Assessment of the toxicity (72h EC_{50}) of FireStopper ® PFE-FR (FFC) to the marine unicellular algae *Skeletonema costatum*

Final report
Study no 1577c-3

Contents

Confidentiality statement

The information contained in this document is confidential and proprietary and is the property of FireStopper International Limited. The contents must not be disclosed to any third party without the express and written approval of FireStopper International Limited.

Study director's statement

I hereby state, that this study was conducted in accordance with the OECD principles of Good Laboratory Practice (GLP) as administered by the UK Dept of Health and that the report fully and accurately reflects the raw data generated during the study.

All raw data and a copy of the final report will be archived within Opus Plus Ltd's facility, on Flotta, for a period of three and a half years from the date of issue of the final report.

(Signed)

09 Nov 2011

(Date)

Melanie Anderson
Study Director Ecotoxicology
Opus Plus Limited

Quality assurance statement

The conduct of this study has been subjected to inspections by Opus Plus Ltd Quality Assurance Unit. Short term studies are not inspected individually but are subject to process based inspections. The dates of inspection are given below.

Date of QA Inspection	Type of Inspection	Date of Report to Management
10 August 2011	Study Plan audit	N/A
25 April – 09 May 2011	Facility Inspection	09 May 2011
23 – 26 August 2011	Algal test process inspection	29 August 2011
28 October 2011	Report audit	28 October 2011

This report has been audited by the Quality Assurance Personnel according to the appropriate Standard Operating Procedure. The report is considered to describe accurately the methods and procedures used in the study and the original data generated during the study.

(Signed)

28/October/2011

(Date)

Summary

Sponsor name	FireStopper International Limited	**Test personnel**	Miss Melanie Anderson, Study Director Mr Will Scott, Technician Mr Will Clouston, Technician Mrs Brenda Hudson, Ecotox Supervisor
Sponsor address	P.O Box 655 Pacific Palisades CA 90272-0655 USA	**Test facility**	Opus Plus Limited Flotta, STROMNESS Orkney, KW16 3NP **t** +44 1856 702 000 **f** +44 1856 701 473 admin@opus-results.com www.opus-results.com
Sponsor contact	Mr Ranjit Bedi	**Test guidelines**	ISO 10253 2006 Water quality - Marine algal growth inhibition test with *Skeletonema costatum*

Study number 1577c-3 was commissioned by FireStopper International Limited to determine the aquatic phase toxicity of Firestopper ® PFE-FR (FFC) to the marine unicellular algae *Skeletonema costatum*. A summary of the testing conducted is given below:

Test material	FireStopper ® PFE-FR (FFC)
Behaviour in seawater	Soluble
Preparation method	Dilution Series
Range finding test period	12 – 15 Aug 2011
Provisional 72h EC_{50} ($mg.l^{-1}$)	728.20
Definitive test period	13 – 16 Sept 2011
24h EC_{50} ($mg.l^{-1}$)	617.73
48h EC_{50} ($mg.l^{-1}$)	779.46
72h EC_{50} ($mg.l^{-1}$)	778.52
72h EC_{90} ($mg.l^{-1}$)	2537.76
NOEC ($mg.l^{-1}$)	100

Tests were assessed for compliance with the following guideline criteria:

Parameter	Guideline criterion	Observed values
Salinity at pre 0h/0h of ISO culture media (ppt)	36 ± 4	36
pH at pre 0h/0h in the ISO culture medium	8 ± 0.2	8.16 – 8.17
pH at 0h in the test material stocks	8 ± 0.2	8.02 – 8.12
pH increase during the test in the control	≤ 1	8.11 – 8.65
Temperature incubation (°C)	20 ± 2	19.8 – 21.6
Light intensity (Lux)	6000 - 10000	6100 - 7730
Reference toxicant 72h EC_{50} ($mg.l^{-1}$)	2.0 - 4.0	2.51
Control growth rate (d^{-1})	≥ 0.90	1.22
Coefficient of Variation at 72h	≤ 7%	0.58

Section 1

Pre-cultures in the exponential growth phase were prepared from stock laboratory cultures by inoculating nutrient medium (culture medium) to a cell density of approximately 10^4 cells per millilitre. Details of culture methods, in addition to test methods, procedures, guidelines and statistical methods are given in Appendix A. Appendix B indicates the nature of test material preparation methods. Appendix C contains the raw cell count data and Appendix D contains the quality control data.

