

# **TOXICITY TESTING OF THE CONCENTRATE DISCHARGE OF THE CARLSBAD SEAWATER DESALINATION PLANT**

## **BACKGROUND**

The Encina Power Plant has been selected as the site for the development of the Carlsbad Seawater Desalination Plant. The source water for the seawater reverse osmosis (SWRO) desalination plant will be collected from the existing cooling water discharge canal of the power plant. The power plant withdraws cooling water from the Pacific Ocean via the Agua Hedionda Lagoon. The concentrate and the treated waste filter backwash water from the desalination plant will be discharged into the existing cooling water discharge channel downstream of the point of interconnection for complete mixing with the cooling water discharge from the power plant prior to its ultimate disposal to the ocean.

In the summer of 2003, Poseidon Resources completed toxicity testing on the combined concentrate/cooling water discharge to identify if the discharge will exhibit acute and chronic toxicity and will meet the 2001 California Ocean Plan (COP) toxicity requirements.

## **SAMPLING WATER SOURCES**

For the purposes of the toxicity testing, the following samples were required: (1) concentrate from the SWRO plant; (2) Encina Power Plant cooling water effluent. A representative sample of the seawater desalination plant concentrate was obtained using Poseidon's Carlsbad pilot SWRO plant.

The Carlsbad pilot plant is a 25 gpm seawater desalination facility located at the Encina power plant site. The plant consists of the same treatment facilities and uses the same chemicals as these planned to be used at the full-scale Carlsbad desalination plant. Under average conditions, the pilot desalination plant intake pump diverts up to 55 gpm of seawater from the Carlsbad power plant cooling water discharge. The intake seawater is treated using a two-stage sand filtration system followed by cartridge filter and reverse osmosis (RO) seawater desalination system. The basic design criteria of the pilot plant are the same as these used for the full-scale facility. The pilot plant uses the same type of cartridge filters, and number and type of reverse osmosis membranes as the full-scale facility. The pilot project generates 25 gpm of permeate of low salinity (300 to 400 mg/L), and the following waste streams:

- Filter Backwash Water – 3 to 5 gpm
- RO system concentrate – 20 to 25 gpm

The waste streams indicated above are generated continuously and the volumetric ratios of these streams are similar to these that will be generated at the full-scale desal plant in Carlsbad.

The Encina cooling water effluent samples for the toxicity tests were collected at the power plant sampling station designated for NPDES permit sample collection. This sampling station is located adjacent at the power plant discharge outfall.

## **COOLING WATER/CONCENTRATE BLENDING RATIO TESTED**

In order to get a very conservative estimate of the toxicity of worst-case combination of discharge conditions and hydrodynamic conditions in the ocean, the actual dilution ratio between power plant cooling water and desalination plant concentrate tested was 10:1. This ratio also reflects the results from a hydrodynamic modeling effort entitled "Hydrodynamic Modeling of Dispersion and Dilution of Concentrated Seawater Produced by the Ocean Desalination Project at the Encina Power Plant, Carlsbad, CA (Scott A Jenkins, Ph.D., and Joseph Wasyl, 2003)", which indicate that under most extreme conditions, the seawater salinity in the point of the combined discharge is expected to increase with approximately 10 percent over the background ocean salinity.

Therefore, the cooling water/concentrate test sample referred to in the attached toxicity testing results as "Encina Cooling Water Discharge/Desal RO Brine" was created by combining 10 volumetric parts of Encina cooling water with 1 volumetric part of the Carlsbad desalination plant concentrate (brine).

Under typical operating circumstances, the power plant discharge will be 750 MGD and the Carlsbad desalination plant will be operating at an intake flow of 100 MGD and will be discharging concentrate of 50 MGD. Under such circumstances, the desalination plant's 50 MGD concentrate discharge will be diluted by a ratio of 650 MGD: 50 MGD (13:1) prior to ocean discharge. When combined by the minimum 15:1 ocean dilution allotted to the power plant in its NPDES permit, the total dilution achieved will be 195:1. If the Regional Water Quality Control Board were to establish acute toxicity limits on the basis of the total achieved dilution, a combined dilution of 195:1 would result in an acute toxicity limit of approximately 6 TUa, based on the formula for calculation of this parameter defined in the 2001 California Ocean Plan.

Under worst-case scenario, it is anticipated that the minimum power plant cooling discharge flow will be 270 MGD. For such scenario, the 50 MGD desalination plant concentrate discharge would be diluted by a factor of 170:50 (3.4:1) prior to ocean discharge. With a minimum ocean discharge initial dilution of 15:1, the total combined dilution would be 51:1. Such worst-case dilution of 51:1 would result in an acute toxicity limit of approximately 1.8 TUa.

