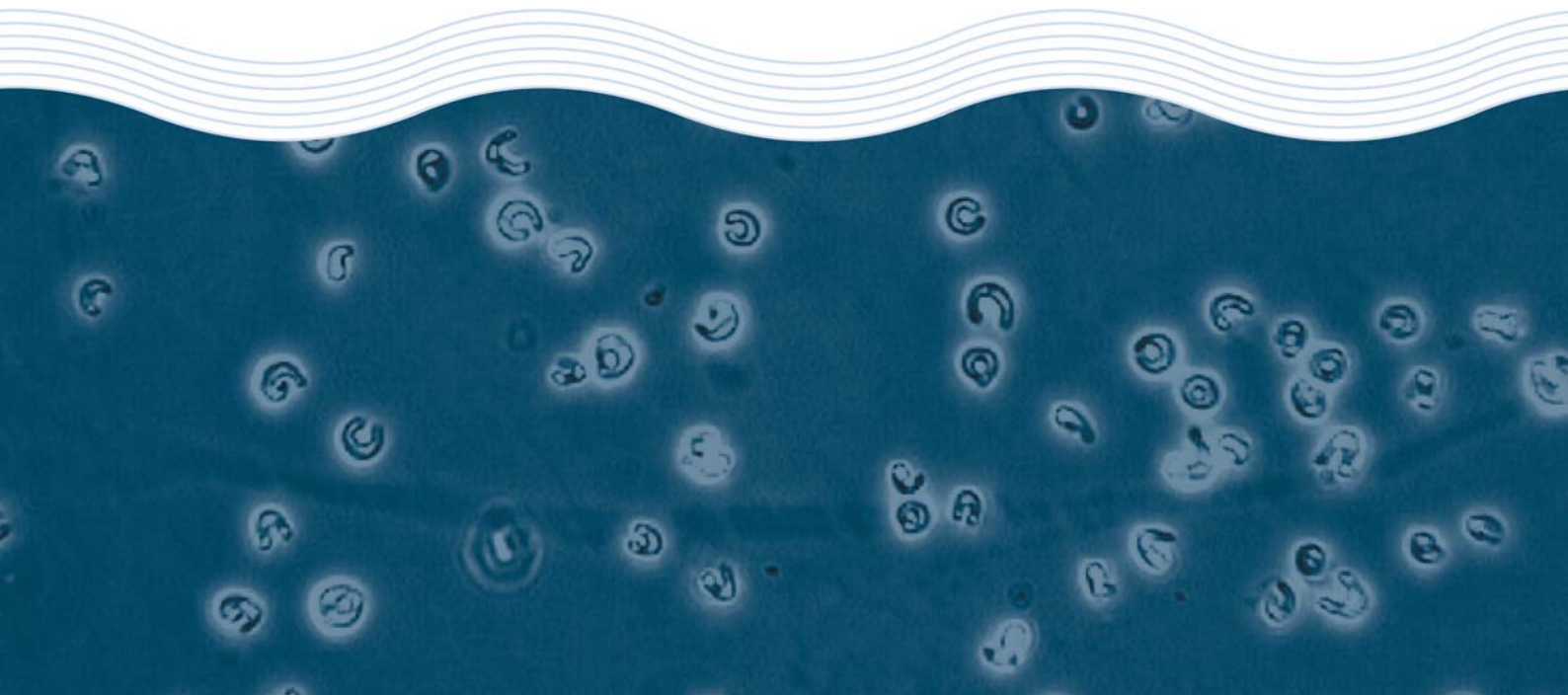


**Toxicity Assessment of S200
OILGONE**

Gary Pearse Enterprises Pty Ltd

Test Report

April 2014



Toxicity Assessment of S200 OILGONE

Gary Pearse Enterprises Pty Ltd

Test Report

April 2014

Toxicity Test Report: TR1147/1

(Page 1 of 2)

This document is issued in accordance with NATA's accreditation requirements

Client:	Gary Pearse Enterprises Pty Ltd 2962 Tathra Bermagui Road Murrah NSW 2550	ESA Job #:	PR1147
Attention:	Gary Pearse	Date Sampled:	Not supplied
Client Ref:	Not supplied	Date Received:	28 February 2014
		Sampled By:	Client
		ESA Quote #:	PL1147_q01

Lab ID No.:	Sample Name:	Sample Description:
6517	S200 Oilgone	Chemical sample received at room temperature in apparent good condition