Characterisation of FireStopper ® PFE-FR (FFC)

Table 1.1 Description & characterisation (SOP 402)

Property	MSDS supplied	Observed
Form	Liquid	Liquid
Colour	Clear to Slightly Hazy	Clear To Slightly Hazy
Density	1.210 – 1.260	1.1802g/cm^3 @ 20°C
Odour	Mild	Acidic
Viscosity	Not Stated	Slight
pH	Not Stated	TSW=6.44, DiW=4.02 (1000 mg.l^{-1} stock)
Aqueous solubility	Soluble	Soluble at 1000 mg.l^{-1} in sea water after 1 hour stirring
Preparation method		Dilution Series
Flash point	Not Flammable	
Melting point	Not Stated	
Boiling point	Not Applicable	
	Name, CAS No., Percentage composition	
	A Proprietary aqueous solution Composed of organic and inorganic compounds	

Firestopper ® PFE-FR (FFC) was characterised as soluble and was therefore prepared by dilution series.

Rangefinding test preparation

Table 1.2 Test material preparation (dilution series SOP 403)

Diluent	Preparation volumes (ml)	Nominal concentrations ($mg.l^{-1}$)	Weight (g) or volume (ml) added	pH of Main Stock at 0h	pH of Main Stock at 0h if adjustment required
ISO culture medium	250	1	0.25 ml from 1000$mg.l^{-1}$	8.09	N/A
	250	10	2.5 ml from 1000$mg.l^{-1}$	8.11	N/A
	250	100	25 ml from 1000$mg.l^{-1}$	8.11	N/A
	500	1000	0.4998g	6.76	8.06

Rangefinding test results

Table 1.3 Calculated growth rates and effects after 72h

Nominal concentrations ($mg.l^{-1}$)	72h growth
control 1	0.99
control 2	0.99
control 3	1.00
control 4	1.02
1	1.02
10	1.03
100	1.03
1000	0.29

The Rangefinding test exhibited a 72h EC_{50} of 728.20 $mg.l^{-1}$ (dilution series).

Definitive test preparation

Table 1.4 Test material preparation (dilution series SOP 403)

Diluent	Preparation volumes (ml)	Nominal concentrations ($mg.l^{-1}$)	Weight (g) or volume (ml) added	Actual nominal concentration ($mg.l^{-1}$)
ISO culture medium	250	100	2.5 ml from 10000$mg.l^{-1}$	100
	250	320	8.0 ml from 10000$mg.l^{-1}$	320
	250	1000	25 ml from 10000$mg.l^{-1}$	1000
	250	3200	80 ml from 10000$mg.l^{-1}$	3200
	500	10000	5.001g	10002

Definitive test results

Table 1.5 Calculated growth rates and effects after 24h, 48h and 72h

Nominal concentration	24h Rep growth	24h Mean growth	48h Rep growth	48h Mean growth	72h Rep growth	72h Mean growth
100a	0.90	0.90	1.00	0.96	1.02	1.03
100b	0.89		0.92		1.04	
320a	0.88	0.89	0.92	0.92	0.83	0.86
320b	0.90		0.92		0.89	
1000a	0.00	0.00	0.27	0.30	0.33	0.34
1000b	0.00		0.33		0.35	
3200a	0.00	0.00	0.00	0.00	0.00	0.00
3200b	0.00		0.00		0.00	
10000a	0.00	0.00	0.00	0.00	0.00	0.00
10000b	0.00		0.00		0.00	

Table 1.6 Initial and final pH values in the test media

Test material	Nominal concentration ($mg.l^{-1}$)	0h pH before adjustment	0h pH after adjustment	72h pH replicate a	72h pH replicate b
Firestopper ® PFE-FR (FFC)	100	8.02	N/A	8.34	8.39
	320	8.04	N/A	8.41	8.33
	1000	8.05	N/A	8.41	8.43
	3200	8.05	N/A	8.39	8.41
	10000	4.66	8.12	8.27	8.31

