ECOTOXICOLOGY LABORATORY
TEST REPORT

CLIENT	JODY CELLAR LEICHHARDT SALT PTY LTD UNIT A7, LEVEL 1 435 ROBERTS ROAD SUBIACO WA 6008 AUSTRALIA

JOB INFORMATION	JOB REFERENCE	:	2024-MDTN-000077
	SAMPLE REFERENCE	:	001
	CLIENT ORDER NO.	:	LEI-S-PO-0214
	PROJECT	:	BITTERNS ASSESSMENT
	DATE SAMPLED	:	24/01/2024
	DATE RECEIVED	:	30/1/2024
	DATE REPORTED	:	12/04/2024

REPORT NOTES	Sample received in good condition at a temperature of 24.7°C.

TESTED BY

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DR TRISTAN STRINGER
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NATA Accreditation Number: 5646
Accredited for compliance with ISO/IEC 17025 – Testing

List of Revisions

REV.	DATE	REVISION DETAILS	AUTHOR	ISSUE
0	28/03/2024	Document prepared	Dr Tristan Stringer	Dr Tristan Stringer
1	12/04/2024	Additional 8-species SSD added. The 9 species SSD was recalculated using the proper application of the default ACR on the *A. sinjiensis* bioassay result for the SSD.	Dr Tristan Stringer	Dr Tristan Stringer

This document replaces and supersedes all previous versions.

Sample Information

SAMPLE REFERENCE	SAMPLE DATE	MATRIX	PRESERVATION	INTERTEK REFERENCE
B6	24/01/2024	Bitterns	Chilled	2024-MDTN-000077 - 001

Additional Sample Information

One (1) bitterns sample was received on 30/1/2024 for testing as per quote QU23462 REV1

Methodology

Following the protocol outlined in ANZECC/ARMCANZ (2000) and ANZG (2018), a suite of bioassays was used to assess the toxicity of one bitterns sample.

For quality assurance purposes reference toxicant) bioassays were tested simultaneously. Statistically calculated effect concentrations (EC_{10}, EC_{50}, NOEC and LOEC) have been reported the samples and reference toxicants.

Bioassay Details

BIOASSAY	PROTOCOL	REFERENCE	TEST SPECIES	TEMP.
15-min Acute Microtox Bioassay	WIECX-17	Microbics 1992	*V. fischeri*	15°C
72-hour Microalgal Growth Bioassay	WIECX-06	Stauber et al. 1994	*I. galbana*	22°C
72-hour Macroalgal Zoospore Germination Bioassay	WIECX-08	Burridge et al. 1999	*E. radiata*	22°C
72-hr Sea Urchin Larval Development Bioassay	WIECX-25	ASTM E1563	*E. mathaei*	25°C
5-7 day Copepod Early Life Stage Bioassay [1]	WIECX-26	ISO 16778	*G. imparipes*	22°C
48-hr Copepod Immobilisation Bioassay [1]	WIECX-28	Gissi et al. 2013	*A. sinjiensis*	30°C
48-hr Mollusc Larval Development Bioassay[2]	ESA Protocol	APHA 1998	*S. echinata*	29°C
1-hr Sea Urchin Fertilisation Bioassay [2]	ESA Protocol	USEPA 2002	*H. tuberculata*	20°C
8-day Sea Anemone pedal lacerate development [2]	ESA Protocol	Howe et al. 2014	*A. pulchella*	25°C
7-day Fish Larval Development Bioassay	WIECX-16	USEPA 1004.0	*S. lalandi*	22°C

Note [1] – Non-NATA Accredited Method
Note [2]: Subcontracted test to Ecotox Services Australia

RESULTS

Sample Information

SAMPLE REFERENCE	INTERTEK REFERENCE
B6	2024-MDTN-000077 - 001

Physicochemistry

PARAMETER	CONTROL	0.08%	0.16%	0.31%	0.63%	1.25%
pH [1]	8.01	8.06	8.07	8.08	8.12	8.18
Salinity (‰) [1]	35.0	35.2	35.5	35.9	36.8	38.7
DO (%) [1]	100	100	100	100	100	100

PARAMETER	2.5%	5%	10%
pH [1]	8.22	8.26	8.25
Salinity (‰) [1]	47 [3]	56 [3]	77 [3]
DO (%) [1]	100	100	100

Note [1] – Non-NATA Accredited Method.
Note [3] – Refractometer reading for indicative purposes only.

Concentration-Response Data

BIOASSAY (% CONTROL)	CONTROL	0.08%	0.16%	0.31%	0.63%	1.25%	2.5%	5%	10%
Microtox Bioassay	100 ± 4	98 ± 2	93 ± 8	94 ±2	92 ± 4	81 ± 0	71 ± 1	55 ± 2	30 ± 2
Microalgal Growth Bioassay	100 ± 1	-	-	103 ± 3	108 ± 5	106 ± 4	99 ± 9	18 ± 0	0 ± 0
Macroalgal Zoospore Germination Bioassay	100 ± 2	-	-	-	100 ± 2	99 ± 2	94 ± 1	75 ± 2	4 ± 0
Sea Urchin Larval Development Bioassay	100 ± 4	100 ± 2	98 ± 5	88 ± 4	15 ± 2	1 ± 1	0 ± 0	0 ± 0	-
Copepod Early Life Stage Bioassay [1]	100 ± 15	-	100 ± 9	95 ± 9	103 ± 11	59 ± 3	13 ± 9	-	-
Copepod Immobilisation Bioassay [1]	100 ± 8	-	103 ± 0	93 ± 12	88 ± 10	67 ± 10	0 ± 0	-	-
Mollusc Larval Development Bioassay [2]	100 ± 5		99 ± 3	101 ± 5	99 ± 3	101 ± 2	0 ± 0	0 ± 0	-
Sea Urchin Fertilisation Bioassay [2]	100 ± 1	-	100 ± 1	99 ± 2	100 ± 2	87 ± 7	0 ± 0	0 ± 0	-
Sea Anemone pedal lacerate development [2]	100 ± 10	-	100 ± 10	95 ± 10	105 ± 12	63 ± 0	0 ± 0	0 ± 0	-
Fish Larval Development Bioassay	100 ± 9	-	100 ± 7	88 ± 4	78 ± 9	35 ± 15	0 ± 0	-	-

Note [1] – Non-NATA Accredited Method
Note [2]: Subcontracted test to Ecotox Services Australia

Statistical Effects Data

BIOASSAY	EC_{10} (%)	EC_{50} (%)	NOEC (%)	LOEC (%)
Microtox Bioassay	0.84	5.60	0.31	0.63
Microalgal Growth Bioassay	2.95	4.04	2.5	5.0
Macroalgal Zoospore Germination Bioassay	3.60	6.32	1.25	2.5
Sea Urchin Larval Development Bioassay	0.30	0.46	0.16	0.31
Copepod Early Life Stage Bioassay [1]	0.84	1.50	0.63	1.25
Copepod Immobilisation Bioassay [1]	0.82 (0.143) [4]	1.43	0.63	1.25
Mollusc Larval Development Bioassay [2]	1.1	1.8	1.25	2.5
Sea Urchin Fertilisation Bioassay [2]	1.1	1.6	0.63	1.25
Sea Anemone pedal lacerate development [2]	0.9	1.4	0.63	1.25
Fish Larval Development Bioassay	0.44	1.02	0.31	0.63

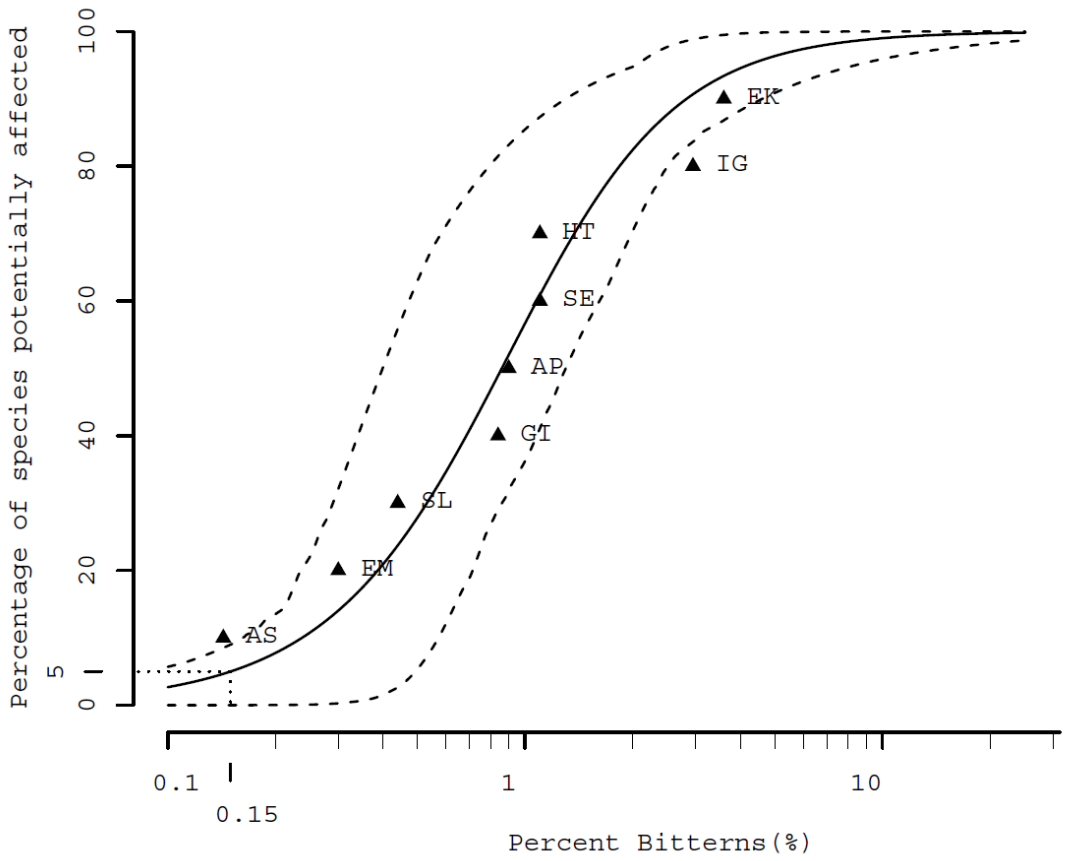
Note [1] – Non-NATA Accredited Method
Note [2] – Subcontracted test to Ecotox Services Australia
Note [4] – The acute EC_{50} converted into a sorrugate chronic endpoint using the default acute to chronic ratio (ACR) of 10 for use in the SSD following methodology in Warne et al. 2018
EC_{10} - The effective concentration where a 10% effect is observed compared to the control group.
EC_{50} - The effective concentration where a 50% effect is observed compared to the control group.
NOEC- No observed effect concentration
LOEC - Lowest observed effect concentration

Quality Assurance Limits

BIOASSAY	REFERENCE TOXICANT	OBSERVED EC_{50}	CUSUM CHART LIMITS	COEFFICIENT OF VARIANCE	CONTROL RESPONSE	TEST ACCEPTABILITY
Microtox Bioassay	Phenol	26. 2 mg/L	15.7 – 32.9 mg/L	18.4%	> 90%	PASS
Microalgal Growth Bioassay	Copper	25.8 µg/L	21.1 – 37.6 µg/L	15.4%	> 3 x cell divisions	PASS
Macroalgal Zoospore Germination Bioassay	Copper	130 µg/L	115 -203 µg/L	14.3%	>80% Germination	PASS
Sea Urchin Larval Development Bioassay	Copper	13.8 µg/L	9.5 – 21.5 µg/L	22.9%	>80% Development	PASS
Copepod Early Life Stage Bioassay [1]	Copper	16.3 µg/L	9.3 – 21.9 µg/L	24.0%	60-90% Development	PASS
Copepod Immobilisation Bioassay [1]	Copper	29.5 µg/L	19.2 – 36.0 µg/L	16.1%	>80% Survival	PASS
Mollusc Larval Development Bioassay[2]	Copper	15.3 µg/L	13.8 – 16.5 µg/L	-	>70% Development	PASS
Sea Urchin Fertilisation Bioassay [2]	Copper	56.9 µg/L	54.8 – 58.1 µg/L	-	>70% Fertilised	PASS
Sea Anemone pedal lacerate development [2]	Copper	8.8 µg/L	6.7 – 12.8µg/L	-	>90% Development	PASS
Fish Larvae Development	Copper	33.2 µg/L	27.2 µg/L	17.4%	>80% Development	PASS

Note [1] – Non-NATA Accredited Method

Figure 1 –Species Sensitivity Distribution with 9 Species [5]