Test Performed:	48-hr acute survival test using the copepod <i>Parvocalanus crassirostris</i>
Test Protocol:	ESA SOP 124 (2014)
Test Temperature:	The test was performed at 27±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest test concentration was prepared by adding a weighed aliquot of sample 6517 'S200 Oilgone' into filtered seawater (FSW). The remaining test concentrations were achieved by serially diluting the highest test concentration with FSW. A FSW control was tested concurrently with the prepared sample.
Source of Test Organisms:	In house culture
Age of Test Organisms:	<7 days
Test Initiated:	13 March 2014 at 1300h

Sample 6517: S200 Oilgone Concentration (mg/L)	% Survival (Mean ± SD)	Vacant	Vacant
FSW Control	100 ± 0.0		
1.25	100 ± 0.0		
2.5	95.0 ± 10.0		
5.0	95.0 ± 10.0		
10.0	75.0 ± 10.0 *		
20.0	55.0 ± 37.9 *		
48-hr EC10 = 5.4 (2.4-8.0) mg/L			
48-hr EC50 = >20.0 mg/L			
NOEC = 5.0 mg/L			
LOEC = 10.0 mg/L			


*Significantly lower percent survival compared with the FSW Control (Steel's Many-One Rank Test, 1-tailed, P=0.05)

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % survival	≥80.0%	100%	Yes
Reference Toxicant within cusum chart limits	4.3-27.6µg Cu/L	5.1µg Cu/L	Yes



Toxicity Test Report: TR1147/1

(Page 2 of 2)

Test Report Authorised by: 

Dr Rick Krassoi, Director on 4 April 2014

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

ESA (2014) *SOP 124 – Acute toxicity test using the copepod *Gladioferens imparipes**. Issue No. 3. Ecotox Services Australasia, Sydney, New South Wales.

Toxicity Test Report: TR1147/2

(Page 1 of 2)

This document is issued in accordance with NATA's accreditation requirements

Client:	Gary Pearse Enterprises Pty Ltd 2962 Tathra Bermagui Road Murrah NSW 2550	ESA Job #:	PR1147
Attention:	Gary Pearse	Date Sampled:	Not supplied
Client Ref:	Not supplied	Date Received:	28 February 2014
		Sampled By:	Client
		ESA Quote #:	PL1147_q01

Lab ID No.:	Sample Name:	Sample Description:
6517	S200 Oilgone	Chemical sample received at room temperature in apparent good condition

Test Performed:	96-hr acute toxicity test using the amphipod <i>Melita plumulosa</i>
Test Protocol:	ESA SOP 108 (ESA 2011), based on USEPA (2002) and Department of Transport and Communications (1990)
Test Temperature:	The test was performed at 20±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest test concentration was prepared by adding a weighed aliquot of sample 6517 'S200 Oilgone' into filtered seawater (FSW). The remaining test concentrations were achieved by serially diluting the highest test concentration with FSW. A FSW control was tested concurrently with the prepared sample.
Source of Test Organisms:	In-house culture, originally sourced from Hawkesbury River, NSW
Test Initiated:	5 March 2014 at 1345h

Sample 6517: S200 Oilgone	Vacant		Vacant	
Concentration (mg/L)	% Unaffected (Mean ± SD)			
FSW Control	95.0 ± 10.0			
1.25	100 ± 0.0			
2.5	90.0 ± 20.0			
5.0	90.0 ± 20.0			
10.0	95.0 ± 10.0			
20.0	95.0 ± 10.0			
96-hr EC10 = >20.0mg/L				
96-hr EC50 = >20.0mg/L				
NOEC = 20.0mg/L				
LOEC = >20.0mg/L				

Toxicity Test Report: TR1147/2

(Page 2 of 2)

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % unaffected	≥90.0%	95.0%	Yes
Reference Toxicant within cusum chart limits	60.3-367.7µg Cu/L	138.8µg Cu/L	Yes

Test Report Authorised by:



Dr Rick Krasso, Director on 4 April 2014

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

Department of Transport and Communications (1990) Guidelines for Acceptance of Oil Spill Dispersants in Australian Waters. Pollution Prevention Section, Department of Transport and Communications, Canberra ACT.

ESA (2011) SOP 108 – *Amphipod Acute Toxicity Test*. Issue No 8. Ecotox Services Australasia, Sydney, NSW.