Table 1.7 Calculated EC_{50} values with 95% confidence limits, and 72h EC_{90} and NOEC values

Test material		EC_{50} ($mg.l^{-1}$)	95% Confidence limits ($mg.l^{-1}$) Lower	Upper	72h EC_{90} ($mg.l^{-1}$)	72h NOEC ($mg.l^{-1}$)
Firestopper ® PFE-FR (FFC)	24h	617.73	522.59	696.79		
	48h	779.46	677.42	888.89		
	72h	779.52	682.32	867.84	2537.76	100

Interpretation

The test was conducted in accordance with the study plan and met all relevant validity criteria.

Firestopper ® PFE-FR (FFC) exhibited a 72h EC_{50} value of 779.52 $mg.l^{-1}$(dilution series) to the marine phytoplankton *Skeletonema costatum* in the aqueous phase.

The result is based on nominal concentrations and was calculated by Linear Interpolation within the Toxcalc suite of statistical analysis.

There were no interferences in this test.

Retention and archiving of test documentation

The study plan and all data and records generated during the test are archived at Opus Plus Ltd's offices, and will be retained for a period of three and a half years from the date of the study.

References

ISO 10253 (2006) Water quality – Marine algal growth inhibition test with *Skeletonema costatum.*

ISO 5667-16 (1998) Water quality sampling – guidance on biotesting on samples.

ToxCalc Version 5 Tidepool Scientific Software.

Appendix A

Test organism/seawater

Pre-cultures in the exponential growth phase were prepared from stock laboratory cultures by inoculating treated seawater with nutrient medium (culture medium) to a cell density of approximately 2 x 10^3 to 10^4 cells per millilitre. The pre-cultures were incubated at approximately 20±2 °C under constant illumination for 3d ±1d, and were used as the inoculum source for subsequent toxicity tests.

Clean natural seawater with a salinity of 36‰ ± 4‰ at 0h is used in the test.

Test method and guidelines

The test was conducted in accordance with SOP 104 and ISO 10253 (2006) Water Quality – marine algal growth inhibition test. ISO 5667-16 (2006) Water quality-sampling – guidance on biotesting of samples. The method assesses the growth rate of cultures in solutions of test material in enriched seawater in comparison to the growth rate of cultures in enriched seawater alone. Growth rate was measured in terms of increase in cell number or in biomass.

Test procedure

The test was conducted in 100 ml borosilicate glass conical flasks, to which 80 ml of test medium seawater was added. Each treatment was prepared in duplicate, and inoculated with cells from the pre-cultures (in exponential growth phase) to give an initial cell density of approximately 10,000 cells per ml.

The initial inoculum was checked microscopically using a haemocytometer. Following inoculation, all flasks were loosely covered with aluminium foil caps and mounted on an orbital shaker at approximately 150 rpm. The illumination consisted of 40W cool white tubes (as specified in the ISO guidelines) which were mounted at a distance of approximately 40 cm directly above the test area. Light intensity values were measured daily during the test. Flasks were assigned positions on the shaker. The controlled temperature room temperature was 20±2 °C.

Rangefinding tests were conducted over 72h to determine the approximate concentrations at which effects were observed.

Definitive tests were conducted over 72h at concentrations determined from the results of the Rangefinding tests. Definitive tests employ five concentrations and two replicates per concentration. A reference test with 3,5 Dichlorophenol was conducted concurrently with the definitive test. In the definitive test, counts of algal cell numbers were carried out daily by microscope using either haemocytometer or Sedgewick-Rafter chamber, depending upon cell numbers present or by Fluorometer measurements. Three readings were performed on each test vessel (SOP 106).