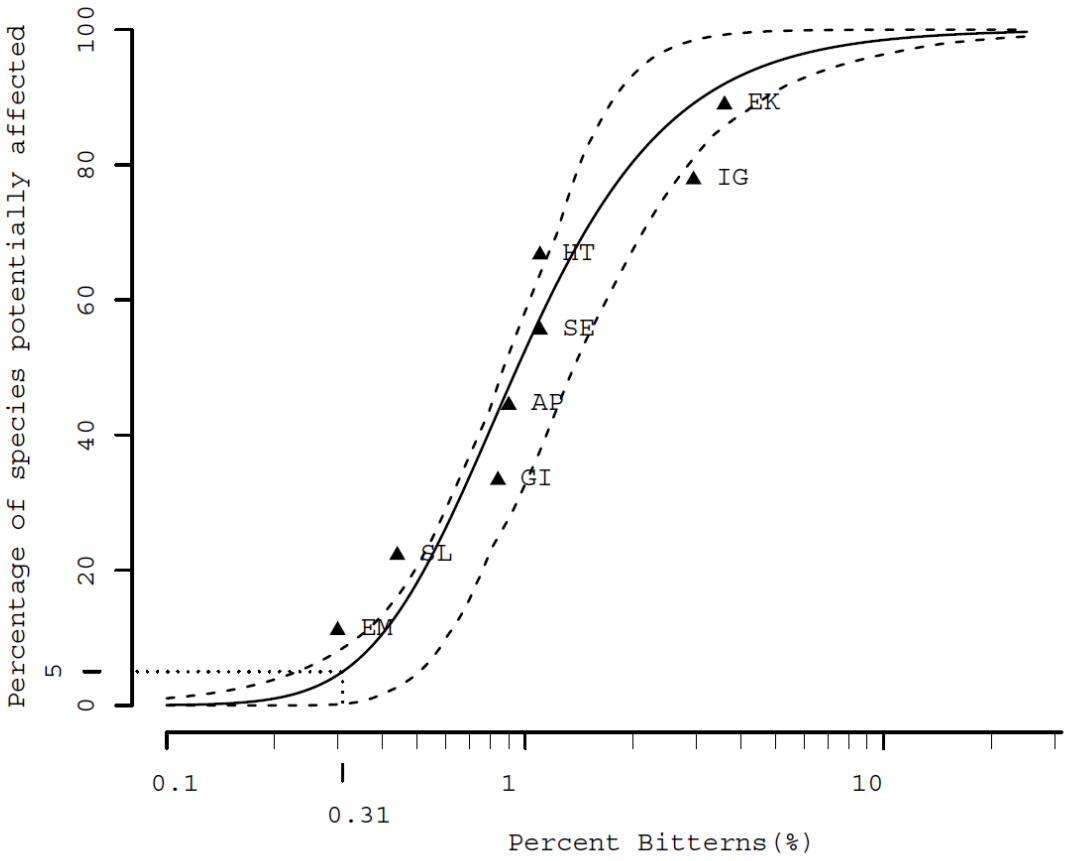


Species Protection Trigger Values and Dilution Factors – 9 Species SSD

LEVEL OF SPECIES PROTECTION	TRIGGER VALUE (% SAMPLE)	DILUTION FACTOR REQUIRED
99%	0.053	1887
95%	0.15	667
90%	0.24	416
80%	0.39	256

Note [5] – The species sensitivity distribution analysis is conducted in accordance with ANZG (2018) and Warne et al. (2018) using BurrliOZ 2.0 to derive trigger values, due to the nature of this type of analysis, it falls outside the scope of NATA accreditation.

Figure 2 –Species Sensitivity Distribution with 8 Species [5]



Species Protection Trigger Values and Dilution Factors – 8 Species SSD

LEVEL OF SPECIES PROTECTION	TRIGGER VALUE (% SAMPLE)	DILUTION FACTOR REQUIRED
99%	0.20	500
95%	0.31	323
90%	0.39	256
80%	0.52	192

Note [5] – The species sensitivity distribution analysis is conducted in accordance with ANZG (2018) and Warne et al. (2018) using BurrliOZ 2.0 to derive trigger values, due to the nature of this type of analysis, it falls outside the scope of NATA accreditation.

General Comments

Quality assurance and quality control criteria were within acceptable limits for all bioassays.

Using the methods outlined in Warne et al. (2018) and ANZG (2018) and using the statistical package BurrliOZ v2.0 a species sensitivity distribution (SSD) was preformed to calculate species protection guideline values for 99, 95, 90 and 80% species protection.

The SSD was derived using the EC_{10} results from the 5 chronic bioassays and the converted acute EC_{50} (using the default ACR of 10) for the one acute bioassay used in this assessment. It noted that the Microtox bioassay is not included in the SSD as per recommendations in ANZG 2018 & Batley et al. 2018. The resulting dilution factors for 99, 95, 90 and 80% species protection resulting from 9 species test suite for the bitterns sample were 1,887x, 667x, 416x, and 256x dilution respectively.

When reviewing the data used in the SSD it is noted that the most sensitive data point is the converted acute *A. sinjiensis* bioassay with a surrogate chronic EC_{10} value of 0.143%. the use of acute data in SSD is not ideal as it must be converted into a surrogate chronic first (i.e. by dividing the acute EC_{50} by 10 (Warn et al 2018)). Using converted acute data like this in SSDs is generally only used where there is no other chronic bioassay alternative. The justification for including the acute *A. sinjiensis* bioassay in this assessment is that it is widely distributed across tropical and sub-tropical marine waters of Australia (Gissi et al. 2013) and thus is a species relevant to the receiving environment. Currently there is no chronic bioassay for A. sinjiensis commercially available.

To understand the effect that this acute data point is having on the SSD and the resulting dilution factors, another SSD was run excluding the converted *A. sinjiensis* data point. This SSD still meets the minimum species requirement of 8 chronic bioassays for a high reliability SSD. This SSD (Fig. 2) resulted in dilution factors for 99, 95, 90 and 80% species protection of 500x, 323x, 256x, and 192x dilution.

The difference between the two 99% species protection dilution factors is significant, approximately a factor of four. When considering this, it shows that the 9 species SSD is heavily influenced by the converted acute data point and thus makes the SSD much more conservative when compared to the 8 species SSD. In addition, when considering reliability of the SSD (using Table 7 of Warne et al. 2018) the use of combined converted acute and chronic data set for the 9 species SSD results in a reliability of “Moderate”, but for the 8 species SSD the resulting reliability of the SSD is considered “High”.

It is beyond the scope of this report to determine the most suitable dilution factors for this bitterns sample.

APPENDIX A

Statistical Data

MICROTOX BIOASSAY

Start Date:	1/02/2024	Test ID:	VF240201	Sample ID:	2024-MDTN-000077-001
End Date:	1/02/2024	Lab ID:	ECOTOX-Intertek Geotech	Sample Type:	Bitterns
Sample Date:	24/01/2024	Protocol:	WIECX-17-Microtox	Test Species:	MICROTOX-Vibrio fischeri
Comments:					

Conc-%	1	2	3
B-Control	1.0000	0.9594	0.9955
0.08	0.9724	0.9980	0.9713
0.16	1.0000	0.8599	0.9009
0.31	0.9679	0.9394	0.9267
0.63	0.9652	0.9057	0.8972
1.25	0.8161	0.8112	0.8084
2.5	0.7101	0.7027	0.7129
5	0.5311	0.5591	0.5669
10	0.2812	0.3060	0.3113

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
B-Control	0.9850	1.0000	1.4641	1.3679	1.5208	5.719	3				4	300
0.08	0.9805	0.9955	1.4433	1.4005	1.5257	4.942	3	0.334	2.580	0.1607	6	300
0.16	0.9203	0.9343	1.3195	1.1871	1.5208	13.427	3	2.321	2.580	0.1607	24	300
0.31	0.9447	0.9591	1.3364	1.2966	1.3906	3.636	3	2.049	2.580	0.1607	16	300
*0.63	0.9227	0.9368	1.2954	1.2444	1.3832	5.893	3	2.708	2.580	0.1607	22	300
*1.25	0.8119	0.8243	1.1222	1.1177	1.1276	0.448	3	5.488	2.580	0.1607	56	300
*2.5	0.7086	0.7194	1.0006	0.9941	1.0054	0.582	3	7.440	2.580	0.1607	88	300
*5	0.5524	0.5608	0.8379	0.8165	0.8525	2.254	3	10.052	2.580	0.1607	134	300
*10	0.2995	0.3040	0.5790	0.5589	0.5919	3.045	3	14.207	2.580	0.1607	210	300

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.92897	0.894	0.95011	3.18186
Bartlett's Test indicates unequal variances (p = 1.00E-03)	26.1136	20.0902		

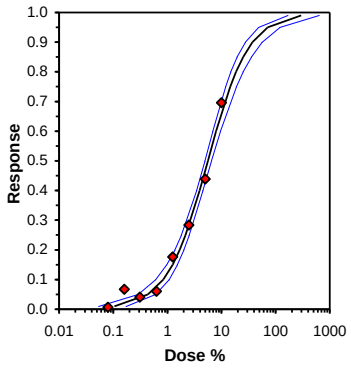
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.31	0.63	0.44193	322.581	0.05849	0.05916	0.26838	0.00582	2.0E-10	8, 18

Maximum Likelihood-Logit

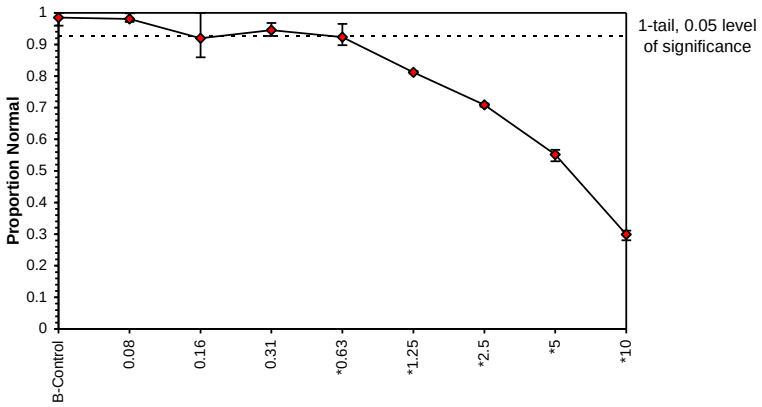
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.66099	0.1633	2.26141	3.06057	0.01333	19.3558	12.5916	3.6E-03			11
Intercept	-1.9893	0.10755	-2.2525	-1.7262							
TSCR	0.02358	0.0114	-0.0043	0.05148							

Point	Logits	%	95% Fiducial Limits	
EC01	-4.595	0.10489	0.05376	0.17257
EC05	-2.944	0.4376	0.28606	0.60291
EC10	-2.197	0.83537	0.60685	1.06705
EC15	-1.735	1.24661	0.96323	1.525
EC20	-1.386	1.6851	1.35911	2.00248
EC25	-1.099	2.1614	1.79919	2.51742
EC40	-0.405	3.9375	3.44123	4.49064
EC50	0.000	5.59235	4.896	6.4708
EC60	0.405	7.9427	6.83825	9.49801
EC75	1.099	14.4695	11.8163	18.7535
EC80	1.386	18.5594	14.7602	24.9852
EC85	1.735	25.0875	19.2863	35.4286
EC90	2.197	37.4379	27.4565	56.4529
EC95	2.944	71.4685	48.4367	120.148
EC99	4.595	298.154	168.782	640.973

Significant heterogeneity detected (p = 3.60E-03)



Dose-Response Plot



MICROALGAL GROWTH BIOASSAY

Start Date:	23/02/2024	Test ID:	ER240223	Sample ID:	2024-MDTN-000077-001
End Date:	26/02/2024	Lab ID:	ECOTOX-Intertek Geotech	Sample Type:	Bitterns
Sample Date:	24/01/2024	Protocol:	WIECX-08-Macroalgae Germ	Test Species:	EK-Ecklonia radiata
Comments:					

Conc-%	1	2	3
B-Control	1.0000	0.9914	0.9914
0.31	1.0000	0.9877	1.0000
0.63	1.0000	1.0000	1.0000
1.25	1.0000	1.0000	1.0000
2.5	0.9767	0.9103	1.0000
5	0.1806	0.1880	0.1806

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
B-Control	0.9943	1.0000	1.4922	1.4779	1.5208	1.658	3				2	300
0.31	0.9959	1.0016	1.5004	1.4597	1.5208	2.349	3	-0.183	2.500	0.1125	1	300
0.63	1.0000	1.0058	1.5208	1.5208	1.5208	0.000	3	-0.635	2.500	0.1125	0	300
1.25	1.0000	1.0058	1.5208	1.5208	1.5208	0.000	3	-0.635	2.500	0.1125	0	300
2.5	0.9623	0.9679	1.4016	1.2667	1.5208	9.117	3	2.014	2.500	0.1125	11	300
*5	0.1830	0.1841	0.4421	0.4389	0.4484	1.241	3	23.343	2.500	0.1125	245	300

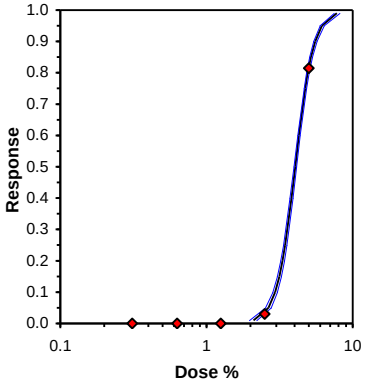
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.7628	0.858	-0.5304	6.1937
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	2.5	5	3.53553	40	0.0299	0.03008	0.55196	0.00304	7.1E-11	5, 12

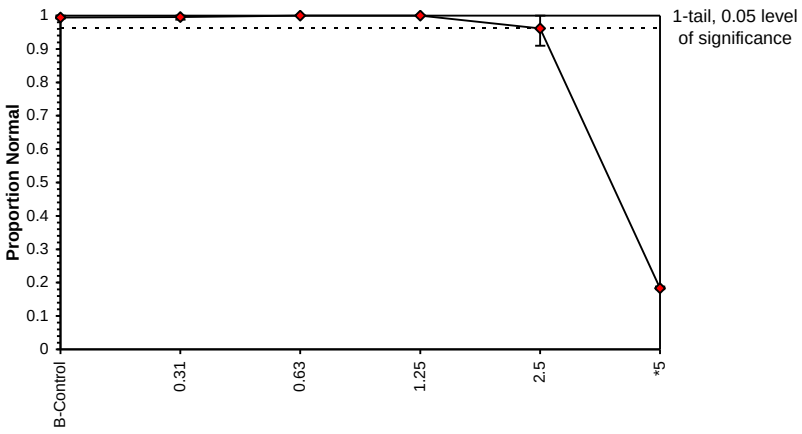
Maximum Likelihood-Logit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	16.1154	0.61035	14.9192	17.3117	0.00667	1.67784	7.81473	0.64			6
Intercept	-9.7717	0.40153	-10.559	-8.9846							
TSCR	0.00251	0.00147	-0.0004	0.00539							