## **TOXICITY TEST SPECIES**

The COP requires a minimum of three test species for use in measuring compliance with toxicity limits. The COP has approved one plant species, six invertebrate, and two fish species for toxicity testing. Where possible, the COP requires the toxicity testing to include a fish, an invertebrate, and an aquatic plant.

Table 1 represents test species used and the types of toxicity tests completed.

**Table 1**  
**Toxicity Test Species**

Species	Chronic Toxicity Test	Acute Toxicity Test
Giant Kelp ( <i>Macrocystis pyrifera</i> )	Percent germination; germ tube length	NA
Abalone ( <i>Halilotis rufescens</i> )	Growth & fecundity	NA
Topsmelt ( <i>Atherinops affinis</i> ) <sup>2</sup>	Larval growth rate	Percent survival

The species shown on Table 1 and used in this toxicity testing study are the same the Encina Power Plant uses to fulfill its toxicity testing obligations under plant's current NPDES permit requirements.

## **TOXICITY TEST RESULTS**

The chronic and acute toxicity testing results are attached with this document. These results indicate that under worst-case discharge conditions, the blend of power plant cooling water and desalination plant concentrate will not exhibit acute or chronic toxicity.

Analytical Report

Client: Poseidon  
 Project: Poseidon 1-Discharge Samples  
 Client Sample ID: Enc. Cooling Water Discharge/Brine RO Desal  
 MEC Test ID: C030721.0862, C030723.0162  
 Date Received: 21, 23 Jul 03  
 Date Test Started: 22 Jul 03  
 Date Test Ended: 26 Jul 03  
 Matrix: Liquid

96 Hour Acute Effluent Toxicity Bioassay  
 MEC Testing Protocol No. BIO 062C  
 EPA-821-R-02-012

Test Organism: *Atherinops affinis*  
 Age: 10 day(s) old

Concentration	Number of Test Organisms at Start of Test	Number of Test Organisms at End of Test	Percent Survival
Control	40	40	100
12.5	40	40	100
25	40	39	97.5
50	40	40	100
75	40	38	95
100	40	39	97.5

Acute Toxicity Statement for Sample Enc. Cooling Water Discharge/Brine RO Desal

Statistical Method	Result	Reference Interval	Conclusion
Shapiro-Wilk's Test	Non-Normal; $p \leq 0.01$	N/A	Could Not Be Confirmed

Statistical Method	NOEC	LOEC	LC50	Point Estimation Method	Result
Steel's Many-One Rank Test	100%	>100%	0.23	Maximum Likelihood-Probit	> 100%

**Acute Toxicity Statement:** Test substance Enc. Cooling Water Discharge/Brine RO Desal did not have a toxic effect on the survival of Topsmelt exposed for a duration of 96 hours. Test substance Enc. Cooling Water Discharge/Brine RO Desal produced 97.5 percent survival in the 100 percent concentration at 96 hours. The LC<sub>50</sub> at 96 hours was calculated to be greater than 100 percent test substance.

Toxicity, expressed as toxic units acute(TUa), was calculated to be 0.23

**Protocol Deviations:** Test substance was renewed and test animals were fed daily to ensure acceptable control performance. Test substance was renewed at greater than two hours from feeding time on Day 2. Since there was no significant mortality in the Control, this deviation is not expected to impact the significance of the test results

*Lisa Craft*  
 QA Officer

8/26/03  
 Date

*[Signature]*  
 Approved

9/17/03  
 Date

# MEC Analytical Systems, Inc.

## Analytical Report

Client	Poseidon	Date Received:	21, 23 Jul 03
Project:	Poseidon 1-Discharge Samples	Date Test Started:	22 Jul 03
Client Sample ID:	Enc. Cooling Water Discharge/Brine RO Desal	Date Test Ended:	26 Jul 03
MEC Test ID:	C030721.0862, C030723.0162	Matrix:	Liquid

**96 Hour Acute Effluent Toxicity Bioassay**  
 MEC Testing Protocol No.: BIO 062C  
 EPA-821-R-02-012

**Test Organism: *Atherinops affinis***

**Test Solution Physical and Chemical Data**

Total Chloride (mg/L)			
Concentration (%)	Mean	Min	Max
Control	0.02	0.01	0.04
100	0.01	0.00	0.03