Statistical methods

The raw data for each duplicate vessel and time period were averaged, to give values for each concentration of cell volume. Growth rate was calculated on the basis of these measurements. Daily intrinsic growth rate was calculated for each duplicate for each time period, using an exponential model:

$N_t = N_o .e^{kt}$ where N_o = volume or number at beginning of test
N_t = volume or number at time t
t = time in days
k = growth rate ($.d^{-1}$)

The average value of k for each time interval was calculated for each concentration. Since the criterion of effect was the concentration causing 50% reduction in growth rate with respect to the controls, the response for each concentration was estimated from; effect = 1- (control k/treatment k)

The resulting values represent proportional reduction in growth rate. The EC_{50} for each time interval and the 72h EC_{90} and NOEC values were calculated using an appropriate statistical method from the ToxCalc Version 5 software.

Appendix B

Test material preparation

The test materials were assessed for risk to health, and appropriate handling and containing procedures implemented. Comparisons of the reported and observed physical characteristics (eg form, colour, odour, pH and density) of the test material were made.

In order to determine an appropriate test preparation method, an assessment was made of the material's behaviour in seawater. A 1000 $mg.l^{-1}$ stock was prepared in filtered seawater, and the resulting mixture was stirred for one hour. If the material was observed to be soluble a dilution series was prepared, where an appropriate weight of test material was added to prepare an initial stock. Appropriate volumes were taken from this stock to prepare subsequent test concentrations which were brought to volume with culture medium. If it was poorly soluble then it was stirred again for approximately 19 hrs, then left to settle for one hour and its behaviour assessed (SOP 402). If, the material produced floating, settled or neutrally buoyant particles or films, it was classified as poorly soluble and exposures were carried out with Water Accommodated Fractions (WAFs). WAFs were prepared by the direct addition of the required nominal weights or volumes to seawater followed by gentle stirring for approximately 20 hours and a settling period of approximately one hour. After this settling period, the middle phase of the preparation is siphoned, avoiding incorporation of undissolved particles, if present.

A reference test was conducted concurrently using 3,5 Dichlorophenol at 3.2, 1.8 and 1.0 $mg.l^{-1}$ which were prepared from a main stock of 100 $mg.l^{-1}$. The 100 $mg.l^{-1}$ stock was stirred for a minimum of one hour, or until completely dissolved.

Culture medium is prepared from natural seawater supplied by pump from Scapa Flow, Orkney. All seawater was UV sterilised and filtered to 0.2 µm. The filtered treated seawater was then enriched with nutrients and vitamins in accordance with ISO guidelines. The salinity of the enriched natural seawater at 0h was 36‰ ± 4‰.

At pre 0h or 0h, the pH of the culture medium was adjusted if required, by adding 1M HCl, or NaOH to give a pH of 8±0.2.

If, at 0h the pH of the test material stock(s) was outwith the pH range of 8±0.2 then the pH was returned to within these limits by adjustment with either 1M HCl or NaOH as was appropriate. If the pH requires adjustment, a stirring period was required to ensure the pH remained constant.

Appendix C

Fluorescence measurements (Relative Fluorescence Units (RFU)) in test vessels after 24, 48 and 72h

Conc (mg/l)	24h Measurement	48h Measurement	72h Measurement
100a	102.97	949.20	1084.33
	100.59	936.92	1055.51
	99.66	934.22	1031.19
100b	103.32	743.40	1169.99
	97.80	705.77	1157.32
	95.55	697.41	1138.18
320a	99.28	739.48	530.13
	97.28	720.18	530.30
	96.76	711.11	527.62
320b	104.04	706.16	676.48
	99.41	706.57	665.36
	96.76	702.31	654.21
1000a	20.36	69.78	83.52
	19.61	66.06	85.16
	19.65	65.90	83.18
1000b	24.31	86.91	92.36
	23.92	82.87	91.65
	23.71	81.75	88.59
3200a	6.14	11.50	9.59
	6.05	11.40	9.59
	6.04	11.38	9.44
3200b	6.52	11.93	12.03
	6.47	11.87	12.07
	6.45	11.81	11.91
10000a	5.82	12.47	14.02
	5.79	12.38	13.61
	5.76	12.29	13.33
10000b	6.54	13.28	14.22
	6.46	13.17	14.29
	6.44	13.16	14.58