Point	Logits	%	95% Fiducial Limits	
EC01	-4.595	2.09515	1.96572	2.21449
EC05	-2.944	2.65243	2.53277	2.7618
EC10	-2.197	2.95128	2.83953	3.05342
EC15	-1.735	3.15295	3.0471	3.24997
EC20	-1.386	3.31384	3.21277	3.40684
EC25	-1.099	3.45289	3.35588	3.54263
EC40	-0.405	3.81236	3.72438	3.89563
EC50	0.000	4.03974	3.95538	4.12137
EC60	0.405	4.28069	4.1974	4.36364
EC75	1.099	4.72634	4.63584	4.8217
EC80	1.386	4.92466	4.82715	5.02965
EC85	1.735	5.17595	5.06671	5.29626
EC90	2.197	5.52964	5.39957	5.67633
EC95	2.944	6.15266	5.97717	6.35591
EC99	4.595	7.78918	7.46341	8.17953



Dose-Response Plot



MACROALGAL ZOOSPORE GERMINATION BIOASSAY

Start Date:	23/02/2024	Test ID:	ER240223	Sample ID:	2024-MDTN-000077-001
End Date:	26/02/2024	Lab ID:	ECOTOX-Intertek Geotech	Sample Type:	Bitterns
Sample Date:	24/01/2024	Protocol:	WIECX-08-Macroalgae Germ	Test Species:	EK-Ecklonia radiata
Comments:					

Conc-%	1	2	3
B-Control	1.0000	1.0000	0.9759
0.63	1.0000	0.9794	1.0000
1.25	0.9794	0.9759	1.0000
2.5	0.9488	0.9398	0.9398
5	0.7229	0.7590	0.7590
10	0.0361	0.0350	0.0361

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
B-Control	0.9920	1.0000	1.4855	1.4149	1.5208	4.114	3				2	300
0.63	0.9931	1.0012	1.4894	1.4268	1.5208	3.644	3	-0.114	2.500	0.0864	2	300
1.25	0.9851	0.9931	1.4542	1.4149	1.5208	3.988	3	0.907	2.500	0.0864	4	300
*2.5	0.9428	0.9504	1.3294	1.3228	1.3425	0.856	3	4.518	2.500	0.0864	17	300
*5	0.7470	0.7530	1.0439	1.0164	1.0577	2.283	3	12.781	2.500	0.0864	76	300
*10	0.0358	0.0360	0.1902	0.1881	0.1913	0.955	3	37.490	2.500	0.0864	289	300

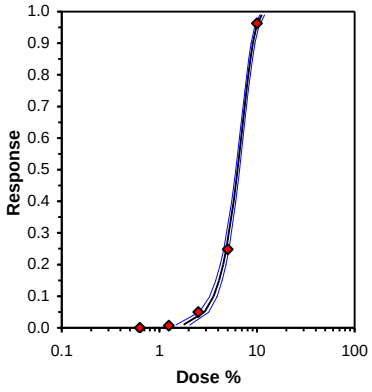
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96569	0.858	-0.3642	-0.0162
Bartlett's Test indicates equal variances (p = 0.02)	13.7421	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1.25	2.5	1.76777	80	0.02192	0.02209	0.77007	0.00179	4.3E-13	5, 12

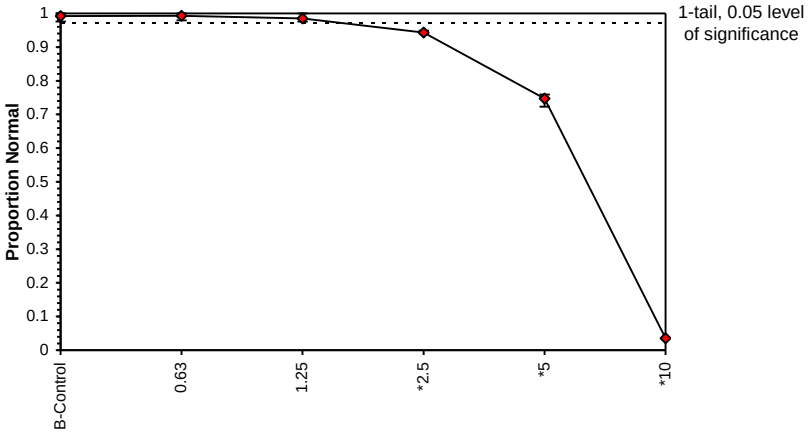
Maximum Likelihood-Weibull

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	7.66066	0.41526	6.84675	8.47457	0.00667	3.69193	7.81473	0.3			4
Intercept	-6.502	0.36746	-7.2222	-5.7818							
TSCR	0.00863	0.00327	0.00222	0.01504							

Point	Weibull	%	95% Fiducial Limits	
EC01	-4.600	1.77117	1.48396	2.04539
EC05	-2.970	2.89088	2.56087	3.19306
EC10	-2.250	3.58917	3.25561	3.89077
EC15	-1.817	4.08855	3.75967	4.38493
EC20	-1.500	4.4973	4.1753	4.78779
EC25	-1.246	4.85416	4.53954	5.13915
EC40	-0.672	5.76852	5.47326	6.04324
EC50	-0.367	6.32276	6.03489	6.59842
EC60	-0.087	6.87604	6.58843	7.1618
EC75	0.327	7.78732	7.47889	8.11523
EC80	0.476	8.14461	7.82025	8.49822
EC85	0.640	8.55732	8.20938	8.94668
EC90	0.834	9.07031	8.68616	9.51242
EC95	1.097	9.81688	9.36844	10.35
EC99	1.527	11.1713	10.5797	11.9035



Dose-Response Plot



SEA URCHIN LARVAL DEVELOPMENT BIOASSAY

Start Date:	8/03/2024	Test ID:	SD240308	Sample ID:	2024-MDTN-000077-001
End Date:	11/03/2024	Lab ID:	ECOTOX-Intertek Geotech	Sample Type:	Bitterns
Sample Date:	24/01/2024	Protocol:	WIECX-25-Sea Urchin Larval	Test Species:	EM-Echinometra mathaei
Comments:					

Conc-%	1	2	3
B-Control	1.0000	0.9597	1.0000
0.08	0.9991	0.9876	1.0000
0.16	0.9716	1.0000	0.9316
0.31	0.9258	0.8636	0.8596
0.63	0.1241	0.1690	0.1637
1.25	0.0177	0.0000	0.0102
2.5	0.0000	0.0000	0.0000
5	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
B-Control	0.9866	1.0000	1.4701	1.3687	1.5208	5.972	3				4	300
0.08	0.9956	1.0091	1.5072	1.4593	1.5416	2.838	3	-0.669	2.500	0.1389	1	300
0.16	0.9678	0.9809	1.4095	1.3063	1.5208	7.625	3	1.090	2.500	0.1389	10	300
*0.31	0.8830	0.8950	1.2247	1.1868	1.2949	4.965	3	4.416	2.500	0.1389	35	300
*0.63	0.1523	0.1543	0.4001	0.3600	0.4236	8.708	3	19.262	2.500	0.1389	255	300
*1.25	0.0093	0.0094	0.0949	0.0500	0.1336	44.369	3	24.754	2.500	0.1389	297	300
2.5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	3				300	300
5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	3				300	300

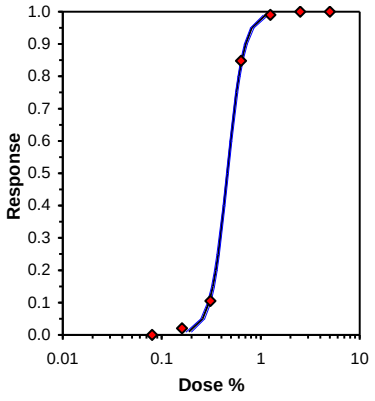
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.97133	0.858	-0.1404	-0.2446
Bartlett's Test indicates equal variances (p = 0.64)	3.393	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.16	0.31	0.22271	625	0.0462	0.04668	1.1242	0.00463	1.3E-11	5, 12

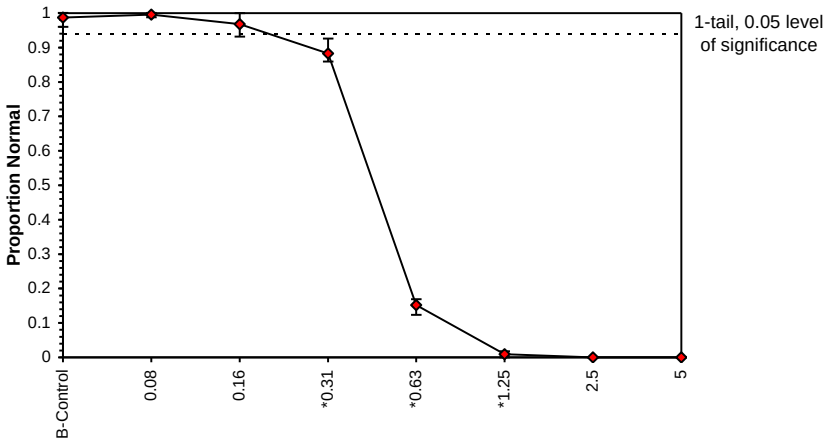
Maximum Likelihood-Logit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	11.8627	0.36698	11.1434	12.582	0.01333	8.22339	11.0705	0.14			11
Intercept	4.01093	0.13132	3.75354	4.26832							
TSCR	0.01361	0.00413	0.00551	0.0217							

Point	Logits	%	95% Fiducial Limits	
EC01	-4.595	0.18816	0.17614	0.19959
EC05	-2.944	0.25922	0.24724	0.27052
EC10	-2.197	0.29969	0.28808	0.31063
EC15	-1.735	0.32784	0.31656	0.33852
EC20	-1.386	0.35077	0.33976	0.36126
EC25	-1.099	0.37092	0.36011	0.38127
EC40	-0.405	0.42433	0.41379	0.4347
EC50	0.000	0.45908	0.44835	0.46984
EC60	0.405	0.49667	0.48535	0.50832
EC75	1.099	0.5682	0.55456	0.58282
EC80	1.386	0.60083	0.58568	0.6173
EC85	1.735	0.64285	0.62543	0.6621
EC90	2.197	0.70325	0.68202	0.72709
EC95	2.944	0.81301	0.78367	0.84663
EC99	4.595	1.12007	1.06284	1.18763



Dose-Response Plot



COPEPOD EARLY LIFE STAGE BIOASSAY

Start Date:	6/02/2024	Test ID:	GI240206	Sample ID:	2024-MDTN-000077-001
End Date:	14/02/2024	Lab ID:	ECOTOX-Intertek Geotech	Sample Type:	Bitterns
Sample Date:	24/01/2024	Protocol:	WIECX-26-Copepod Larval D	Test Species:	GI-Gladioferens Imparipes
Comments:					

Conc-%	1	2	3	4	5
B-Control	1.0000	1.0000	0.9091	0.7955	1.0000
0.16	0.9091	1.0000	1.0000		
0.31	0.8523	0.9659	1.0000		
0.63	1.0000	0.9091	1.0000		
1.25	0.6250	0.5682	0.5682		
2.5	0.2273	0.1136	0.0568		

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
B-Control	0.9409	1.0000	1.3857	1.1015	1.5208	13.984	5				29	500
0.16	0.9697	1.0306	1.4354	1.2645	1.5208	10.308	3	-0.441	2.624	0.2958	9	300
0.31	0.9394	0.9984	1.3607	1.1763	1.5208	12.753	3	0.221	2.624	0.2958	18	300
0.63	0.9697	1.0306	1.4354	1.2645	1.5208	10.308	3	-0.441	2.624	0.2958	9	300
*1.25	0.5871	0.6240	0.8731	0.8538	0.9117	3.832	3	4.548	2.624	0.2958	124	300
*2.5	0.1326	0.1409	0.3605	0.2407	0.4969	35.767	3	9.096	2.624	0.2958	260	300

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.903	0.868	-0.6566	-0.7198
Bartlett's Test indicates equal variances (p = 0.53)	4.14342	15.0863		

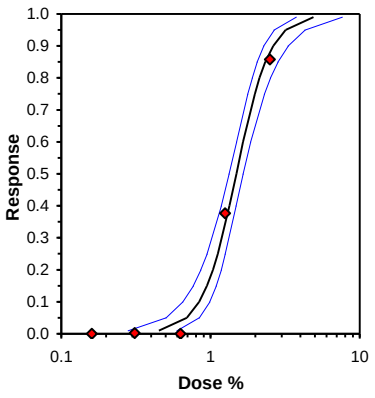
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	0.63	1.25	0.88741	158.73	0.18012	0.18644	0.59885	0.02382	1.6E-06	5, 14