USEPA (2002) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fifth Edition. United States Environmental Protection Agency, Office of Research and Development, Washington DC, EPA/600/4-90/027F.

Toxicity Test Report: TR1147/3

(Page 1 of 2)

This document is issued in accordance with NATA's accreditation requirements

Client:	Gary Pearse Enterprises Pty Ltd 2962 Tathra Bermagui Road Murrah NSW 2550	ESA Job #:	PR1147
Attention:	Gary Pearse	Date Sampled:	Not supplied
Client Ref:	Not supplied	Date Received:	28 February 2014
		Sampled By:	Client
		ESA Quote #:	PL1147_q01

Lab ID No.:	Sample Name:	Sample Description:
6517	S200 Oilgone	Chemical sample received at room temperature in apparent good condition

Test Performed:	96-hr fish imbalance toxicity test using barramundi <i>Lates calcarifer</i>
Test Protocol:	ESA SOP 117 (ESA 2013), based on USEPA (2002)
Test Temperature:	The test was performed at 25±2°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest test concentration was prepared by adding a weighed aliquot of sample 6517 'S200 Oilgone' into filtered seawater (FSW). The remaining test concentrations were achieved by serially diluting the highest test concentration with FSW. A FSW control was tested concurrently with the prepared sample.
Source of Test Organisms:	Hatchery reared, SA
Test Initiated:	27 March 2014 at 1530h

Sample 6517: S200 Oilgone	Concentration		% Unaffected		Vacant	Vacant
	(mg/L)		(Mean ± SD)			
FSW Control		100	± 0.0			
1.25		100	± 0.0			
2.5		100	± 0.0			
5.0		100	± 0.0			
10.0		95.0	± 10.0			
20.0		100	± 0.0			
96-hr EC10 = >20.0mg/L						
96-hr EC50 = >20.0mg/L						
NOEC = 20.0mg/L						
LOEC = >20.0mg/L						

Toxicity Test Report: TR1147/3

(Page 2 of 2)

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % unaffected	≥80.0%	100%	Yes
Reference Toxicant within cusum chart limits	569.0-3247.9µg Cu/L	1309.4µg Cu/L	Yes

Test Report Authorised by:



Dr Rick Krassoi, Director on 4 April 2014

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

This document is issued in accordance with NATA's accreditation requirements. Accredited for compliance with ISO/IEC 17025. NATA is a signatory to the APLAC mutual recognition arrangement for the mutual recognition of the equivalence of testing, calibration and inspection reports. This document shall not be reproduced except in full.

Citations:

ESA (2013) SOP 117 –*Freshwater and Marine Fish Imbalance Test*. Issue No 10. Ecotox Services Australasia, Sydney, NSW

USEPA (2002) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fifth edition EPA-821-R-02-012. United States Environmental Protection Agency, Office of Research and Development, Washington FC, USA

Statistical Printouts for the Juvenile Copepod Tests

Marine Copepod Acute Test-48-hr Survival

Start Date: 13/03/2014 13:00 Test ID: PR1147 Sample ID: S200
 End Date: 15/03/2014 12:30 Lab ID: 6517 Sample Type: CP-Chemical product
 Sample Date: Protocol: ESA 124 Test Species: PC-Parvocalanus crassirostris
 Comments:

Conc-mg/L	1	2	3	4
FSW	1.0000	1.0000	1.0000	1.0000
1.25	1.0000	1.0000	1.0000	1.0000
2.5	1.0000	1.0000	0.8000	1.0000
5	1.0000	1.0000	0.8000	1.0000
10	0.6000	0.8000	0.8000	0.8000
20	0.8000	0.6000	0.0000	0.8000

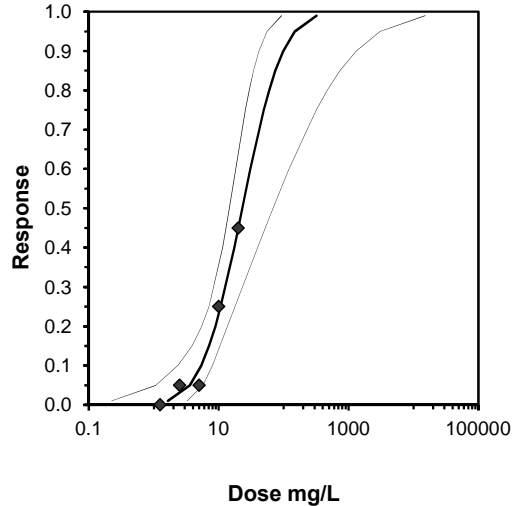
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root				Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%				
FSW	1.0000	1.0000	1.3503	1.3453	1.3652	0.739	4	0	21	
1.25	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	0	20	
2.5	0.9500	0.9500	1.2857	1.1071	1.3453	9.261	4	1	20	
5	0.9500	0.9500	1.2857	1.1071	1.3453	9.261	4	1	20	
*10	0.7500	0.7500	1.0519	0.8861	1.1071	10.508	4	5	20	
*20	0.5500	0.5500	0.8315	0.2255	1.1071	50.176	4	9	20	