Concentration (%)	Salinity	D.O. (%)	Temp. (°C)	Salinity (ppt)	pH
Control	Mean	85	20.2	32.0	8.0
	Minimum	79	19.9	31.7	7.8
	Maximum	98	20.4	32.4	8.3
12.5	Mean	84	20.1	32.4	8.0
	Minimum	73	20.1	32.3	7.8
	Maximum	100	20.3	32.7	8.2
25	Mean	85	20.2	32.9	8.1
	Minimum	77	20.1	32.7	7.9
	Maximum	103	20.6	33.1	8.3
50	Mean	85	20.2	33.7	8.1
	Minimum	75	20.0	33.5	7.9
	Maximum	104	20.5	33.9	8.2
75	Mean	87	20.1	34.6	8.1
	Minimum	79	20.0	34.3	7.9
	Maximum	107	20.3	34.8	8.3
	Mean	86	20.2	35.5	8.1

Analytical Report

Client: Poseidon Date Received: 21, 23 Jul 03  
Project: Poseidon 1-Discharge Samples Date Test Started: 22 Jul 03  
Client Sample ID: Enc. Cooling Water Discharge/Brine RO Desal Date Test Ended: 26 Jul 03  
MEC Test ID: C030721.0862, C030723.0162 Matrix: Liquid

**TEST:** 96 Hour Acute Effluent Toxicity Bioassay, MEC Protocol No. BIO 062C, EPA-821-R-02-012

**LAB CONTROL WATER:** Sea Water collected from Scripps Institute of Oceanography.  
Dissolved Oxygen 98 %  
Temperature 20.1° C  
pH 8

**TEST ORGANISM:** Topsmelt, *Atherinops affinis* Age: 10 day(s) old  
Supplier: Aquatic BioSystems  
Feeding: Fed *Artemia* nauplii *ad libitum* daily prior to testing.

**TEST CHAMBER:** Half liter containers, 4 replicate samples, 5 concentrations, and 4 replicate controls, brought to a 250mL final volume.

**EXPERIMENTAL DESIGN:**

1. Test substance was collected on July 21 and 23, 2003 at 0900 and 0800 hours respectively by Encina Power Station personnel and delivered to MEC at 1230 and 0925 hours on the same days. Temperatures upon arrival were 8.7 and 6.7°C.
2. The salinity of the samples was adjusted prior to receipt by MEC personnel. Encina Power Station personnel diluted the Poseidon brine sample by using 10 parts discharge water of Encina Power Station to every 1 part Poseidon brine sample with resulting salinities of 35.1 and 35.2 ppt for the two samples.
3. The temperature of the effluent was adjusted to 21±1°C.
4. 10 test organisms were placed in each test container.
5. Test chambers were held at 21±1°C for 96 hours with a photoperiod of 16 hours light: 8 hours darkness.
6. Test chambers were renewed daily.
7. Each test chamber was fed 1000 freshly hatched *Artemia* nauplii daily for the duration of the test.

**MORTALITY CRITERIA:** Lack of respiratory movement and lack of reaction to gentle prodding

**ACCEPTIBILITY CRITERIA** ≥ 90% survival in controls

**REFERENCE TOXICITY:** Toxicant: CuSO<sub>4</sub>, Lot No.: 2351-09, Received: 3/14/03, Opened:  
(Control Chart Included) 3/25/03, Expires: 12/17/04.  
96 Hour LC<sub>50</sub>: 257.65 ppb  
Laboratory Mean: 238.84 ppb  
Test Date: 7/22/03 Within 95 % Confidence Limits

**STUDY DIRECTOR:** T. Staker  
**INVESTIGATORS:** J. Word, M. Irwin, A. Margolis, L. Sequeira, C. Osuch, R. Marshall, B. Hester, T. Staker

**Acute Fish Test-96 Hr Survival**

Start Date: 7/22/03 16:05 ✓ Test ID: C030721.0862 ✓ Sample ID: Brine/Discharge ✓  
 End Date: 7/26/03 14:30 ✓ Lab ID: CAMEC-MEC Analytical, Car Sample Type: EFF3-Power Plant ✓  
 Sample Date: 7/21/03 09:00 ✓ Protocol: EPAA 91-EPA Acute ✓ Test Species: AA-Atherinops affinis ✓

Comments:

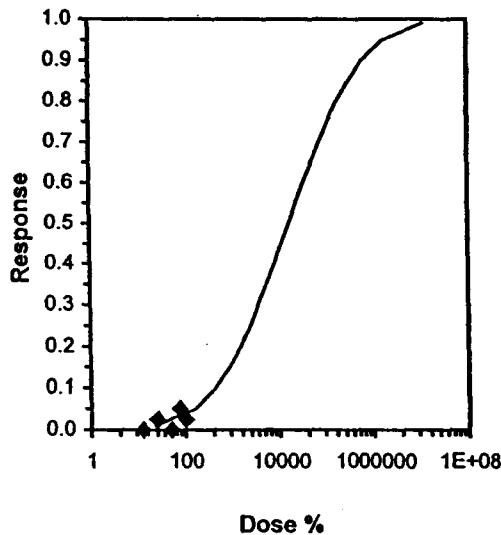
Conc-%	1	2	3	4
Control	1.0000	1.0000	1.0000	1.0000
12.5	1.0000	1.0000	1.0000	1.0000
25	1.0000	1.0000	1.0000	0.9000
50	1.0000	1.0000	1.0000	1.0000
75	1.0000	1.0000	0.9000	0.9000
100	1.0000	0.9000	1.0000	1.0000

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root				Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%				
Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	0	40	
12.5	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	0	40	
25	0.9750	0.9750	1.3713	1.2490	1.4120	5.942	4	1	40	
50	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	0	40	
75	0.9500	0.9500	1.3305	1.2490	1.4120	7.072	4	2	40	
100	0.9750	0.9750	1.3713	1.2490	1.4120	5.942	4	1	40	

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.82981	0.884	-0.9927	0.8961
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Stee's Many-One Rank Test	100	>100		1

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	0.81567	0.76623	-0.6861	2.31748	0	2.61806	7.81472	0.45	4.2143	1.22599	5
Intercept	1.56253	1.35863	-1.1004	4.22545							

Point	Probits	%	95% Fiducial Limits
EC01	2.674	23.0263	
EC05	3.355	157.663	
EC10	3.718	439.677	
EC15	3.964	878.307	
EC20	4.158	1522.24	
EC25	4.326	2439.98	
EC40	4.747	8011.28	
EC50	5.000	16379.6	
EC60	5.253	33489.1	
EC75	5.674	109956	
EC80	5.842	176247	
EC85	6.036	305463	
EC90	6.282	610199	
EC95	6.645	1701672	
EC99	7.326	1.2E+07	



TU<sub>a</sub> = 0.23 ✓

96 Hour Acute Toxicity Test with Topsmelt

Client	Pescidon
Project:	Pilot Study
Client Sample ID:	Discharge/Brine
MEC Sample ID:	Q030721.08
MEC Protocol:	BIO062
Study Director:	TS

Date Received:	7-21-03
Date Test Started:	7-22-03
Date Test Ended:	7-26-03
Matrix:	liquid
Species:	A-affinis
Organisms/Chamber:	10

Conc.	Rep	24 Hours Date: 7/23/03 Time: 1720		48 Hours Date: 7/24/03 Time: 1149		72 Hours Date: 7/25/03 Time: 1246		96 Hours Date: 7-26-03 Time: 1430	
		# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead
Control	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
12.5	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
25	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	9	1
50	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
75	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	9	1	9	1
	4	10	0	10	0	10	0	9	1
100	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	9	1
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
Initials:		am		JW/Par		JW/Par		TS	



**MEC ANALYTICAL SYSTEMS, INC.**

Analytical Report

<b>Client:</b>	Poseidon	<b>Date Received:</b>	23 Jul 03
<b>Project:</b>	Poseidon 1-Discharge Samples 2 <sup>nd</sup> Set	<b>Date Test Started:</b>	23 Jul 03
<b>Sample Matrix:</b>	Liquid	<b>Date Test Ended:</b>	25 Jul 03
<b>Sample Name/ID:</b>	Encina Discharge/Desal RO Brine Poseidon Desal RO Brine/DI	<b>Test ID No.:</b>	C030723.0147 C030723.0247

**Giant Kelp Germination and Growth Bioassay**

MEC Testing Protocol BIO047

EPA/600/R-95/136

Test Organism: *Macrocystis pyrifera*

**Concentrations Tested:** Control, 3, 6, 12, 50 and 100%

**Summary of Results – Germination**

	NOEC	LOEC	EC <sub>50</sub>	TU
<b>Encina Discharge/Desal RO Brine</b>	100%	>100%	>100%	1
<b>Poseidon Desal RO Brine/DI</b>	100%	>100%	>100%	1
<b>Ref Tox</b>	100	180	166.35	N/A

**Summary of Results – Growth**

	NOEC	LOEC	EC <sub>50</sub>	TU
<b>Encina Discharge/Desal RO Brine</b>	100%	>100%	>100%	1
<b>Poseidon Desal RO Brine/DI</b>	100%	>100%	>100%	1
<b>Ref Tox</b>	10	18	224.08	N/A

**Chronic Toxicity Statement - Germination:**



Test substances Encina Discharge/Desal RO Brine and Poseidon Desal RO Brine/DI both produced a NOEC of 100 percent. The EC<sub>50</sub> was greater than 100 percent for both samples.