Maximum Likelihood-Logit

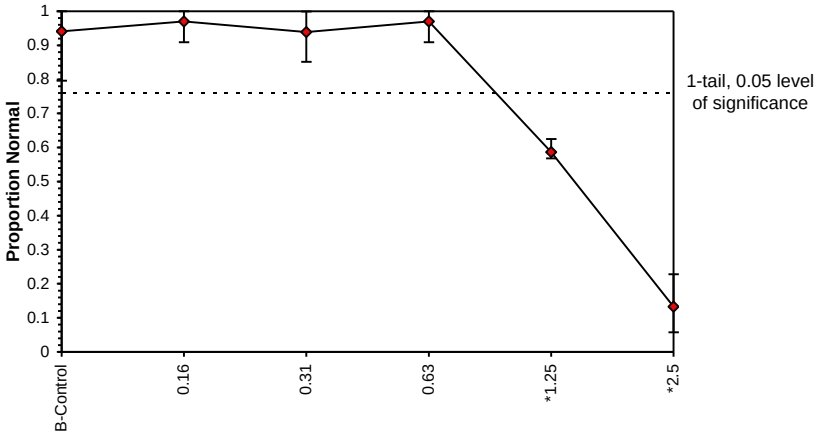
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	8.87776	0.758	6.46546	11.2901	0.058	16.0161	7.81473	1.1E-03			5
Intercept	-1.5263	0.18263	-2.1075	-0.9451							
TSCR	0.04391	0.01407	-0.0009	0.08869							

Point	Logits	%	95% Fiducial Limits	
EC01	-4.595	0.45116	0.28102	0.59545
EC05	-2.944	0.69225	0.50213	0.83998
EC10	-2.197	0.84029	0.65114	0.9844
EC15	-1.735	0.94741	0.76333	1.08807
EC20	-1.386	1.03698	0.85903	1.17513
EC25	-1.099	1.11732	0.94563	1.25414
EC40	-0.405	1.33738	1.18111	1.48041
EC50	0.000	1.48568	1.33298	1.64617
EC60	0.405	1.65043	1.49126	1.84658
EC75	1.099	1.97549	1.77334	2.28946
EC80	1.386	2.12853	1.89611	2.51558
EC85	1.735	2.32977	2.05111	2.82641
EC90	2.197	2.62677	2.2703	3.3088
EC95	2.944	3.18852	2.66401	4.2852
EC99	4.595	4.89242	3.76231	7.64833

Significant heterogeneity detected (p = 1.13E-03)



Dose-Response Plot



COPEPOD IMMOBILISATION BIOASSAY

Start Date:	27/02/2024	Test ID:	AS240227	Sample ID:	2024-MDTN-000077-001
End Date:	29/02/2024	Lab ID:	ECOTOX-Intertek Geotech	Sample Type:	Bitterns
Sample Date:	24/01/2024	Protocol:	WIECX-28-Copepod Immobil	Test Species:	AS-Acartia sinjiensis
Comments:					

Conc-%	1	2	3	4	5	6
B-Control	1.0000	0.8276	1.0000	1.0000	1.0000	1.0000
0.16	1.0000	1.0000	1.0000	1.0000		
0.31	0.8276	1.0000	1.0000	0.8276		
0.63	1.0000	0.8276	0.8276	0.8276		
1.25	0.8276	0.6207	0.6207	0.6207		
2.5	0.0100	0.0000	0.0000	0.0000		

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.9713	1.0000	1.4577	1.1426	1.5208	10.591	6			17	600
0.16	1.0000	1.0296	1.5208	1.5208	1.5208	0.000	4	24.00	11.00	0	400
0.31	0.9138	0.9408	1.3317	1.1426	1.5208	16.396	4	18.00	11.00	34	400
0.63	0.8707	0.8964	1.2371	1.1426	1.5208	15.284	4	15.00	11.00	51	400
*1.25	0.6724	0.6923	0.9661	0.9073	1.1426	12.178	4	10.50	11.00	131	400
*2.5	0.0025	0.0026	0.0626	0.0500	0.1002	40.080	4	10.00	11.00	399	400

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9641	0.891	-0.0987	0.85115
Equality of variance cannot be confirmed				

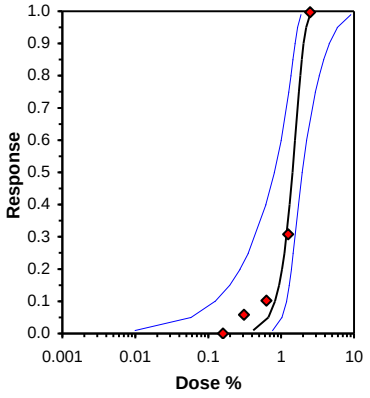
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Wilcoxon Rank Sum Test	0.63	1.25	0.88741	158.73

Maximum Likelihood-Weibull

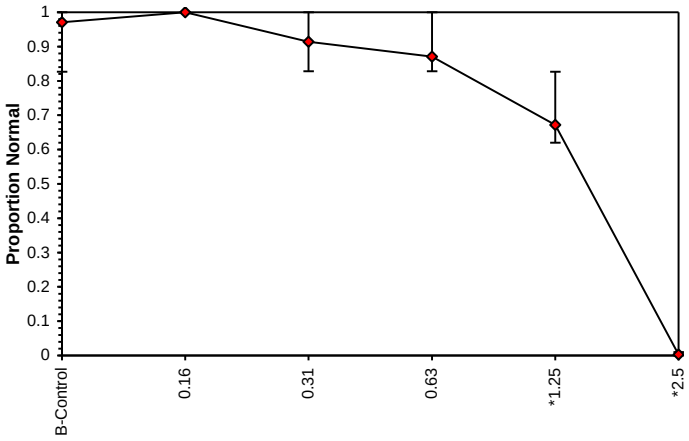
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	7.85608	1.80381	2.11556	13.5966	0.02833	52.6784	7.81473	2.2E-11			10
Intercept	-1.5893	0.46189	-3.0592	-0.1194							
TSCR	0.0398	0.02185	-0.0297	0.10934							

Point	Weibull	%	95% Fiducial Limits	
EC01	-4.600	0.41376	0.00997	0.76222
EC05	-2.970	0.66716	0.05765	1.0242
EC10	-2.250	0.82386	0.1241	1.17649
EC15	-1.817	0.93545	0.19583	1.28601
EC20	-1.500	1.02654	0.2721	1.37896
EC25	-1.246	1.10589	0.35258	1.46485
EC40	-0.672	1.30858	0.616	1.72624
EC50	-0.367	1.43103	0.80458	1.94013
EC60	-0.087	1.55301	0.9951	2.22836
EC75	0.327	1.7534	1.26882	2.94186
EC80	0.476	1.8318	1.35734	3.31787
EC85	0.640	1.92226	1.44802	3.82477
EC90	0.834	2.03454	1.54696	4.56776
EC95	1.097	2.19768	1.67147	5.8861
EC99	1.527	2.49286	1.86139	9.0775

Significant heterogeneity detected (p = 2.15E-11)



Dose-Response Plot



FISH LARVAL DEVELOPMENT BIOASSAY

Start Date:	20/03/2024	Test ID:	SL240320	Sample ID:	2024-MDTN-000077-001
End Date:	27/03/2024	Lab ID:	ECOTOX-Intertek Geotech	Sample Type:	Bitterns
Sample Date:	24/01/2024	Protocol:	WIECX-16-Fish Developmen	Test Species:	SL-Seriola lalandi
Comments:					

Conc-%	1	2	3
B-Control	0.9796	0.9184	1.0000
0.16	1.0000	0.9184	1.0000
0.31	0.8571	0.9184	0.8571
0.63	0.7959	0.8571	0.6735
1.25	0.1837	0.3673	0.4898
2.5	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
B-Control	0.9660	1.0000	1.4098	1.2810	1.5208	8.572	3				10	300
0.16	0.9728	1.0070	1.4409	1.2810	1.5208	9.606	3	-0.306	2.470	0.2512	8	300
0.31	0.8776	0.9085	1.2158	1.1832	1.2810	4.646	3	1.907	2.470	0.2512	36	300
*0.63	0.7755	0.8028	1.0826	0.9626	1.1832	10.309	3	3.216	2.470	0.2512	67	300
*1.25	0.3469	0.3592	0.6231	0.4429	0.7752	26.948	3	7.734	2.470	0.2512	196	300
2.5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	3				300	300

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.92902	0.835	-0.4677	-1.0144
Bartlett's Test indicates equal variances (p = 0.78)	1.75851	13.2767		

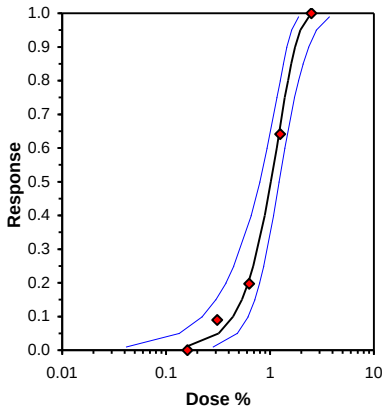
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.31	0.63	0.44193	322.581	0.13485	0.13841	0.32887	0.01552	7.2E-05	4, 10

Maximum Likelihood-Weibull

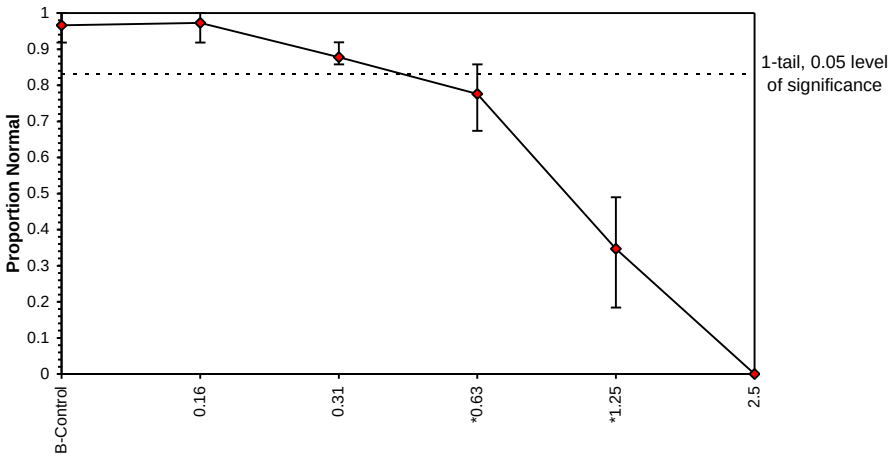
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	5.17359	0.62329	3.19	7.15717	0.03333	12.5797	7.81473	5.6E-03			3
Intercept	-0.416	0.13458	-0.8443	0.01225							
TSCR	0.03427	0.0164	-0.0179	0.08647							

Point	Weibull	%	95% Fiducial Limits	
EC01	-4.600	0.15533	0.04137	0.28314
EC05	-2.970	0.32086	0.13286	0.48308
EC10	-2.250	0.44202	0.22152	0.61406
EC15	-1.817	0.53606	0.30059	0.71135
EC20	-1.500	0.6173	0.37497	0.79383
EC25	-1.246	0.69119	0.44676	0.86852
EC40	-0.672	0.89244	0.65608	1.07674
EC50	-0.367	1.02229	0.79536	1.22131
EC60	-0.087	1.1575	0.93707	1.38708
EC75	0.327	1.39172	1.16212	1.72295
EC80	0.476	1.48731	1.24543	1.87865
EC85	0.640	1.60025	1.33832	2.07557
EC90	0.834	1.74432	1.44968	2.34535
EC95	1.097	1.96106	1.60597	2.78614
EC99	1.527	2.37468	1.87973	3.72835

Significant heterogeneity detected (p = 5.64E-03)



Dose-Response Plot



APPENDIX B

ECOTOX SERVICES REPORT

Toxicity Assessment of 2024-MDTN-000077

Intertek

Test Report

March 2024

T +61 2 9420 9481
Unit 27, 2 Chaplin Drive
Lane Cove NSW 2066 Australia
www.ecotox.com.au
ECOTOX SERVICES AUSTRALIA PTY LIMITED
ABN 95 619 426 201

Toxicity Test Report: TR2231/1

Accredited for compliance with ISO/IEC 17025 - Testing

Client:	Intertek 1 Fleet street Fremantle WA 6160	**ESA Job #:** **Date Sampled:** **Date Received:**	PR2231 20 February 2024 22 February 2024
Attention:	Tristan Stringer	**Sampled By:**	Client
Client Ref:	AUS05-0000152984	**ESA Quote #:**	PL2231_q01

Lab ID No.:	**Sample Name:**	**Sample Description:**
11455	MDTN_000077	Aqueous sample, pH 6.7*, salinity 312‰ (estimate)*. Sample received at 16ºC* in apparent good condition.

*NATA accreditation does not cover the performance of this service

Test Performed:	1-hr sea urchin fertilisation success test using *Heliocidaris tuberculata*
Test Protocol:	ESA SOP 104 (ESA 2014), based on USEPA (2002) and Simon and Laginestra (1996)
Test Temperature:	The test was performed at 20±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The sample was serially diluted with filtered seawater (FSW) to achieve the test concentrations. A FSW control was tested concurrently with the sample.
Source of Test Organisms:	Field collected from South Maroubra, NSW.
Test Initiated:	01 March 2024 at 1920h

Sample 11455*: MDTN_000077*		*Vacant*	*Vacant*
Concentration (%)	**% Fertilised Eggs (Mean ± SD)**		
FSW Control	91.0 ± 1.4		
0.16	91.0 ± 0.8		
0.31	90.5 ± 2.1		
0.63	91.3 ± 1.9		
1.25	79.8 ± 6.7 *		
2.5	0.0 ± 0.0		
5	0.0 ± 0.0		
IC10 = 1.1 (0.91-1.35)%			
EC50 =1.6 (1.58-1.66)%			
NOEC = 0.63%			
LOEC = 1.25%			

*Significantly lower percentage fertilised eggs compared with the FSW Control (Dunnett's Test, 1-tailed, P=0.05)

QA/QC Parameter	**Criterion**	**This Test**	**Criterion met?**
Control mean % fertilised eggs	≥70.0%	91.0%	Yes
Reference Toxicant within cusum chart limits	54.8-58.1µg Cu/L	56.9µg Cu/L	Yes

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Lane Cove NSW 2066 Australia
www.ecotox.com.au
ECOTOX SERVICES AUSTRALIA PTY LIMITED
ABN 95 619 426 201

Test Report Authorised by: Dr Rick Krassoi, Director on 18 March 2024

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

NATA Accredited Laboratory Number: 14709

Citations:

ESA (2014) ESA SOP 104 - *Sea Urchin Fertilisation Success Test*. Issue No. 13. Ecotox Services Australasia, Sydney NSW.