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05) Equality of variance cannot be confirmed	0.740776	0.916	-1.96931	7.310441

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test Treatments vs FSW	5	10	7.071068	

Parameter	Value	SE	95% Fiducial Limits	Maximum Likelihood-Probit						
				Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.033408	0.540501	0.974027 3.092789	0	1.028993	7.814728	0.79	1.361992	0.491785	3
Intercept	2.230515	0.558995	1.134885 3.326146							
TSCR										

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1.651688	0.224751	3.266677
EC05	3.355	3.573362	1.067118	5.722832
EC10	3.718	5.391922	2.355625	8.020007
EC15	3.964	7.116871	3.857174	10.49351
EC20	4.158	8.873498	5.461492	13.57923
EC25	4.326	10.72229	7.060126	17.66043
EC40	4.747	17.27426	11.60419	39.78696
EC50	5.000	23.01398	14.74373	68.82824
EC60	5.253	30.66085	18.36993	121.4184
EC75	5.674	49.39647	25.92384	318.5921
EC80	5.842	59.68823	29.60118	469.086
EC85	6.036	74.42083	34.48955	737.6839
EC90	6.282	98.22904	41.72015	1306.534
EC95	6.645	148.2199	55.16246	3056.895
EC99	7.326	320.668	92.64583	15139.63



Marine Copepod Acute Test-48-hr Survival

Start Date:	13/03/2014 13:00	Test ID:	PR1147	Sample ID:	S200
End Date:	15/03/2014 12:30	Lab ID:	6517	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 124	Test Species:	PC-Parvocalanus crassirostris
Comments:					

Auxiliary Data Summary

Conc-mg/L	Parameter	Mean	Min	Max	SD	CV%	N
FSW	% Survival	100.00	100.00	100.00	0.00	0.00	4
1.25		100.00	100.00	100.00	0.00	0.00	4
2.5		95.00	80.00	100.00	10.00	3.33	4
5		95.00	80.00	100.00	10.00	3.33	4
10		75.00	60.00	80.00	10.00	4.22	4
20		55.00	0.00	80.00	37.86	11.19	4
FSW	pH	8.30	8.30	8.30	0.00	0.00	1
1.25		8.30	8.30	8.30	0.00	0.00	1
2.5		8.30	8.30	8.30	0.00	0.00	1
5		8.20	8.20	8.20	0.00	0.00	1
10		8.20	8.20	8.20	0.00	0.00	1
20		8.10	8.10	8.10	0.00	0.00	1
FSW	DO %	108.70	108.70	108.70	0.00	0.00	1
1.25		104.00	104.00	104.00	0.00	0.00	1
2.5		102.90	102.90	102.90	0.00	0.00	1
5		102.60	102.60	102.60	0.00	0.00	1
10		101.70	101.70	101.70	0.00	0.00	1
20		101.20	101.20	101.20	0.00	0.00	1
FSW	Salinity ppt	35.10	35.10	35.10	0.00	0.00	1
1.25		35.10	35.10	35.10	0.00	0.00	1
2.5		35.20	35.20	35.20	0.00	0.00	1
5		35.30	35.30	35.30	0.00	0.00	1
10		35.40	35.40	35.40	0.00	0.00	1
20		35.40	35.40	35.40	0.00	0.00	1

**Statistical Printouts for the
Juvenile *Melita plumulosa* Tests**

Amphipod Survival and Growth Test-96 hr survival

Start Date:	5/03/2014 13:45	Test ID:	PR1147/02	Sample ID:	S200 OILGONE
End Date:	9/03/2014 10:15	Lab ID:	6517	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 108	Test Species:	ML-Melita Plumulosa