Toxicity for germination, expressed as toxic units chronic (TUc), was calculated to be 1 for both samples.

**Chronic Toxicity Statement - Growth:**

Test substances Encina Discharge/Desal RO Brine and Poseidon Desal RO Brine/DI both produced a NOEC of 100 percent. The EC<sub>50</sub> was greater than 100 percent for both samples.

Toxicity for growth, expressed as toxic units chronic (TUc), was calculated to be 1 for both samples.

	9/10/03		9/17/03
QA Unit	Date	Approved	Date

# MEC ANALYTICAL SYSTEMS, INC.

## Analytical Report

**Client:** Poseidon  
**Project:** Poseidon 1-Discharge Samples 2<sup>nd</sup> Set  
**Sample Matrix:** Liquid  
**Sample Name/ID:** Encina Discharge/Desal RO Brine  
 Poseidon Desal RO Brine/DI

**Date Received:** 23 Jul 03  
**Date Test Started:** 23 Jul 03  
**Date Test Ended:** 25 Jul 03  
**Test ID No.:** C030723.0147  
 C030723.0247

### Giant Kelp Germination and Growth Bioassay MEC Testing Protocol BIO047 EPA/600/R-95/136

Test Organism: *Macrocystis pyrifera*

### Test Solution Physical and Chemical Data

	DO (% Sat)		Temperature (°C)		Salinity (ppt)		pH	
	Start	End	Start	End	Start	End	Start	End
Control (0)	94	99	16.3	16.7	31.9	31.8	7.9	8.1
3	91	96	16.1	16.0	32.1	31.9	8.1	8.2
6	94	95	16.0	15.6	32.2	31.9	8.2	8.2
12	94	95	16.4	15.3	32.4	31.9	8.2	8.3
50	99	97	15.9	15.0	33.7	33.5	8.2	8.3
100	102	99	15.3	15.3	35.5	35.5	8.2	8.3

	DO (% Sat)		Temperature (°C)		Salinity (ppt)		pH	
	Start	End	Start	End	Start	End	Start	End
Control (0)	94	99	16.3	16.7	31.9	31.8	7.9	8.1
3	96	96	16.4	16.3	32.1	31.9	8.1	8.3
6	93	93	16.2	15.6	32.2	31.9	8.1	8.3
12	98	94	16.3	16.0	32.4	32.4	8.1	8.3
50	86	94	16.3	16.0	34.1	33.8	8.1	8.3
100	77	92	16.2	15.6	36.1	35.5	8.2	8.4

**Protocol Deviations:** The temperature of the Control surrogate chamber was above protocol limits on day 2. As there was no adverse effect observed in the test, this deviation is not expected to impact the

# MEC ANALYTICAL SYSTEMS, INC.

## Analytical Report

<b>Client:</b>	Poseidon	<b>Date Received:</b>	23 Jul 03
<b>Project:</b>	Poseidon 1-Discharge Samples 2 <sup>nd</sup> Set	<b>Date Test Started:</b>	23 Jul 03
<b>Sample Matrix:</b>	Liquid	<b>Date Test Ended:</b>	25 Jul 03
<b>Sample Name/ID:</b>	Encina Discharge/Desal RO Brine Poseidon Desal RO Brine/DI	<b>Test ID No.:</b>	C030723.0147 C030723.0247

### APPENDIX

#### Pertinent Test Data

**TEST:** Giant Kelp Germination and Growth Bioassay, MEC Testing Protocol BIO047, EPA/600/R-95/136

**DILUTION WATER:** Control water (zero time), Scripps Institution of Oceanography, La Jolla, CA

Salinity	31.9 ppt
pH	7.9
Dissolved Oxygen	94% saturation
Temperature	16.3°C

**TEST ORGANISM:** Giant kelp, *Macrocystis pyrifera*, from Kim Siewers, Santa Cruz, CA.

**TEST CHAMBER:** 60-mL sterile, disposable petri dish. Five replicates, concentrations of Control (0), 3, 6, 12, 50, and 100 percent. Test substance volume per replicate = 40 mL.