Simon, J. and Laginestra, E.(1997) Bioassay for testing sublethal toxicity in effluents, using gametes of sea urchin *Heliocidaris tuberculata*. National Pulp Mills Research Program Technical Report No. 20. CSIRO, Canberra ACT

USEPA (2002) Short-term methods for measuring the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. Third Edition. United States Environmental Protection Agency, Office of Water, Washington DC, EPA-821-R-02-014.

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Toxicity Test Report: TR2231/2

Accredited for compliance with ISO/IEC 17025 - Testing

Client:	Intertek	**ESA Job #:**	PR2231
	1 Fleet street	**Date Sampled:**	20 February 2024
	Fremantle WA 6160	**Date Received:**	22 February 2024
Attention:	Tristan Stringer	**Sampled By:**	Client
Client Ref:	AUS05-0000152984	**ESA Quote #:**	PL2231_q01

Lab ID No.:	**Sample Name:**	**Sample Description:**
11455	MDTN_000077	Aqueous sample, pH 6.7*, salinity 312‰ (estimate)*. Sample received at 16ºC* in apparent good condition.

*NATA accreditation does not cover the performance of this service

Test Performed:	48-hr larval development test using the milky oyster *Saccostrea scyphophilla* (formerly referred to as *Saccostrea echinata)*
Test Protocol:	ESA SOP 106 (ESA 2016), based on APHA (1998) and Krassoi (1995)
Test Temperature:	The test was performed at 29±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The sample was serially diluted with filtered seawater (FSW) to achieve the test concentrations. A FSW control was tested concurrently with the sample.
Source of Test Organisms:	Field collected from Mackay, QLD.
Test Initiated:	01 March 2024 at 1900h

Sample 11455*: MDTN_000077*	*Vacant*	*Vacant*
Concentration (%) / **% Normal larvae (Mean ± SD)**		
FSW Control: 73.0 ± 4.7		
0.16: 72.5 ± 2.9		
0.31: 74.3 ± 5.0		
0.63: 72.5 ± 2.7		
1.25: 73.8 ± 2.2		
2.5: 0.0 ± 0.0		
5: 0.0 ± 0.0		
48-hr IC10 = 1.1 (0.91-1.35)% **48-hr EC50 = 1.8 (1.76-1.77)%** **NOEC = 1.25%** **LOEC = 2.5%**		

QA/QC Parameter	**Criterion**	**This Test**	**Criterion met?**
FSW Control mean % normal	≥70%	73.0%	Yes
Reference Toxicant within cusum chart limits	15.3µg Cu/L	13.8-16.5µg Cu/L	Yes

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Toxicity Test Report: TR2231/2

Test Report Authorised by: Dr Rick Krassoi, Director on 18 March 2024

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

NATA Accredited Laboratory Number: 14709

Citations:

APHA (1998) Standard Methods for the Examination of Water and Wastewater. 20th Ed. American Public Health Association, American Water Works Association and the Water Environment Federation, Washington, DC.

ESA (2016) SOP 106 – *Bivalve Larval Development Test*. Issue No. 15. Ecotox Services Australasia, Sydney, NSW.

Krassoi, R (1995) Salinity adjustment of effluents for use with marine bioassays: effects on the larvae of the doughboy scallop Chlamys asperrimus and the Sydney rock oyster *Saccostrea commercialis*. *Australasian Journal of Ecotoxicology*, 1: 143-148.

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Toxicity Test Report: TR2231/3

Client:	Intertek	**ESA Job #:**	PR2231
	1 Fleet street	**Date Sampled:**	20 February 2024
	Fremantle WA 6160	**Date Received:**	22 February 2024
Attention:	Tristan Stringer	**Sampled By:**	Client
Client Ref:	AUS05-0000152984	**ESA Quote #:**	PL2231_q01

Lab ID No.:	**Sample Name:**	**Sample Description:**
11455	MDTN_000077	Aqueous sample, pH 6.7*, salinity 312‰ (estimate)*. Sample received at 16ºC in apparent good condition.

Test Performed:	8-day Sea anemone pedal lacerate development test using *Aiptasia pulchella*
Test Protocol:	ESA SOP 128 (ESA 2014), based on Howe *et al* (2014)
Test Temperature:	The test was performed at 25±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The sample was serially diluted with filtered seawater (FSW) to achieve the test concentrations. A FSW control was tested concurrently with the sample.
Source of Test Organisms:	In-house culture, originally sourced from Marine Ecology Research Centre at Southern Cross University, Lismore, NSW
Test Initiated:	01 March 2024 at 2030h

Sample 11455*: MDTN_000077* **Concentration (%)**	**% Normal (Mean ± SD)**	*Vacant*	*Vacant*
FSW Control	95.0 ± 10.0		
0.16	95.0 ± 10.0		
0.31	90.0 ± 11.6		
0.63	100 ± 0.0		
1.25	60.0 ± 0.0 *		
2.5	0.0 ± 0.0		
5.0	0.0 ± 0.0		
8-d EC10 = 0.9 (0.56-0.95)%			
8-d EC50 = 1.4 (1.18-1.59)%			
NOEC = 0.63%			
LOEC = 1.25%			

*Significantly lower percent normally developed juveniles 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 % unaffected	≥90.0%	95.0%	Yes
Reference Toxicant within cusum chart limits	6.7-12.8µg Cu/L	8.8µg Cu/L	Yes

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Toxicity Test Report: TR2231/3

Test Report Authorised by: Dr Rick Krassoi, Director on 18 March 2024

Citations:

ESA (2014) SOP 128 – *Sea Anemone Pedal Lacerate Development Toxicity Test*. Issue No 1. Ecotox Services Australasia, Sydney, NSW.

Howe, Pelli L., Reichelt-Brushett, Amanda J. and Clark, Malcolm W (2014) Development of a chronic, early life-stage sub-lethal toxicity test and recovery assessment for the tropical zooxanthellate sea anemone Aiptasia pulchella. Ecotoxicology and Environmental Safety 100: 138-147.

Chain-of-Custody Documentation

Datasheet ID: 601.2
Last Revised: 21 September 2018

Sample Receipt Notification

Attention : Tristan Stringer

Client : Intertek
1 Fleet Street
Fremantle WA 6160

Email : tristan.stringer@intertek.com
Telephone : 08 9336 5071
Facsimile :

Date : 29/02/2024

Re : Receipt of Samples **Pages : 2**

ESA Project : PR2231 ☑ For Review ☐ Additional Documentation Required - Please Respond

Sample Delivery Details

Completed Chain of Custody accompanied samples: YES
Samples received in apparent good condition and correctly bottled: YES
Security seals on sample bottles and esky intact: YES

Date samples received : 22/02/2024
Time samples received : 14:15
No. of samples received : 1
Sample matrix : Aqueous
Sample temperature : 16-20°C

Comments : 4 x 0.5L sample received at 16 degrees C in apparent good condition

Contact Details

Projects Manager : Dr Rick Krassoi
Telephone : 61 2 9420 9481
Facsimile : 61 2 9420 9484
Email : rkrassoi@ecotox.com.au

Please contact customer services officer for all queries or issues regarding samples

Note that the chain-of-custody provides definitive information on the tests to be performed

Ecotox Services Australia
ABN 95619426201
Unit 27, 2 Chaplin Drive
Lane Cove NSW 2066 Australia

Phone : 61 2 9420 9481
Fax : 61 2 9420 9484
Email : info@ecotox.com.au

Chain-of-Custody / Service Request Form

Datasheet ID: 601.1
Last Revised: 20 September 2018

Customer: Intertek Geotech
Contact Name: Tristan Stringer
Phone: 0448161528 Email: Tristan.Stringer@intertek.com (please provide an email address for sample receipt notification)
Sampled by: JUSTYNA RYBAK

Ship To: ESA
Attention:

16°C

Sample Date (day/month /year)	Sample Time	Sample Name (exactly as written on the sample vessel)	Sample Method (eg. Grab, composite etc.)	Number and Volume of Containers (eg 2 x 1L)	Tests Requested (See reverse for guidance)						Comments / Instructions
					Milky oyster Dev.	Sea urchin Fert.	8-d Anemone Dev.				**Note that testing will be delayed if an incomplete chain of custody is received** • Additional treatment of samples (i.e. spiking) • Sub-contracted services (i.e. chemical analyses) • Dilutions required (if different than 100% down to 6.25%) • Sample holding time restriction (if applicable) • Sample used for litigation (if applicable) *Note: An MSDS must be attached if Available* **ESA Project Number: PR** 2231
11455 [redacted] 20/2/24	Sub	2024-MDN-000077	Sb	4x0.5L	X	X	X				Conc. range 0.16 - 5%

1) Released By:	Date:	2) Received By:	Date: 22/2/24	3) Released By:	Date:	4) Received By:	Date:
Of:	Time:	Of: ESA	Time: 1415	Of:	Time:	Of:	Time:

Note that the chain-of-custody documentation will provide definitive information on the tests to be performed.

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Statistical Printouts for the Sea Urchin Fertilisation Test

Sea Urchin Fertilisation Test-Proportion Fertilised

Start Date:	1/03/2024 19:20	Test ID:	PR2231/02	Sample ID:	MDTN_000077
End Date:	1/03/2024 20:40	Lab ID:	11455	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 104	Test Species:	-HELIOCIDARIS TUBERCULATA
Comments:					

Conc-%	1	2	3	4
FSW Control	0.9100	0.8900	0.9200	0.9200
0.16	0.9000	0.9100	0.9200	0.9100
0.31	0.9300	0.9000	0.9100	0.8800
0.63	0.9400	0.9000	0.9100	0.9000
1.25	0.7600	0.8000	0.8900	0.7400
2.5	0.0000	0.0000	0.0000	0.0000
5	0.0000	0.0000	0.0000	0.0000

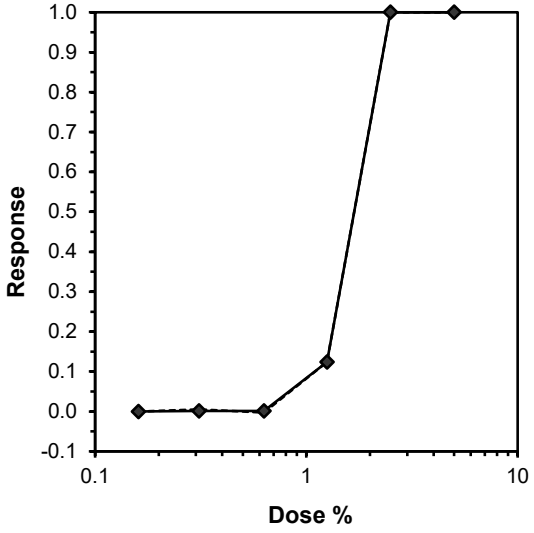
			Transform: Arcsin Square Root					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
FSW Control	0.9100	1.0000	1.2667	1.2327	1.2840	1.910	4				0.9100	1.0000
0.16	0.9100	1.0000	1.2663	1.2490	1.2840	1.128	4	0.012	2.360	0.0784	0.9100	1.0000
0.31	0.9050	0.9945	1.2588	1.2171	1.3030	2.845	4	0.238	2.360	0.0784	0.9088	0.9986
0.63	0.9125	1.0027	1.2719	1.2490	1.3233	2.770	4	-0.155	2.360	0.0784	0.9088	0.9986
*1.25	0.7975	0.8764	1.1086	1.0357	1.2327	7.932	4	4.758	2.360	0.0784	0.7975	0.8764
2.5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4				0.0000	0.0000
5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.91513	0.905	1.20321	3.2968
Bartlett's Test indicates equal variances (p = 0.05)	9.46979	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs FSW Control	0.63	1.25	0.88741	158.73	0.04967	0.05456	0.01989	0.00221	6.5E-04	4, 15

Log-Logit Interpolation (200 Resamples)