Appendix D – CR104396

Control data

Table D1 Source of inoculum

Species:	*Skeletonema costatum*	Culture number:	C5C3 A

Table D2 Initial cell density of inoculum

Pre-culture density (cells/ml)	Initial inoculation volume (ml)	Estimated initial test culture density (cells/ml)	Fluorometer measurement (RFU)
367.5×10^4	0.22	10106	25.39

Table D3 Initial and final pH values of control and 3,5 DCP vessels

Test material		Concentration ($mg.l^{-1}$)	0h pH	72h pH replicate a	72h pH replicate b
Control	1	0	8.11	8.29	
	2	0		8.37	
	3	0		8.65	
	4	0		8.61	
3,5 DCP		1.0	8.10	8.46	8.58
		1.8	8.11	8.84	8.72
		3.2	8.12	8.14	8.18

Table D4 Environmental conditions in the study

	0h	24h	48h	72h
Temp (°C)	20.1	19.8 – 21.5	19.8 – 21.6	19.9 – 21.4
Light intensity (Lux)	7000 - 7730	6100 - 7140	6600 - 7320	6290 - 7490

Table D5 72h control and 3,5-DCP data (Fluorometer measurements)

Replicate	72h Measurement 1	72h Measurement 2	72h Measurement 3	Rep growth	Mean growth
control 1	1024.05	986.37	987.41	1.01	1.00
control 2	986.80	954.22	946.45	1.00	
control 3	1013.61	987.93	986.02	0.99	
control 4	1030.35	1003.61	998.28	1.00	
1.0a	1108.25	1078.70	1071.26	1.03	1.02
1.0b	1084.13	1059.75	1059.16	1.02	
1.8a	1115.75	1103.25	1091.41	1.03	1.04
1.8b	1236.80	1206.60	1194.47	1.06	
3.2a	21.69	21.34	21.30	0.00	0.01
3.2b	26.95	26.69	26.63	0.01	

Table D6 3,5 DCP EC_{50} values and 95% confidence limits

Test material	72h EC_{50} ($mg.l^{-1}$)	95% confidence limits ($mg.l^{-1}$) lower	upper
3,5 DCP	2.51	2.49	2.53

Assessment of aerobic degradability of Firestopper ® PFE-FR in seawater

Final report
Study no 1577c-9

Contents

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Study director's statement

I hereby state, that this study was conducted in accordance with the OECD principles of Good Laboratory Practice (GLP) as administered by the UK Dept of Health and that the report fully and accurately reflects the raw data generated during the study.

All raw data and a copy of the final report will be archived within Opus Plus Ltd facility, on Flotta, for a period of three and a half years from the date of issue of the final report.

Mark Forrest
(Signed)

19 Oct 11
(Date)

Mark Forrest
Study Director, Biodegradation Studies
Opus Plus Limited

Quality assurance statement

The conduct of this study has been subjected to inspections by Opus Plus Ltd Quality Assurance Unit. Short term studies are not inspected individually but are subject to process based inspections. The dates of inspection are given below.

Date of QA Inspection	Type of Inspection	Date of Report to Management
16 August 2011	Study Plan audit	N/A
25 April – 09 May 2011	Facility Inspection	09 May 2011
09 – 16 September 2011	Biodegradation test process inspection	22 September 2011
18 October 2011	Report audit	18 October 2011

This report has been audited by the Quality Assurance Personnel according to the appropriate Standard Operating Procedure. The report is considered to describe accurately the methods and procedures used in the study and the original data generated during the study.

(Signed)

18/October/2011
(Date)

Summary

Sponsor name	Firestopper International Limited	**Test personnel**	Mark Forrest, Study Director Savanna Joyce, Technician Sam Archibald, Technician
Sponsor address	P.O Box 655 Pacific Palisades CA 90272-0655 USA	**Test facility**	Opus Plus Limited Flotta, STROMNESS Orkney, KW16 3NP **t** +44 1856 702 000 **f** +44 1856 701 473 admin@opusplus-results.com www.opusplus-results.com
Sponsor contact	Ranjit Bedi	**Test guidelines**	OECD guideline 306, 'Biodegradability in Seawater- Closed Bottle Method' (OECD 1992)