**EXPERIMENTAL DESIGN:**

1. A. Encina Power Station (E.P.S.) personnel collected a 24-hour composite sample of Encina Cooling Water Discharge at 0800 hours on July 23<sup>rd</sup>, 2003. E.P.S. personnel prepared a mixture of the Encina Cooling Water Discharge with a grab sample of the Desal RO Brine in a 10 to 1 ratio, respectively. MEC personnel received approximately 10 liters of sample in 1 container at 0925 hours on the same day. The temperature of the sample was 6.7°C upon arrival at the MEC laboratory.  
B. Encina Power Station (E.P.S.) personnel collected a grab sample of Poseidon Desal RO Brine at 0800 hours on July 23<sup>rd</sup>, 2003. MEC personnel received approximately 10 liters of sample in 1 container at 0925 hours on the same day. The temperature of the sample was 8.2°C upon arrival at the MEC laboratory. The salinity of sample Poseidon Desal RO Brine was reduced to 36 ppt with the addition of deionized water by MEC personnel. Proper Chain of Custody procedures were implemented.
2. Temperatures of the effluents were adjusted to  $15 \pm 1^\circ\text{C}$ , and the initial dissolved oxygen levels were greater than 40% saturation.
3. 7,500 spores per mL were placed into each chamber.
4. Test chambers were held at  $15 \pm 1^\circ\text{C}$  for 48 hours with a photoperiod of 16 hours light: 8 hours darkness.
5. Dishes were randomly placed according to a computer-generated chart.

MEC ANALYTICAL SYSTEMS, INC.

Analytical Report

<b>Client:</b>	Poseidon	<b>Date Received:</b>	23 Jul 03
<b>Project:</b>	Poseidon 1-Discharge Samples 2 <sup>nd</sup> Set	<b>Date Test Started:</b>	23 Jul 03
<b>Sample Matrix:</b>	Liquid	<b>Date Test Ended:</b>	25 Jul 03
<b>Sample Name/ID:</b>	Encina Discharge/Desal RO Brine	<b>Test ID No.:</b>	C030723.0147
	Poseidon Desal RO Brine/DI		C030723.0247

APPENDIX

Pertinent Test Data

REFERENCE TOXICITY

Material:  $\text{CuCl}_2 \cdot 2 \text{Hydrate}$ , Lot #13318LO, received 7/1/02, opened 7/24/02. Expires: 7/1/04.

Species: *M. pyrifer* spores

48 hour  $\text{EC}_{50}$  (germination): 166.35 ppb

48 hour  $\text{EC}_{50}$  (growth): 224.08 ppb

Laboratory Mean  $\text{EC}_{50}$  for Germination: 174.98 ppb

Laboratory Mean  $\text{EC}_{50}$  for Growth: 170.57 ppb

Germination NOEC: 100 ppb

Tube growth NOEC: 10 ppb

Mean Control Germination: 76.6%

Mean Control Tube Length: 13.79  $\mu\text{m}$

Germination %MSD: 11.13

Tube Growth %MSD: 7.02

Test Date: 7/23/03

Control charts attached

STUDY DIRECTOR:

C. Osuch

INVESTIGATORS:

B. Hester, A. Margolis, and M.A. Irwin

**Macrocyctis Germination and Growth Test-Proportion Germinated**

Start Date: 7/23/2003 16:35 ✓ Test ID: C030723.0177 ✓ Sample ID: Encina Discharge/Desal RO Brine ✓  
 End Date: 7/25/2003 18:30 ✓ Lab ID: CAMEC-MEC Analytical Syst Sample Type: EFF3-Power Plant ✓  
 Sample Date: 7/23/2003 08:00 ✓ Protocol: EPAW 95-EPA West Coast ✓ Test Species: MP-Macrocyctis pyrifer a ✓

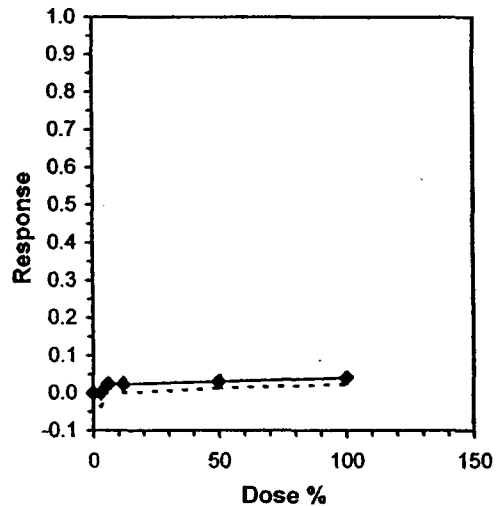
Comments:

Conc-%	1	2	3	4	5
Control	0.8100	0.7000	0.8300	0.7600	0.7300
3	0.7200	0.7600	0.8200	0.8100	0.8600
6	0.6600	0.6800	0.7900	0.8900	0.7600
12	0.7300	0.7700	0.8300	0.7800	0.7200
50	0.8100	0.6800	0.7600	0.8800	0.6500
100	0.7900	0.7400	0.7100	0.7700	0.7300 ✓