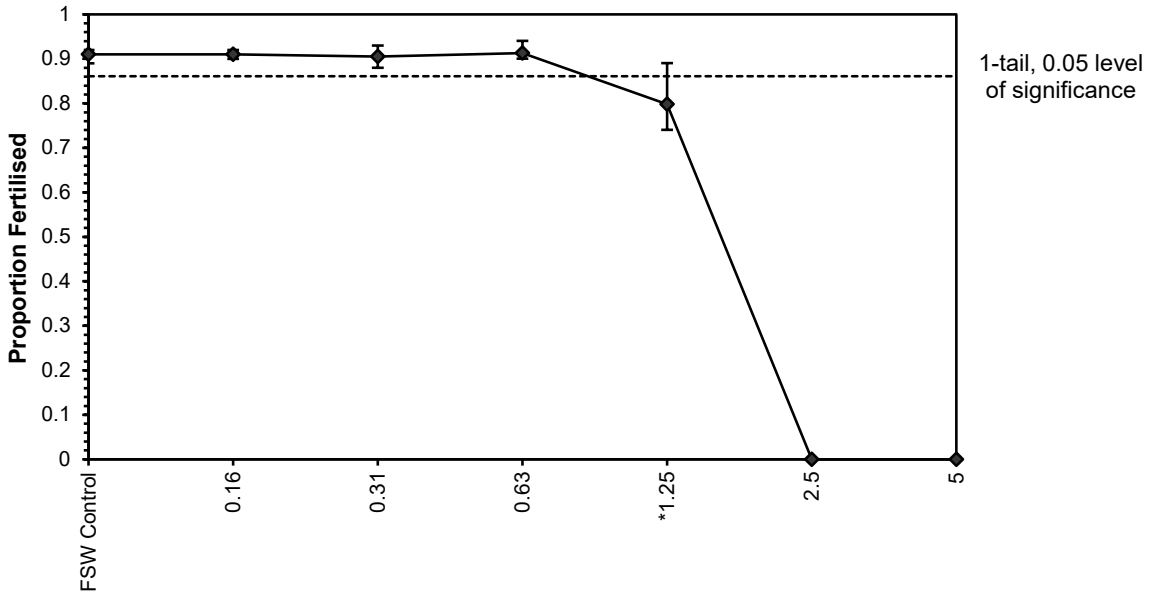
Point	%	SD	95% CL(Exp)		Skew
IC05	0.9028	0.0811	0.7277	1.2725	1.4122
IC10	1.1440	0.0904	0.9136	1.3521	-0.0275
IC15	1.2634	0.0343	1.1110	1.3222	-1.4009
IC20	1.2866	0.0167	1.2450	1.3441	0.3478
IC25	1.3076	0.0164	1.2671	1.3647	0.3535
IC40	1.3643	0.0158	1.3262	1.4200	0.3634
IC50	1.4006	0.0154	1.3638	1.4552	0.3669



Sea Urchin Fertilisation Test-Proportion Fertilised

Start Date:	1/03/2024 19:20	Test ID:	PR2231/02	Sample ID:	MDTN_000077
End Date:	1/03/2024 20:40	Lab ID:	11455	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 104	Test Species:	-HELIOCIDARIS TUBERCULATA
Comments:					

Dose-Response Plot



Sea Urchin Fertilisation Test-Proportion Fertilised

Start Date:	1/03/2024 19:20	Test ID:	PR2231/02	Sample ID:	MDTN_000077
End Date:	1/03/2024 20:40	Lab ID:	11455	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 104	Test Species:	-HELIOCIDARIS TUBERCULATA
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Fertilised	91.00	89.00	92.00	1.41	1.31	4
0.16		91.00	90.00	92.00	0.82	0.99	4
0.31		90.50	88.00	93.00	2.08	1.59	4
0.63		91.25	90.00	94.00	1.89	1.51	4
1.25		79.75	74.00	89.00	6.65	3.23	4
2.5		0.00	0.00	0.00	0.00		4
5		0.00	0.00	0.00	0.00		4
FSW Control	pH	8.10	8.10	8.10	0.00	0.00	1
0.16		8.10	8.10	8.10	0.00	0.00	1
0.31		8.10	8.10	8.10	0.00	0.00	1
0.63		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		8.20	8.20	8.20	0.00	0.00	1
5		8.30	8.30	8.30	0.00	0.00	1
FSW Control	Salinity ppt	35.50	35.50	35.50	0.00	0.00	1
0.16		35.60	35.60	35.60	0.00	0.00	1
0.31		36.40	36.40	36.40	0.00	0.00	1
0.63		37.30	37.30	37.30	0.00	0.00	1
1.25		38.90	38.90	38.90	0.00	0.00	1
2.5		42.30	42.30	42.30	0.00	0.00	1
5		48.90	48.90	48.90	0.00	0.00	1
FSW Control	DO %	99.70	99.70	99.70	0.00	0.00	1
0.16		100.00	100.00	100.00	0.00	0.00	1
0.31		99.40	99.40	99.40	0.00	0.00	1
0.63		99.70	99.70	99.70	0.00	0.00	1
1.25		99.70	99.70	99.70	0.00	0.00	1
2.5		99.60	99.60	99.60	0.00	0.00	1
5		99.70	99.70	99.70	0.00	0.00	1

Sea Urchin Fertilisation Test-Proportion Fertilised

Start Date:	1/03/2024 19:20	Test ID:	PR2231/02	Sample ID:	MDTN_000077
End Date:	1/03/2024 20:40	Lab ID:	11455	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 104	Test Species:	-HELIOCIDARIS TUBERCULATA
Comments:					

Conc-%	1	2	3	4
FSW Control	0.9100	0.8900	0.9200	0.9200
0.16	0.9000	0.9100	0.9200	0.9100
0.31	0.9300	0.9000	0.9100	0.8800
0.63	0.9400	0.9000	0.9100	0.9000
1.25	0.7600	0.8000	0.8900	0.7400
2.5	0.0000	0.0000	0.0000	0.0000
5	0.0000	0.0000	0.0000	0.0000

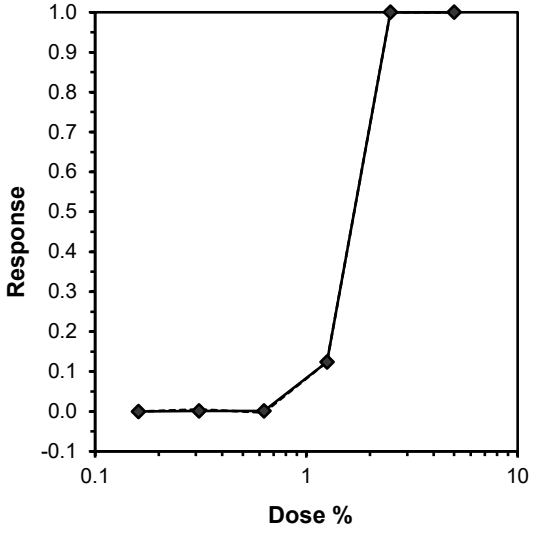
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
FSW Control	0.9100	1.0000	1.2667	1.2327	1.2840	1.910	4				36	400
0.16	0.9100	1.0000	1.2663	1.2490	1.2840	1.128	4	0.012	2.360	0.0784	36	400
0.31	0.9050	0.9945	1.2588	1.2171	1.3030	2.845	4	0.238	2.360	0.0784	38	400
0.63	0.9125	1.0027	1.2719	1.2490	1.3233	2.770	4	-0.155	2.360	0.0784	35	400
*1.25	0.7975	0.8764	1.1086	1.0357	1.2327	7.932	4	4.758	2.360	0.0784	81	400
2.5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4				400	400
5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4				400	400

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.91513	0.905	1.20321	3.2968
Bartlett's Test indicates equal variances (p = 0.05)	9.46979	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs FSW Control	0.63	1.25	0.88741	158.73	0.04967	0.05456	0.01989	0.00221	6.5E-04	4, 15

Trimmed Spearman-Karber

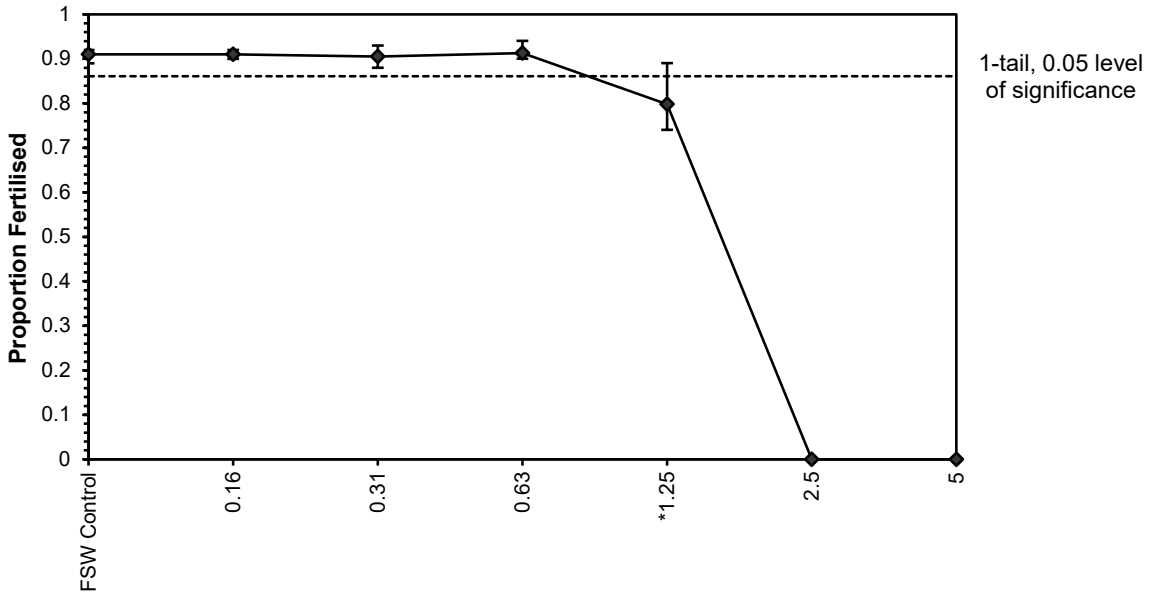
Trim Level	EC50	95% CL	
0.0%	1.6203	1.5835	1.6580
5.0%	1.6592	1.6148	1.7049
10.0%	1.6806	1.6190	1.7445
20.0%	1.6834	1.6586	1.7086
Auto-0.0%	1.6203	1.5835	1.6580



Sea Urchin Fertilisation Test-Proportion Fertilised

Start Date:	1/03/2024 19:20	Test ID:	PR2231/02	Sample ID:	MDTN_000077
End Date:	1/03/2024 20:40	Lab ID:	11455	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 104	Test Species:	-HELIOCIDARIS TUBERCULATA
Comments:					

Dose-Response Plot



Sea Urchin Fertilisation Test-Proportion Fertilised

Start Date:	1/03/2024 19:20	Test ID:	PR2231/02	Sample ID:	MDTN_000077
End Date:	1/03/2024 20:40	Lab ID:	11455	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 104	Test Species:	-HELIOCIDARIS TUBERCULATA
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Fertilised	91.00	89.00	92.00	1.41	1.31	4
0.16		91.00	90.00	92.00	0.82	0.99	4
0.31		90.50	88.00	93.00	2.08	1.59	4
0.63		91.25	90.00	94.00	1.89	1.51	4
1.25		79.75	74.00	89.00	6.65	3.23	4
2.5		0.00	0.00	0.00	0.00		4
5		0.00	0.00	0.00	0.00		4
FSW Control	pH	8.10	8.10	8.10	0.00	0.00	1
0.16		8.10	8.10	8.10	0.00	0.00	1
0.31		8.10	8.10	8.10	0.00	0.00	1
0.63		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		8.20	8.20	8.20	0.00	0.00	1
5		8.30	8.30	8.30	0.00	0.00	1
FSW Control	Salinity ppt	35.50	35.50	35.50	0.00	0.00	1
0.16		35.60	35.60	35.60	0.00	0.00	1
0.31		36.40	36.40	36.40	0.00	0.00	1
0.63		37.30	37.30	37.30	0.00	0.00	1
1.25		38.90	38.90	38.90	0.00	0.00	1
2.5		42.30	42.30	42.30	0.00	0.00	1
5		48.90	48.90	48.90	0.00	0.00	1
FSW Control	DO %	99.70	99.70	99.70	0.00	0.00	1
0.16		100.00	100.00	100.00	0.00	0.00	1
0.31		99.40	99.40	99.40	0.00	0.00	1
0.63		99.70	99.70	99.70	0.00	0.00	1
1.25		99.70	99.70	99.70	0.00	0.00	1
2.5		99.60	99.60	99.60	0.00	0.00	1
5		99.70	99.70	99.70	0.00	0.00	1

Statistical Printouts for the Milky Oyster Larval Development Tests

Bivalve Acute Toxicity Tests-Proportion Normal

Start Date:	1/03/2024 19:00	Test ID:	PR2231/02	Sample ID:	MDTN_000077
End Date:	3/03/2024 19:00	Lab ID:	11455	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Conc-%	1	2	3	4
FSW Control	0.7900	0.6800	0.7400	0.7100
0.16	0.6900	0.7200	0.7600	0.7300
0.31	0.8100	0.7400	0.6900	0.7300
0.63	0.6900	0.7400	0.7200	0.7500
1.25	0.7100	0.7600	0.7500	0.7300
2.5	0.0000	0.0000	0.0000	0.0000
5	0.0000	0.0000	0.0000	0.0000

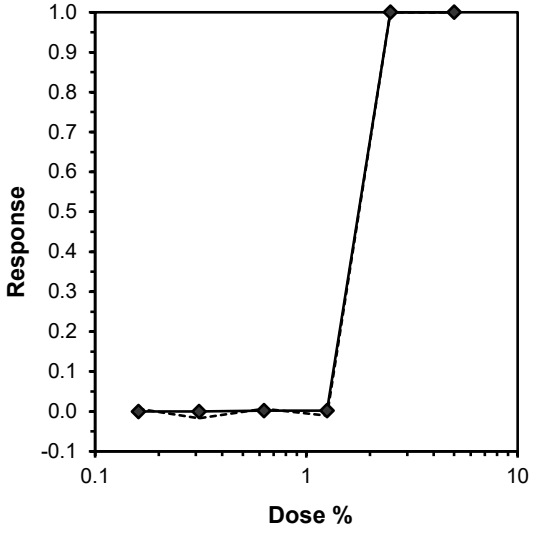
			Transform: Arcsin Square Root					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
FSW Control	0.7300	1.0000	1.0255	0.9695	1.0948	5.215	4				0.7325	1.0000
0.16	0.7250	0.9932	1.0192	0.9803	1.0588	3.178	4	0.214	2.360	0.0700	0.7325	1.0000
0.31	0.7425	1.0171	1.0400	0.9803	1.1198	5.604	4	-0.489	2.360	0.0700	0.7325	1.0000
0.63	0.7250	0.9932	1.0191	0.9803	1.0472	2.892	4	0.217	2.360	0.0700	0.7313	0.9983
1.25	0.7375	1.0103	1.0331	1.0021	1.0588	2.433	4	-0.256	2.360	0.0700	0.7313	0.9983
2.5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4				0.0000	0.0000
5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.96997	0.905	0.44507	0.02703
Bartlett's Test indicates equal variances (p = 0.57)	2.93396	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs FSW Control	1.25	2.5	1.76777	80	0.06417	0.08778	0.00033	0.00176	0.9405	4, 15