Study number 1577c-9 was commissioned by Firestopper International Limited to determine the ready aerobic degradability in seawater of Firestopper ® PFE-FR. A summary of all testing conducted is given below:

Test material	Firestopper ® PFE-FR
Solubility in water	Soluble
COD ($mgO_2.mg^{-1}$)	0.13
Addition rate ($mg.l^{-1}$)	10.00
Preparation method	in stock solution
Test period	17 August – 14 September 2011
% Inhibition (Day 28)	-39
28 day % biodegradation	59
Maximum % biodegradation on day 21	78

Tests were assessed for compliance by the following guideline criteria:

	Test material	Firestopper ® PFE-FR
Guideline validity criteria		**Validity data**
Sodium benzoate	≥60% biodegradation in 14 days	78
Minimum microbial count	$1.0x10^1$ to $1.0x10^3$ CFU's per ml	$1.84x10^3$
Oxygen consumption blank	≤30% of oxygen after 28 days	14
Measurement temperature	20°C ± 1°C	19.3 – 21.0

Test procedure

Unlike similar screening tests for biodegradability in freshwater systems, this method employs no separate bacterial inoculum, and relies upon populations of bacteria which occur naturally in seawater. The test serves only to provide a preliminary level of information on ready degradability in seawater. The raw seawater used for this study was supplied by a submersible pump situated on Sutherland's pier on the west side of Flotta in Scapa Flow. It is pumped continuously from a depth of two metres below low water spring tide level, before passing up 1.8 kilometres of plastic pipe to a 20,000 litre storage tank. Two smaller pumps move the water to three settlement tanks situated nine metres above floor level. The seawater temperature varies between 6 °C in the winter and 14 °C in the summer. The salinity is between 34‰ and 37‰. Five to seven days before test commencement, raw seawater passes by gravity through a 45 µm filter to the ageing tank stored in darkness.

The overall assessment of biodegradability is based upon a comparison between experimentally determined oxygen consumption (BOD measurements) and the oxygen consumption predicted if all carbon present in the test material were completely oxidised (theoretical oxygen demand, ThOD). Where the composition of the test material is known, or can be reasonably inferred, the ThOD can be calculated from the empirical formula and the molecular weight. If neither the empirical formula or chemical composition of the test material can be obtained, then the prediction of maximum potential BOD is obtained from the determination of the chemical oxygen demand (COD) or CHN analysis.

The COD analysis of soluble test materials may be derived by using a COD Colorimeter. The COD value obtained is used directly in calculating the addition rates.

For insoluble test materials a CHN (carbon:hydrogen:nitrogen) analysis is applied. The empirical formula for most organic test material can be derived by this method (excluding muds). The ThOD in mg of oxygen per mg of test substance can be calculated from the empirical formula and molecular weight.

$$\text{ThOD of } C_cH_hO_oN_n = \frac{16\,[2c + 0.5\,(h-3n) - o]}{MW} \text{ mg } O_2.mg^{-1}$$

The table on page 7 summarises the methods and conditions for this test.

Oxygen consumption in test material vessels is corrected for variation in atmospheric pressure, and for any oxygen consumption recorded in blank vessels. A readily degradable soluble reference material, sodium benzoate, is used to provide confirmation of the viability of the naturally occurring seawater bacterial population.

To enable an assessment of potential inhibitory effects of the test material (or its primary degradation products), an inhibition control is used, in which a mixture of the soluble reference compound and the test material is tested. Inhibition is inferred if the degradation rate of the mixture is less than the sum of the independent degradation rates.

In tests conducted with poorly soluble materials, an inert support medium is used to provide a large and controlled surface area, and support medium blank vessels are also prepared. A weighed amount of test material is added to, and homogenised with, a volume of silica powder. A small quantity of the primary homogenate is then added to a larger mass of powder and re-homogenised. The 'dilution' of the test material is controlled by the amount of powder added to the final homogenate. The addition rate of the test substance to the test vessels is determined by the quantity of final homogenate per vessel; the final homogenate is added to the vessel before the addition of the test medium. The ready degradation of the test material is estimated from the theoretical oxygen consumption if 100% of the material were fully mineralised during the test (calculated from the theoretical oxygen demand and the amount added to the test vessel).