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
Control	0.7660	1.0000	1.0680	0.9912	1.1458	6.036	5				0.7800	1.0000
3	0.7940	1.0366	1.1023	1.0132	1.1873	6.133	5	-0.677	2.360	0.1198	0.7800	1.0000
6	0.7560	0.9869	1.0608	0.9483	1.2327	10.718	5	0.141	2.360	0.1198	0.7610	0.9756
12	0.7660	1.0000	1.0673	1.0132	1.1458	4.952	5	0.013	2.360	0.1198	0.7610	0.9756
50	0.7560	0.9869	1.0606	0.9377	1.2171	10.685	5	0.146	2.360	0.1198	0.7560	0.9692
100	0.7480	0.9765	1.0455	1.0021	1.0948	3.540	5	0.443	2.360	0.1198	0.7480	0.9590

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96829	0.9	0.43479	0.05628						
Bartlett's Test indicates equal variances (p = 0.26)	6.48824	15.0863								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100 ✓	>100		1	0.10787	0.14049	0.00179	0.00644	0.92075	5, 24

Linear Interpolation (200 Resamples)				
Point	%	SD	95% CL(Exp)	Skew
IC05	>100			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100 ✓			



**Macrocyctis Germination and Growth Test-Growth-Length**

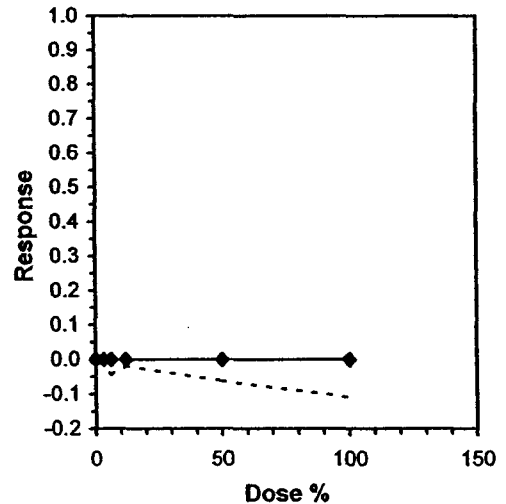
Start Date: 7/23/2003 16:35 ✓ Test ID: C030723.01V7 ✓ Sample ID: Encina Discharge/Desal RO Brine ✓  
 End Date: 7/25/2003 18:30 ✓ Lab ID: CAMEC-MEC Analytical Syst Sample Type: EFF3-Power Plant ✓  
 Sample Date: 7/23/2003 08:00 ✓ Protocol: EPAW 95-EPA West Coast ✓ Test Species: MP-Macrocyctis pyrifera ✓  
 Comments:

Conc-%	1	2	3	4	5
Control	15.587	12.870	13.442	12.870	14.157
3	13.585	13.871	14.157	14.014	13.156
6	14.586	15.587	13.728	13.871	14.157
12	13.871	14.300	14.014	14.157	13.871
50	15.158	15.444	14.300	14.014	14.300
100	15.301	15.015	14.872	15.587	15.587

Conc-%	Transform: Untransformed							1-Tailed			Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	13.785	1.0000	13.785	12.870	15.587	8.253	5				14.314	1.0000
3	13.757	0.9979	13.757	13.156	14.157	2.884	5	0.070	2.360	0.970	14.314	1.0000
6	14.386	1.0436	14.386	13.728	15.587	5.194	5	-1.461	2.360	0.970	14.314	1.0000
12	14.043	1.0187	14.043	13.871	14.300	1.328	5	-0.626	2.360	0.970	14.314	1.0000
50	14.643	1.0622	14.643	14.014	15.444	4.234	5	-2.087	2.360	0.970	14.314	1.0000
100	15.272	1.1079	15.272	14.872	15.587	2.135	5	-3.617	2.360	0.970	14.314	1.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93607	0.9	1.03708	1.93465						
Bartlett's Test indicates equal variances (p = 0.02)	12.9855	15.0863								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100 ✓	>100		1	0.97032	0.07039	1.69604	0.42261	0.00868	5, 24

Linear Interpolation (200 Resamples)				
Point	%	SD	95% CL(Exp)	Skew
IC05	>100			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100 ✓			