Log-Logit Interpolation (200 Resamples)

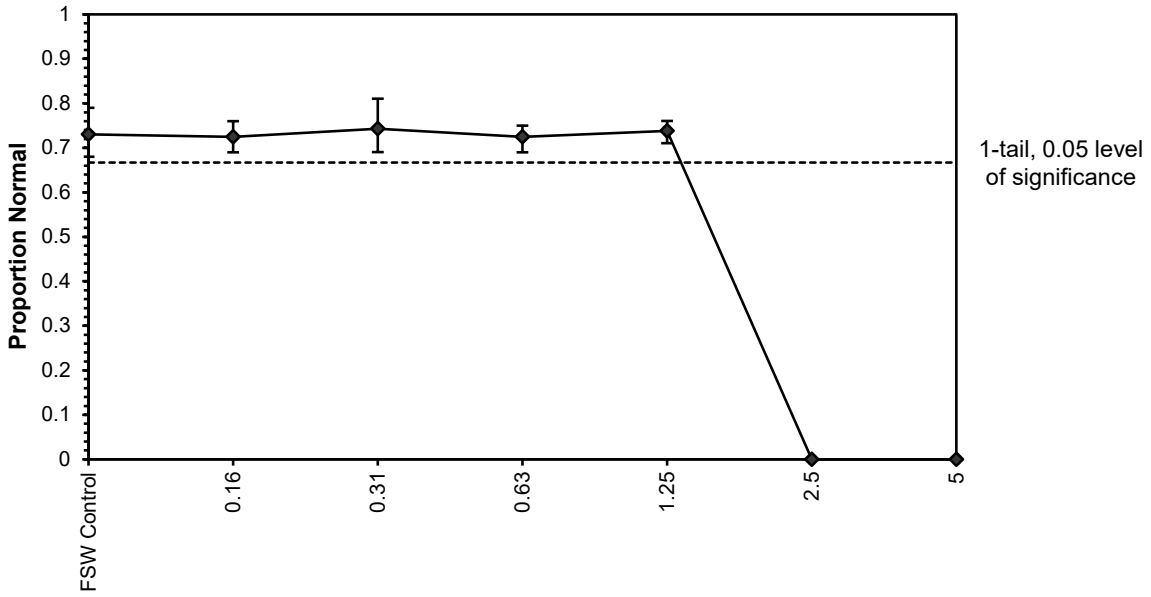
Point	%	SD	95% CL(Exp)		Skew
IC05	1.2669	0.2044	0.0000	1.2681	-4.6137
IC10	1.2834	0.0053	1.2537	1.2847	-1.1660
IC15	1.2992	0.0049	1.2719	1.3006	-1.1070
IC20	1.3145	0.0046	1.2895	1.3160	-1.0478
IC25	1.3295	0.0043	1.3064	1.3310	-0.9891
IC40	1.3744	0.0037	1.3550	1.3761	-0.8258
IC50	1.4060	0.0035	1.3881	1.4079	-0.7336



Bivalve Acute Toxicity Tests-Proportion Normal

Start Date:	1/03/2024 19:00	Test ID:	PR2231/02	Sample ID:	MDTN_000077
End Date:	3/03/2024 19:00	Lab ID:	11455	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Dose-Response Plot



Bivalve Acute Toxicity Tests-Proportion Normal

Start Date:	1/03/2024 19:00	Test ID:	PR2231/02	Sample ID:	MDTN_000077
End Date:	3/03/2024 19:00	Lab ID:	11455	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Normal	73.00	68.00	79.00	4.69	2.97	4
0.16		72.50	69.00	76.00	2.89	2.34	4
0.31		74.25	69.00	81.00	4.99	3.01	4
0.63		72.50	69.00	75.00	2.65	2.24	4
1.25		73.75	71.00	76.00	2.22	2.02	4
2.5		0.00	0.00	0.00	0.00		4
5		0.00	0.00	0.00	0.00		4
FSW Control	pH	8.10	8.10	8.10	0.00	0.00	1
0.16		8.10	8.10	8.10	0.00	0.00	1
0.31		8.10	8.10	8.10	0.00	0.00	1
0.63		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		8.20	8.20	8.20	0.00	0.00	1
5		8.30	8.30	8.30	0.00	0.00	1
FSW Control	Salinity ppt	35.50	35.50	35.50	0.00	0.00	1
0.16		35.60	35.60	35.60	0.00	0.00	1
0.31		36.40	36.40	36.40	0.00	0.00	1
0.63		37.30	37.30	37.30	0.00	0.00	1
1.25		38.90	38.90	38.90	0.00	0.00	1
2.5		42.30	42.30	42.30	0.00	0.00	1
5		48.90	48.90	48.90	0.00	0.00	1
FSW Control	DO %	99.70	99.70	99.70	0.00	0.00	1
0.16		100.00	100.00	100.00	0.00	0.00	1
0.31		99.40	99.40	99.40	0.00	0.00	1
0.63		99.70	99.70	99.70	0.00	0.00	1
1.25		99.70	99.70	99.70	0.00	0.00	1
2.5		99.60	99.60	99.60	0.00	0.00	1
5		99.70	99.70	99.70	0.00	0.00	1

Bivalve Acute Toxicity Tests-Proportion Normal

Start Date:	1/03/2024 19:00	Test ID:	PR2231/02	Sample ID:	MDTN_000077
End Date:	3/03/2024 19:00	Lab ID:	11455	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Conc-%	1	2	3	4
FSW Control	0.7900	0.6800	0.7400	0.7100
0.16	0.6900	0.7200	0.7600	0.7300
0.31	0.8100	0.7400	0.6900	0.7300
0.63	0.6900	0.7400	0.7200	0.7500
1.25	0.7100	0.7600	0.7500	0.7300
2.5	0.0000	0.0000	0.0000	0.0000
5	0.0000	0.0000	0.0000	0.0000

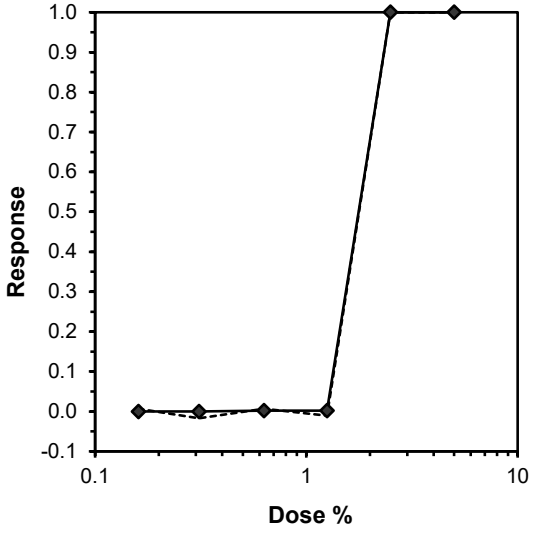
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
FSW Control	0.7300	1.0000	1.0255	0.9695	1.0948	5.215	4				108	400
0.16	0.7250	0.9932	1.0192	0.9803	1.0588	3.178	4	0.214	2.360	0.0700	110	400
0.31	0.7425	1.0171	1.0400	0.9803	1.1198	5.604	4	-0.489	2.360	0.0700	103	400
0.63	0.7250	0.9932	1.0191	0.9803	1.0472	2.892	4	0.217	2.360	0.0700	110	400
1.25	0.7375	1.0103	1.0331	1.0021	1.0588	2.433	4	-0.256	2.360	0.0700	105	400
2.5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4				400	400
5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4				400	400

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.96997	0.905	0.44507	0.02703
Bartlett's Test indicates equal variances (p = 0.57)	2.93396	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1.25	2.5	1.76777	80	0.06417	0.08778	0.00033	0.00176	0.9405	4, 15
Treatments vs FSW Control										

Trimmed Spearman-Karber

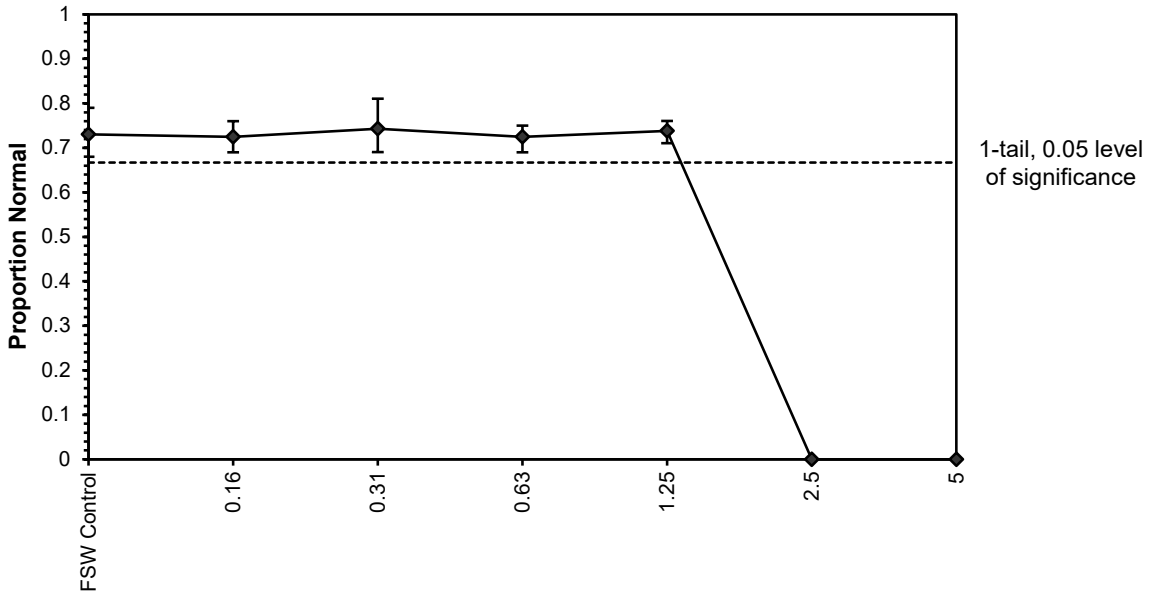
Trim Level	EC50	95% CL	
0.0%	1.7636	1.7565	1.7707
5.0%	1.7667	1.7642	1.7693
10.0%	1.7667	1.7642	1.7693
20.0%	1.7667	1.7642	1.7693
Auto-0.0%	1.7636	1.7565	1.7707



Bivalve Acute Toxicity Tests-Proportion Normal

Start Date:	1/03/2024 19:00	Test ID:	PR2231/02	Sample ID:	MDTN_000077
End Date:	3/03/2024 19:00	Lab ID:	11455	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Dose-Response Plot



Bivalve Acute Toxicity Tests-Proportion Normal

Start Date:	1/03/2024 19:00	Test ID:	PR2231/02	Sample ID:	MDTN_000077
End Date:	3/03/2024 19:00	Lab ID:	11455	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Normal	73.00	68.00	79.00	4.69	2.97	4
0.16		72.50	69.00	76.00	2.89	2.34	4
0.31		74.25	69.00	81.00	4.99	3.01	4
0.63		72.50	69.00	75.00	2.65	2.24	4
1.25		73.75	71.00	76.00	2.22	2.02	4
2.5		0.00	0.00	0.00	0.00		4
5		0.00	0.00	0.00	0.00		4
FSW Control	pH	8.10	8.10	8.10	0.00	0.00	1
0.16		8.10	8.10	8.10	0.00	0.00	1
0.31		8.10	8.10	8.10	0.00	0.00	1
0.63		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		8.20	8.20	8.20	0.00	0.00	1
5		8.30	8.30	8.30	0.00	0.00	1
FSW Control	Salinity ppt	35.50	35.50	35.50	0.00	0.00	1
0.16		35.60	35.60	35.60	0.00	0.00	1
0.31		36.40	36.40	36.40	0.00	0.00	1
0.63		37.30	37.30	37.30	0.00	0.00	1
1.25		38.90	38.90	38.90	0.00	0.00	1
2.5		42.30	42.30	42.30	0.00	0.00	1
5		48.90	48.90	48.90	0.00	0.00	1
FSW Control	DO %	99.70	99.70	99.70	0.00	0.00	1
0.16		100.00	100.00	100.00	0.00	0.00	1
0.31		99.40	99.40	99.40	0.00	0.00	1
0.63		99.70	99.70	99.70	0.00	0.00	1
1.25		99.70	99.70	99.70	0.00	0.00	1
2.5		99.60	99.60	99.60	0.00	0.00	1
5		99.70	99.70	99.70	0.00	0.00	1

Statistical Printouts for the Sea Anemone Pedal Lacerate Development Test

Anemone Larval Toxicity Test-% Unaffected

Start Date:	1/03/2024 20:30	Test ID:	PR2231/04	Sample ID:	MDTN_000077
End Date:	9/03/2024 20:30	Lab ID:	11455	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 128	Test Species:	AI-Aiptasia pulchella
Comments:					