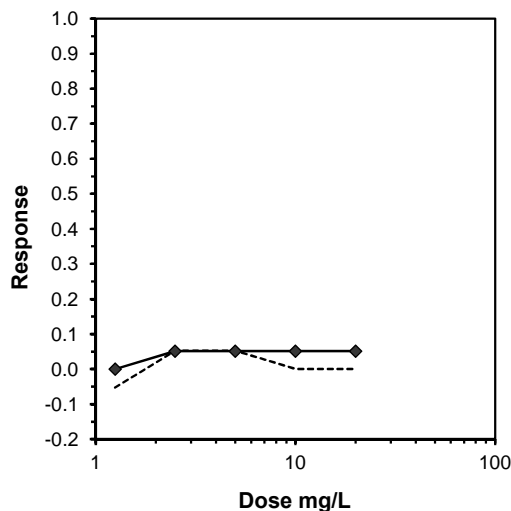
Conc-mg/L	1	2	3	4
FSW Control	1.0000	1.0000	1.0000	0.8000
1.25	1.0000	1.0000	1.0000	1.0000
2.5	0.6000	1.0000	1.0000	1.0000
5	1.0000	0.6000	1.0000	1.0000
10	1.0000	1.0000	1.0000	0.8000
20	1.0000	1.0000	0.8000	1.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Isotonic	
			Mean	Min	Max	CV%	N			Mean	N-Mean
FSW Control	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4			0.9750	1.0000
1.25	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0.9750	1.0000
2.5	0.9000	0.9474	1.2305	0.8861	1.3453	18.660	4	17.50	10.00	0.9250	0.9487
5	0.9000	0.9474	1.2305	0.8861	1.3453	18.660	4	17.50	10.00	0.9250	0.9487
10	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	18.00	10.00	0.9250	0.9487
20	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	18.00	10.00	0.9250	0.9487

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05) Equality of variance cannot be confirmed	0.744108	0.916	-1.55348	1.468341

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test Treatments vs FSW Control	20	>20		

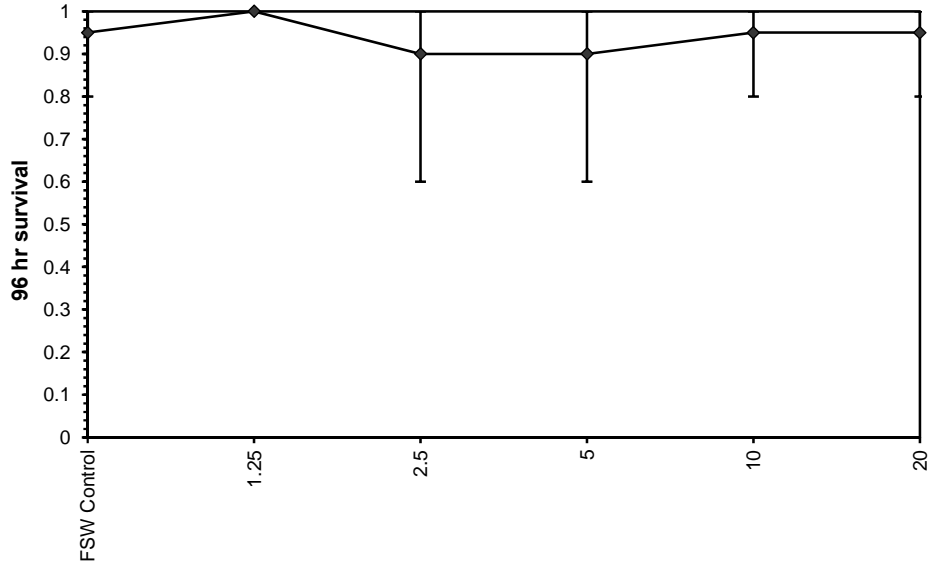
Log-Logit Interpolation (200 Resamples)				
Point	mg/L	SD	95% CL(Exp)	Skew
IC05	2.4757			
IC10	>20			
IC15	>20			
IC20	>20			
IC25	>20			
IC40	>20			
IC50	>20			



Amphipod Survival and Growth Test-96 hr survival

Start Date: 5/03/2014 13:45 Test ID: PR1147/02 Sample ID: S200 OILGONE
End Date: 9/03/2014 10:15 Lab ID: 6517 Sample Type: CP-Chemical product
Sample Date: Protocol: ESA 108 Test Species: ML-Melita Plumulosa
Comments:

Dose-Response Plot



Amphipod Survival and Growth Test-96 hr survival

Start Date:	5/03/2014 13:45	Test ID:	PR1147/02	Sample ID:	S200 OILGONE
End Date:	9/03/2014 10:15	Lab ID:	6517	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 108	Test Species:	ML-Melita Plumulosa
Comments:					

Auxiliary Data Summary

Conc-mg/L	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Non-immobilised	95.00	80.00	100.00	10.00	3.33	4
1.25		100.00	100.00	100.00	0.00	0.00	4
2.5		90.00	60.00	100.00	20.00	4.97	4
5		90.00	60.00	100.00	20.00	4.97	4
10		95.00	80.00	100.00	10.00	3.33	4
20		95.00	80.00	100.00	10.00	3.33	4
FSW Control	pH	8.10	8.10	8.10	0.00	0.00	1
1.25		8.20	8.20	8.20	0.00	0.00	1
2.5		8.20	8.20	8.20	0.00	0.00	1
5		8.20	8.20	8.20	0.00	0.00	1
10		8.20	8.20	8.20	0.00	0.00	1
20		8.30	8.30	8.30	0.00	0.00	1
FSW Control	DO %	100.40	100.40	100.40	0.00	0.00	1
1.25		101.20	101.20	101.20	0.00	0.00	1
2.5		101.90	101.90	101.90	0.00	0.00	1
5		102.50	102.50	102.50	0.00	0.00	1
10		103.00	103.00	103.00	0.00	0.00	1
20		104.20	104.20	104.20	0.00	0.00	1
FSW Control	Salinity ppt	35.50	35.50	35.50	0.00	0.00	1
1.25		35.50	35.50	35.50	0.00	0.00	1
2.5		35.50	35.50	35.50	0.00	0.00	1
5		35.50	35.50	35.50	0.00	0.00	1
10		35.50	35.50	35.50	0.00	0.00	1
20		35.50	35.50	35.50	0.00	0.00	1

Statistical Printouts for the Fish Imbalance Tests

Fish Imbalance Test-96 hr Imbalance

Start Date:	27/03/2014 15:30	Test ID:	PR1147/11	Sample ID:	S200 OILGONE
End Date:	31/03/2014 14:45	Lab ID:	6517	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 117	Test Species:	LT-Lates calcarifer

Comments:

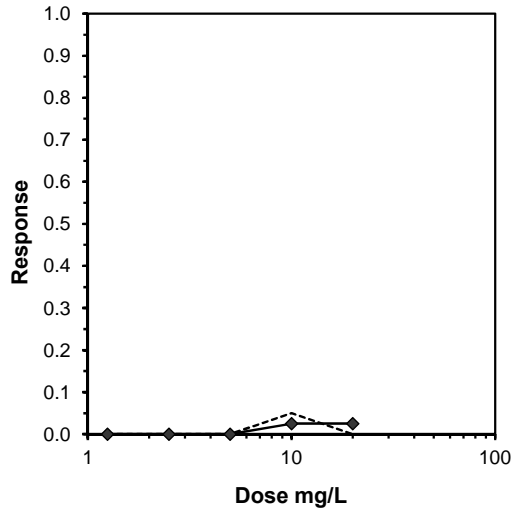
Conc-mg/L	1	2	3	4
FSW Control	1.0000	1.0000	1.0000	1.0000
1.25	1.0000	1.0000	1.0000	1.0000
2.5	1.0000	1.0000	1.0000	1.0000
5	1.0000	1.0000	1.0000	1.0000
10	1.0000	1.0000	1.0000	0.8000
20	1.0000	1.0000	1.0000	1.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Isotonic	
			Mean	Min	Max	CV%	N			Mean	N-Mean
FSW Control	1.0000	1.0000	1.3503	1.3453	1.3652	0.739	4			1.0000	1.0000
1.25	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	16.00	10.00	1.0000	1.0000
2.5	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	16.00	10.00	1.0000	1.0000
5	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	16.00	10.00	1.0000	1.0000
10	0.9500	0.9500	1.2857	1.1071	1.3453	9.261	4	14.50	10.00	0.9750	0.9750
20	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	16.00	10.00	0.9750	0.9750

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05) Equality of variance cannot be confirmed	0.509619	0.916	-2.98733	13.74812

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test Treatments vs FSW Control	20	>20		

