, ≈20 mg/L TOC

6 Definitions:

TOC: total organic carbon

TCO_2: theoretical amount of CO_2 which may be developed from the test item (expressed as mg CO_2 / g test item). This value is calculated from the carbon content of the test item and the relation of molar masses of CO_2 (= 44.01) and carbon (= 12.01).

$ThCO_2$: theoretical amount of CO_2, which may be developed from the test item within the whole test solution (= 3.5 L).

7 Method description:

The test item and the polyvalent inoculum from an activated sewage plant dealing predominantly with domestic sewage are incubated together in a mineral nutrient medium at a temperature range of 19-25 °C. The test item is the sole carbon and energy source. The test solutions are aerated with CO_2-free compressed air and are stirred on a magnetic stirrer. When the test material is mineralised it is converted to CO_2 which is trapped in a system of gas-washing bottles into bariumhydroxide. The CO_2 is quantified by titration of the remaining bariumhydroxide with HCl. Comparing the amount of CO_2 produced upon degradation of the test item with the theoretical amount of CO_2 ($ThCO_2$) the percentage of degradation is calculated. Two Blank controls with inoculum but without any test item are run in

parallel in order to determine the amount of CO_2 derived from the inoculum. The mean of these values have to be subtracted from those values determined for the test solutions with the test item. At the end of the test the reaction in the test solutions is stopped by addition of 1 mL HCl conc. to each of the test solutions, and inorganic carbonates are made volatile. Aeration with CO_2 -free compressed air is continued for another 1 day in order to purge the remaining CO_2 off the test solutions. Two test solutions with the test item are tested in parallel at ≈10 mg/L TOC. The test duration normally is 28 days (+ 1 day purging off the dissolved CO_2 from the test solutions after acidification of the test solutions).

8 Evaluation

$$\% TCO_2 = \frac{\text{mg } CO_2 \text{ produced} \times 100}{(\text{mg Test item in the test solution}) \times (TCO_2)} = \% \text{ Degradation}$$

9 Test report

The test solutions were prepared according to the OECD guideline 301 B.

Carbon content of the test item (calculated from TOC-measurement): **172.9 mg C / g Test item**[1)]

Relation molar masses of CO_2 : C = 44.01 : 12.01 = 3.667

Resulting: TCO_2 = 633.58 mg CO_2 / g Test item

Volume of test solutions: 3500 mL

Amount of test item within:

Test solution 1: 226.7 mg / 3500 mL

$ThCO_2$ = 143.63 mg CO_2/ 3500 mL Test solution

Test Solution 2: 226.6 mg / 3500 mL

$ThCO_2$ = 143.57 mg CO_2/ 3500 mL Test solution

[1)] This measurement was performed at "Analytische Laboratorien Prof. Dr. Malissa und G. Reuter GmbH" being also accredited according DIN EN ISO 17025

Amount of control item sodiumbenzoate within:

Test Solution 3: 120.9 mg / 3500 mL Test solution

$ThCO_2$ = 257.67 mg CO_2 / 3500 mL Test solution

Amount of test and control items in the toxicity control in:

Test solution 4: 227.7 mg test item/ 3500 mL test solution;
+121.1 mg sodiumbenzoate/ 3500 mL test solution
$ThCO_2$ = 402.36 mg CO_2 / 3500 mL test solution

10 Results

The results are given in table 1 and 2, and a plot of the degradation curve is included in the report.

10.1 Total-CO_2-Evolution of the Blank: 89.3 mg CO_2 / 3.5 L in 28 d (+ 1d purging off the dissolved CO_2 from the test solutions after acidification of the test solutions)

10.2 Control: The Control was degraded 91% within 28 days. The threshold of "ready biodegradability" of ≥ 60 % was passed within 8 days (74%).

10.3 Test item: The threshold of "ready biodegradability" of ≥ 60 % was met within both test solutions. The mean value of 80% is higher than the pass value of the OECD Guideline 301B. The "10-Days-Window" was met,too. Thus, the test item **„Dryflex 1"** may be termed "readily biodegradable". There was no toxic effect towards microorganisms at the concentration tested.