Conc-%	1	2	3	4
FSW Control	1.0000	1.0000	0.8000	1.0000
0.16	0.8000	1.0000	1.0000	1.0000
0.31	0.8000	0.8000	1.0000	1.0000
0.63	1.0000	1.0000	1.0000	1.0000
1.25	0.6000	0.6000	0.6000	0.6000
2.5	0.0000	0.0000	0.0000	0.0000
5	0.0000	0.0000	0.0000	0.0000

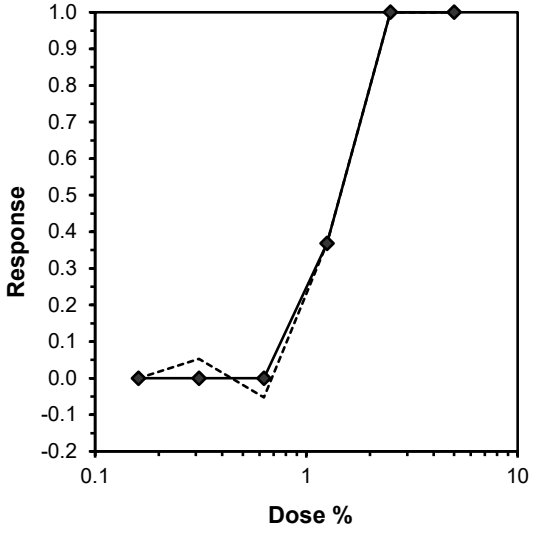
			Transform: Arcsin Square Root					Rank	1-Tailed	Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
FSW Control	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4			0.9500	1.0000
0.16	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	18.00	10.00	0.9500	1.0000
0.31	0.9000	0.9474	1.2262	1.1071	1.3453	11.212	4	16.00	10.00	0.9500	1.0000
0.63	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0.9500	1.0000
*1.25	0.6000	0.6316	0.8861	0.8861	0.8861	0.000	4	10.00	10.00	0.6000	0.6316
2.5	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			0.0000	0.0000
5	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.85319	0.905	-0.91894	0.24216
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	0.63	1.25	0.88741	158.73
Treatments vs FSW Control				

Log-Logit Interpolation (200 Resamples)

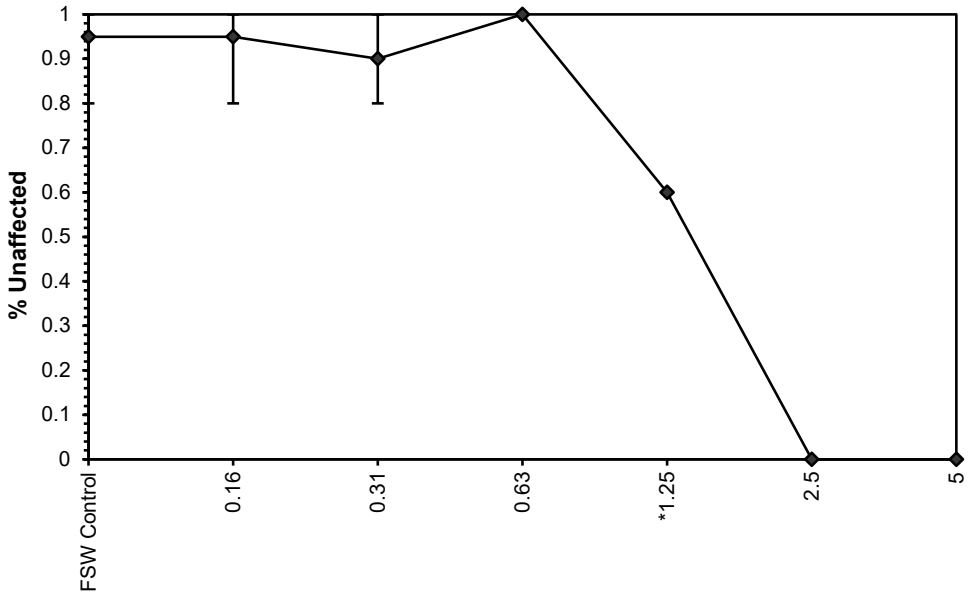
Point	%	SD	95% CL(Exp)		Skew
IC05	0.7858	0.2157	0.0000	0.8459	-1.4756
IC10	0.8911	0.0828	0.5573	0.9494	-3.1816
IC15	0.9746	0.0543	0.7170	1.0213	-1.0668
IC20	1.0464	0.0430	0.8426	1.0785	-1.0968
IC25	1.1110	0.0342	0.9505	1.1287	-1.0687
IC40	1.2628	0.0072	1.2423	1.2780	0.1209
IC50	1.3029	0.0060	1.2860	1.3157	0.1401



Anemone Larval Toxicity Test-% Unaffected

Start Date:	1/03/2024 20:30	Test ID:	PR2231/04	Sample ID:	MDTN_000077
End Date:	9/03/2024 20:30	Lab ID:	11455	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 128	Test Species:	AI-Aiptasia pulchella
Comments:					

Dose-Response Plot



Anemone Larval Toxicity Test-% Unaffected

Start Date:	1/03/2024 20:30	Test ID:	PR2231/04	Sample ID:	MDTN_000077
End Date:	9/03/2024 20:30	Lab ID:	11455	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 128	Test Species:	AI-Aiptasia pulchella
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% unaffacted	95.00	80.00	100.00	10.00	3.33	4
0.16		95.00	80.00	100.00	10.00	3.33	4
0.31		90.00	80.00	100.00	11.55	3.78	4
0.63		100.00	100.00	100.00	0.00	0.00	4
1.25		60.00	60.00	60.00	0.00	0.00	4
2.5		0.00	0.00	0.00	0.00		4
5		0.00	0.00	0.00	0.00		4
FSW Control	pH	8.10	8.10	8.10	0.00	0.00	1
0.16		8.10	8.10	8.10	0.00	0.00	1
0.31		8.10	8.10	8.10	0.00	0.00	1
0.63		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		8.20	8.20	8.20	0.00	0.00	1
5		8.30	8.30	8.30	0.00	0.00	1
FSW Control	DO, %	99.70	99.70	99.70	0.00	0.00	1
0.16		100.00	100.00	100.00	0.00	0.00	1
0.31		99.40	99.40	99.40	0.00	0.00	1
0.63		99.70	99.70	99.70	0.00	0.00	1
1.25		99.70	99.70	99.70	0.00	0.00	1
2.5		99.60	99.60	99.60	0.00	0.00	1
5		99.70	99.70	99.70	0.00	0.00	1
FSW Control	Salinity ppt	35.50	35.50	35.50	0.00	0.00	1
0.16		35.60	35.60	35.60	0.00	0.00	1
0.31		36.40	36.40	36.40	0.00	0.00	1
0.63		37.30	37.30	37.30	0.00	0.00	1
1.25		38.90	38.90	38.90	0.00	0.00	1
2.5		42.30	42.30	42.30	0.00	0.00	1
5		48.90	48.90	48.90	0.00	0.00	1

Anemone Larval Toxicity Test-% Unaffected

Start Date:	1/03/2024 20:30	Test ID:	PR2231/04	Sample ID:	MDTN_000077
End Date:	9/03/2024 20:30	Lab ID:	11455	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 128	Test Species:	AI-Aiptasia pulchella
Comments:					

Conc-%	1	2	3	4
FSW Control	1.0000	1.0000	0.8000	1.0000
0.16	0.8000	1.0000	1.0000	1.0000
0.31	0.8000	0.8000	1.0000	1.0000
0.63	1.0000	1.0000	1.0000	1.0000
1.25	0.6000	0.6000	0.6000	0.6000
2.5	0.0000	0.0000	0.0000	0.0000
5	0.0000	0.0000	0.0000	0.0000

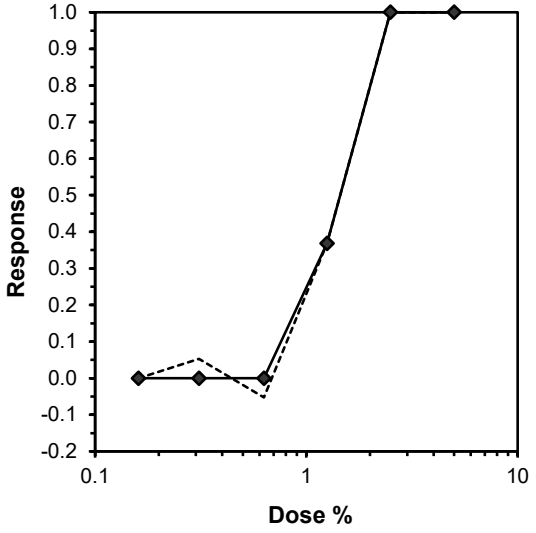
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
FSW Control	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4			1	20
0.16	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	18.00	10.00	1	20
0.31	0.9000	0.9474	1.2262	1.1071	1.3453	11.212	4	16.00	10.00	2	20
0.63	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0	20
*1.25	0.6000	0.6316	0.8861	0.8861	0.8861	0.000	4	10.00	10.00	8	20
2.5	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20
5	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.85319	0.905	-0.91894	0.24216
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	0.63	1.25	0.88741	158.73
Treatments vs FSW Control				

Trimmed Spearman-Karber

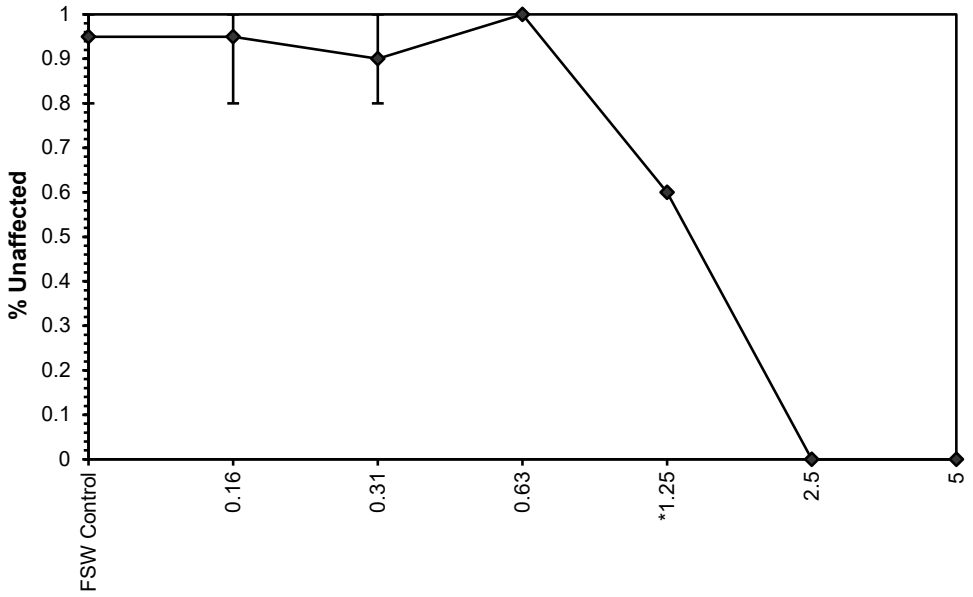
Trim Level	EC50	95% CL	
0.0%	1.3714	1.1819	1.5912
5.0%	1.3835	1.1717	1.6336
10.0%	1.3955	1.1536	1.6880
20.0%	1.4184	1.0811	1.8610
Auto-0.0%	1.3714	1.1819	1.5912



Anemone Larval Toxicity Test-% Unaffected

Start Date:	1/03/2024 20:30	Test ID:	PR2231/04	Sample ID:	MDTN_000077
End Date:	9/03/2024 20:30	Lab ID:	11455	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 128	Test Species:	AI-Aiptasia pulchella
Comments:					

Dose-Response Plot



Anemone Larval Toxicity Test-% Unaffected

Start Date:	1/03/2024 20:30	Test ID:	PR2231/04	Sample ID:	MDTN_000077
End Date:	9/03/2024 20:30	Lab ID:	11455	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 128	Test Species:	AI-Aiptasia pulchella
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% unaffacted	95.00	80.00	100.00	10.00	3.33	4
0.16		95.00	80.00	100.00	10.00	3.33	4
0.31		90.00	80.00	100.00	11.55	3.78	4
0.63		100.00	100.00	100.00	0.00	0.00	4
1.25		60.00	60.00	60.00	0.00	0.00	4
2.5		0.00	0.00	0.00	0.00		4
5		0.00	0.00	0.00	0.00		4
FSW Control	pH	8.10	8.10	8.10	0.00	0.00	1
0.16		8.10	8.10	8.10	0.00	0.00	1
0.31		8.10	8.10	8.10	0.00	0.00	1
0.63		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		8.20	8.20	8.20	0.00	0.00	1
5		8.30	8.30	8.30	0.00	0.00	1
FSW Control	DO, %	99.70	99.70	99.70	0.00	0.00	1
0.16		100.00	100.00	100.00	0.00	0.00	1
0.31		99.40	99.40	99.40	0.00	0.00	1
0.63		99.70	99.70	99.70	0.00	0.00	1
1.25		99.70	99.70	99.70	0.00	0.00	1
2.5		99.60	99.60	99.60	0.00	0.00	1
5		99.70	99.70	99.70	0.00	0.00	1
FSW Control	Salinity ppt	35.50	35.50	35.50	0.00	0.00	1
0.16		35.60	35.60	35.60	0.00	0.00	1
0.31		36.40	36.40	36.40	0.00	0.00	1
0.63		37.30	37.30	37.30	0.00	0.00	1
1.25		38.90	38.90	38.90	0.00	0.00	1
2.5		42.30	42.30	42.30	0.00	0.00	1
5		48.90	48.90	48.90	0.00	0.00	1