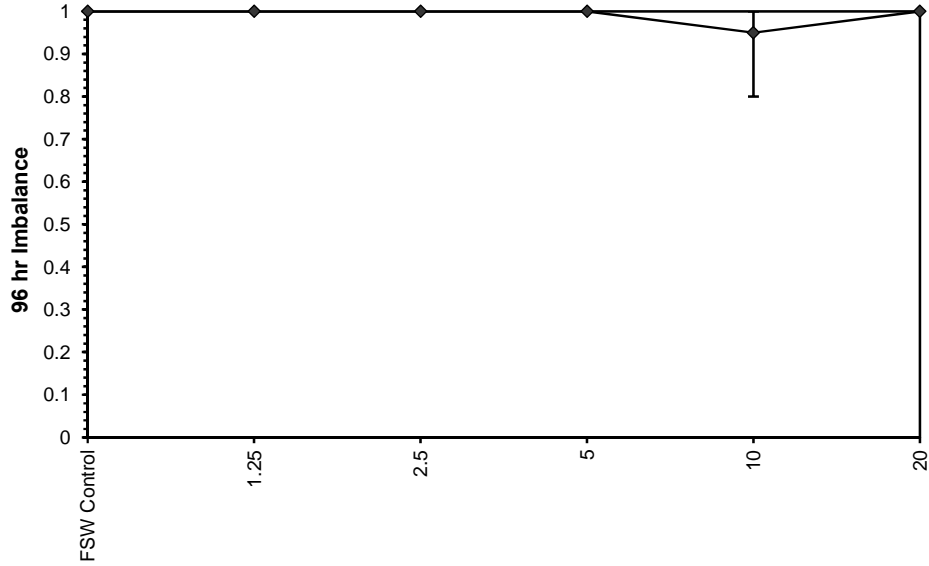
Log-Logit Interpolation (200 Resamples)				
Point	mg/L	SD	95% CL(Exp)	Skew
IC05	>20			
IC10	>20			
IC15	>20			
IC20	>20			
IC25	>20			
IC40	>20			
IC50	>20			



Fish Imbalance Test-96 hr Imbalance

Start Date: 27/03/2014 15:30 Test ID: PR1147/11 Sample ID: S200 OILGONE
End Date: 31/03/2014 14:45 Lab ID: 6517 Sample Type: CP-Chemical product
Sample Date: Protocol: ESA 117 Test Species: LT-Lates calcarifer
Comments:

Dose-Response Plot



Fish Imbalance Test-96 hr Imbalance

Start Date:	27/03/2014 15:30	Test ID:	PR1147/11	Sample ID:	S200 OILGONE
End Date:	31/03/2014 14:45	Lab ID:	6517	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 117	Test Species:	LT-Lates calcarifer

Comments:

Auxiliary Data Summary

Conc-mg/L	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Un-affected	100.00	100.00	100.00	0.00	0.00	4
1.25		100.00	100.00	100.00	0.00	0.00	4
2.5		100.00	100.00	100.00	0.00	0.00	4
5		100.00	100.00	100.00	0.00	0.00	4
10		95.00	80.00	100.00	10.00	3.33	4
20		100.00	100.00	100.00	0.00	0.00	4
FSW Control	pH	8.10	8.10	8.10	0.00	0.00	1
1.25		8.10	8.10	8.10	0.00	0.00	1
2.5		8.10	8.10	8.10	0.00	0.00	1
5		8.10	8.10	8.10	0.00	0.00	1
10		8.10	8.10	8.10	0.00	0.00	1
20		8.00	8.00	8.00	0.00	0.00	1
FSW Control	Salinity ppt	35.60	35.60	35.60	0.00	0.00	1
1.25		35.50	35.50	35.50	0.00	0.00	1
2.5		35.50	35.50	35.50	0.00	0.00	1
5		35.50	35.50	35.50	0.00	0.00	1
10		35.50	35.50	35.50	0.00	0.00	1
20		35.60	35.60	35.60	0.00	0.00	1
FSW Control	DO %	101.10	101.10	101.10	0.00	0.00	1
1.25		100.50	100.50	100.50	0.00	0.00	1
2.5		100.60	100.60	100.60	0.00	0.00	1
5		100.50	100.50	100.50	0.00	0.00	1
10		100.60	100.60	100.60	0.00	0.00	1
20		100.30	100.30	100.30	0.00	0.00	1