SGS Institut Fresenius GmbH
- Bioanalytics -
D-65232 Taunusstein, date: 26-Jun-2008

i.V. (Dr. Weyandt)

i.V. (Dr. Lebertz)

Results of the Study on the Biodegradability according to CO_2 Evolution-Test, OECD 301 B

Test item: **„Dryflex 1“**
Table 1: Test Solution 1 with the test item

Time	Concentration: 226.7 mg / 3.5 L $ThCO_2$: 143.63 mg CO_2/ 3.5 L	
	mg CO_2 produced in the Test solution, cumulative	% TCO_2 (= % Degradation)
2d	0.45	<1
5d	35.64	25
8d	74.25	52
12d	87.20	61
16d	98.53	69
19d	106.73	74
23d	111.52	78
28d	114.90	80
29d	118.41	82 [1)]

Table 1b: Test Solution 2

Time	Concentration: 226.6 mg / 3.5 L $ThCO_2$: 143.57 mg CO_2/ 3.5 L	
	mg CO_2 produced in the Test solution, cumulative	% TCO_2 (= % Degradation)
2d	0.37	<1
5d	38.73	27
8d	72.28	50
12d	84.35	59
16d	94.71	66
19d	102.09	71
23d	106.02	74
28d	108.96	76
29d	112.24	78 [1)]

1) The test solution was stopped at time 28d by the addition of 1 mL conc. HCl. The final titration was performed at time t_{29d}.

Table 3: Toxicity Control

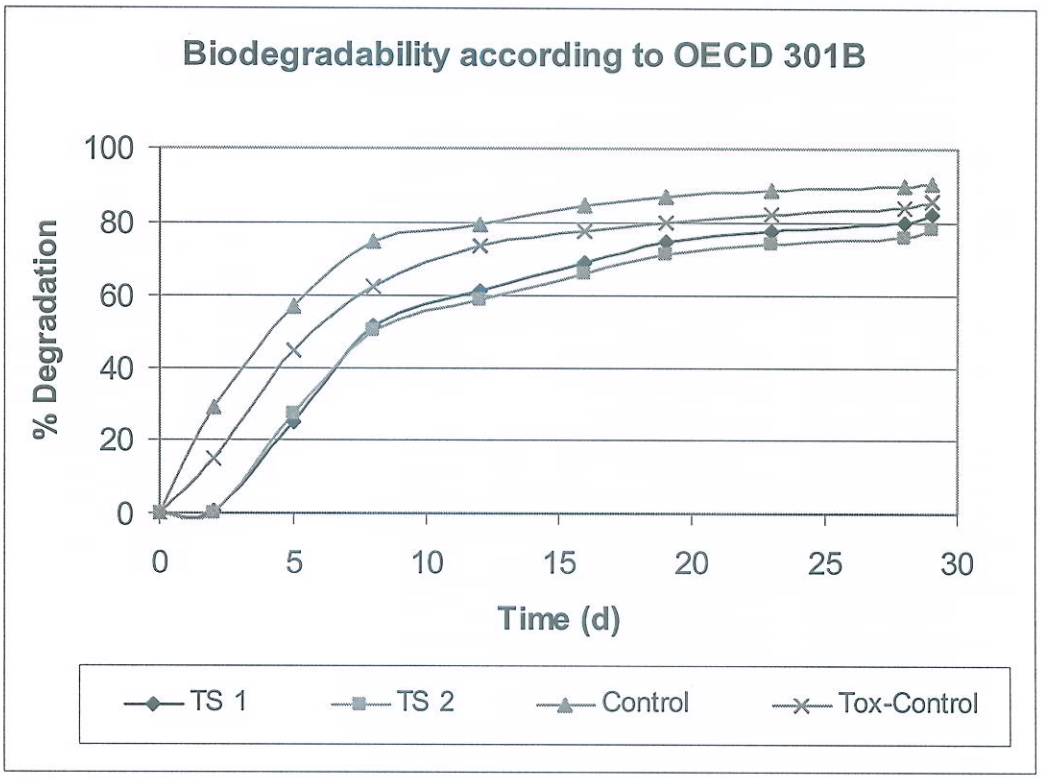
Time	Concentration: 227.7 mg Test Item + 121.1 mg Sodium Benzoate / 3.5 L $ThCO_2$: 402.36 mg CO_2/ 3.5 L	
	mg CO_2 produced in the Test solution, cumulative	% TCO_2 (= % Degradation)
2d	59.84	15
5d	180.85	45
8d	249.39	62
12d	295.32	73
16d	311.08	77
19d	320.83	80
23d	330.53	82
28d	337.40	84
29d	346.12	86 [1)]

Table 4: Positive Control Sodium Benzoate

Time	Concentration: 120.9 mg Sodium Benzoate / 3.5 L $ThCO_2$: 257.67 mg CO_2/ 3.5 L	
	mg CO_2 produced in the Test solution, cumulative	% TCO_2 (= % Degradation)
2d	75.38	29
5d	145.84	57
8d	191.70	74
12d	204.87	80
16d	217.51	84
19d	224.44	87
23d	228.34	89
28d	231.66	90
29d	233.99	91 [1)]

1) The test solution was stopped at time 28d by the addition of 1 mL conc. HCl. The final titration was performed at time t_{29d}.

Graphical Representation of the Results



TS 1 = 1st Test solution with the test item
TS 2 = 2nd Test solution with the test item