Summary of test method and conditions

Guideline	OECD 306: Ready aerobic degradation in seawater	OECD 1992
Test parameters	Measurement	Dissolved oxygen at 7 day intervals
	Measurement method	Polarographic electrode
	Equipment	YSI 58 meter with YSI 5905 BOD probe
	Incubation temperature of bottle	20°C ± 3°C
	Duration	28 days
	Replication	Test material, oxygen blank, reference: 3 per timepoint, minimum of 2 per timepoint for data processing
	Medium	Natural seawater
	Saturation value for dissolved oxygen at normal atmospheric pressure	7.45 mg/litre
	Enrichment ($g.l^{-1}$)	KH_2PO_4 8.5 K_2HPO_4 21.76 $Na_2HPO_4.2H_2O$ 29.92 NH_4Cl 0.5 $CaCl_2$ 31.84 $MgSO_4.7H_2O$ 22.5 $FeCl_3.6H_2O$ 0.25 EDTA, Di-sodium salt 0.4
	Test vessels	270-276 ml glass BOD bottles
Test material preparation	Soluble material	In stock solution
Reference materials	Sodium benzoate	Soluble reference
Blanks and controls	Oxygen consumption blank	Background O_2 consumption in test medium
	Inhibition test	Mixture of sodium benzoate and test material
Formal validity criteria	Bottle temperature when measuring dissolved oxygen	20°C ± 1°C
	Soluble reference	≥60% biodegradation of ThOD in 14 days
	Oxygen consumption blank	≤ 30% of oxygen after 28 days
Informal validity criteria	Microbial count using the spread plate method	A minimum of 1.0×10^1 to 1.0×10^3 colony forming units per ml of aged sea water

Aerobic degradability in seawater of Firestopper ® PFE-FR

Test data

Average barometric pressure corrected dissolved oxygen concentrations (mg $O_2.l^{-1}$)

Material	Day 0	7	14	21	28
Oxygen consumption blank	7.27	7.00	6.77	6.57	6.28
Sodium benzoate	7.26	5.37	4.82	4.65	4.30
Test material	7.27	6.21	5.97	5.53	5.50
Test material + sodium benzoate	7.27	4.80	4.37	3.89	2.44

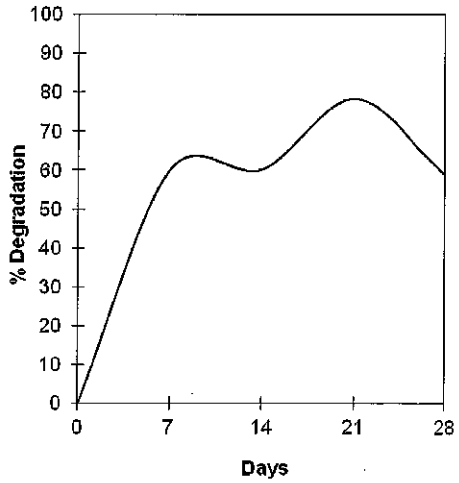
Average net oxygen consumption (BOD, mg $O_2.l^{-1}$)

Material	Day 7	14	21	28
Oxygen consumption blank	0.27	0.50	0.70	0.99
Sodium benzoate	1.63	1.95	1.92	1.98
Test material	0.79	0.79	1.03	0.77
Test material + sodium benzoate	2.20	2.40	2.68	3.84

Percentage degradation of Firestopper ® PFE-FR

Material	100% BOD ($mg.l^{-1}$)	Measured BOD ($mg.l^{-1}$) 7	14	21	28	Percentage degradation 7	14	21	28
Test material	1.3	0.79	0.79	1.03	0.77	60	60	78	59
Test material + Sodium benzoate	3.8	2.20	2.40	2.68	3.84	58	63	70	100

Firestopper® PFE-FR



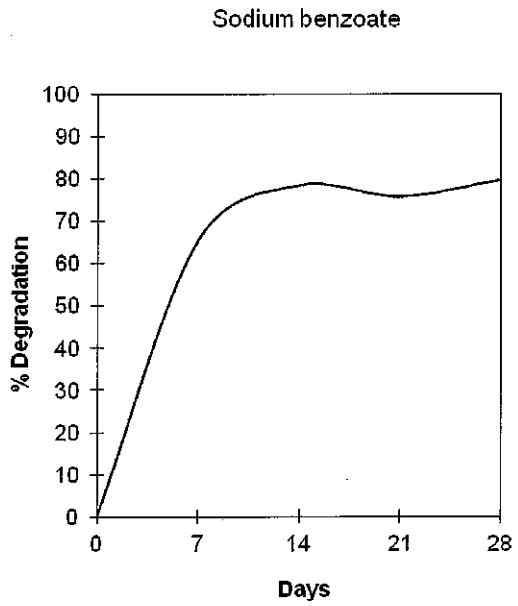
Inhibition due to test material

Day	Test material BOD ($mg.l^{-1}$)	Sodium benzoate BOD ($mg.l^{-1}$)	Sum of separate BODs ($mg.l^{-1}$)	Test material + Sodium benzoate BOD ($mg.l^{-1}$)	Percentage inhibition
7	0.79	1.63	2.42	2.20	9
14	0.79	1.95	2.74	2.40	12
21	1.03	1.92	2.92	2.68	8
28	0.77	1.98	2.75	3.84	-39

Test quality data

Reference material degradation

Material	100% BOD ($mg.l^{-1}$)	Measured BOD ($mg.l^{-1}$)				Percentage degradation			
		7	14	21	28	7	14	21	28
Sodium benzoate	2.5	1.63	1.95	1.88	1.98	65	78	76	80



Blank oxygen demand

Day	Mean Dissolved oxygen ($mg.l^{-1}$)	Mean BOD ($mg.l^{-1}$)	BOD (%)
0	7.27	---	---
7	7.00	0.27	4
14	6.77	0.50	7
21	6.57	0.70	10
28	6.28	0.99	14

Seawater data

Seawater property	Seawater data
Seawater source:	Sutherland Pier, Scapa Flow
Date of collection:	09 August 2011
Depth of collection:	2m below low water Spring tide level
Volume collected (litres):	130
Appearance at collection:	Clear
Salinity at collection (‰):	37
Temperature at collection (°C):	15.4
Temperature on day zero (°C):	20.7
Pre-treatment prior to testing:	Filtered through 45 µm mesh Nutrient enriched Sedimentation and decanting Aged in darkness for: 8 days Aerated for: 20 minutes
Microbial count at collection (CFU/ml):	$1.84x10^3$
Microbial count on day zero of test (CFU/ml):	$1.73x10^3$

Characterisation of test material (SOP 402)

Property	MSDS supplied	Observed
Form	Liquid	Liquid
Colour	Clear to slight hazy	Clear to slight hazy
Density	1.210 – 1.260	$1.1802 g/cm^3$ @ 20°C
Odour	Mild	Acidic
Viscosity	Not stated	Slight
pH	Not stated	TSW = 6.44, DiW= 4.02(1000$mg.l^{-1}$)
Solubility in water	Soluble	Soluble
Flash point	Not flammable	
Melting point	Not stated	
Boiling point	Not applicable	
Chemical description	Name, CAS number, Percentage composition	
	A proprietary aqueous solution composed of organic and inorganic components	

Conclusion

The test was conducted in accordance with the study plan and met all relevant validity criteria. There were no interferences in this test.

Firestopper ® PFE-FR biodegraded by 59% over 28 days and showed an inhibition of -39% to seawater bacteria.

Firestopper ® PFE-FR achieved a maximum biodegradation of 78% on day 21 of the 28 day study.

The oxygen blank degradation was within formal limits of acceptability. The soluble reference material, sodium benzoate, degraded by more than 60% in the first 14 days, indicating that the seawater used in the test contained a satisfactory population of viable bacteria. The seawater data table on page 10 confirms the microbial count for seawater used in this test was within acceptable limits.