Test: MC-Macrocystis Germination and Growth Test ✓

Test ID: C030723.0147 ✓

Species: MP-Macrocystis pyrifera ✓

Protocol: EPAW 95-EPA West Coast ✓

Sample ID: Encina Discharge/Desal RO Brine ✓

Sample Type: EFF3-Power Plant ✓

Start Date: 7/23/2003 16:35 ✓

End Date: 7/25/2003 18:30 ✓

Lab ID: CAMEC-MEC Analytical Systems, Inc ✓

Pos	ID	Rep	Group	Total Counted	Number Germ	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	Cal Factor	Notes
	1	1	Control	100	81	10	10	11	12	14	12	11	10	10	9	1.43	
	2	2	Control	100	70	11	8	10	8	10	8	7	11	8	9	1.43	
	3	3	Control	100	83	10	8	12	9	9	12	9	6	9	10	1.43	
	4	4	Control	100	76	9	7	10	9	7	12	12	6	11	7	1.43	
	5	5	Control	100	73	9	11	10	8	11	8	11	8	11	12	1.43	
	6	1	3.000	100	72	7	13	9	11	10	9	9	7	11	9	1.43	
	7	2	3.000	100	76	9	10	11	11	12	7	8	8	12	9	1.43	
	8	3	3.000	100	82	8	10	8	10	11	8	11	15	8	10	1.43	
	9	4	3.000	100	81	9	10	8	10	11	11	10	9	12	8	1.43	
	10	5	3.000	100	86	10	6	6	10	10	15	8	10	9	8	1.43	
	11	1	6.000	100	66	10	10	9	14	8	9	10	11	13	8	1.43	
	12	2	6.000	100	68	11	9	13	10	10	11	13	12	13	7	1.43	
	13	3	6.000	100	79	12	10	8	11	8	9	7	13	9	9	1.43	
	14	4	6.000	100	89	9	8	9	13	9	10	12	8	9	10	1.43	
	15	5	6.000	100	76	10	10	12	10	13	7	11	7	9	10	1.43	
	16	1	12.000	100	73	9	13	7	9	9	11	9	11	7	12	1.43	
	17	2	12.000	100	77	12	9	8	8	13	9	11	9	13	8	1.43	
	18	3	12.000	100	83	12	9	11	11	10	10	10	9	8	8	1.43	
	19	4	12.000	100	78	10	9	8	11	10	10	8	13	10	10	1.43	
	20	5	12.000	100	72	8	10	9	11	10	9	10	9	10	11	1.43	
	21	1	50.000	100	81	9	9	9	13	10	11	10	14	10	11	1.43	
	22	2	50.000	100	68	12	10	13	13	8	12	8	10	11	11	1.43	
	23	3	50.000	100	76	10	12	8	10	12	12	8	11	8	9	1.43	
	24	4	50.000	100	88	12	8	10	9	10	6	11	11	11	10	1.43	
	25	5	50.000	100	65	11	11	7	8	13	10	10	9	11	10	1.43	
	26	1	100.000	100	79	16	7	9	10	9	10	12	11	10	13	1.43	
	27	2	100.000	100	74	14	13	11	10	7	10	8	11	8	13	1.43	
	28	3	100.000	100	71	9	12	16	7	11	9	8	12	10	10	1.43	
	29	4	100.000	100	77	14	9	11	11	9	8	11	14	9	13	1.43	
	30	5	100.000	100	73	12	12	10	7	12	13	10	13	9	11	1.43	

Comments: ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓

CLIENT:	Poseidon 1
PROJECT:	Discharge Samples
CLIENT SAMPLE ID:	Discharge/Bone
MEC SAMPLE ID:	C030723.01
STUDY DIRECTOR:	Co

DATE RECEIVED:	7.23.03
DATE TEST STARTED:	7.23.03
DATE TEST ENDED:	7.25.03
MEC SOP NO.:	B10047
SPECIES:	M. pyralis

	Concentration	DO (M)	Temp (°C)	Salinity (ppt)	pH
<b>Day 0 (0 Hours)</b>  Date: 7.23.03 Time: 1800 Technician: MM	CONTROL	94	16.3	31.9	7.9
	BRINE CONTROL				
	3	91	16.1	32.1	8.1
	6	94	16.0	32.2	8.2
	12	94	16.4	32.4	8.2
	50	99	15.9	33.7	8.2
	100	102	15.3	35.5	8.2
	<b>48 Hours</b>  Date: 7.25.03 Time: 1805 Technician: MM/OH	CONTROL	99	16.7	31.8
BRINE CONTROL					
3		96	16.0	31.9	8.2
6		95	15.6	31.9	8.2
12		95	15.3	31.9	8.3
50		97	15.0	33.5	8.3
100		99	15.3	35.5	8.3

START TIME:	1635 <sup>MM</sup>
END TIME:	1830 <sup>OH</sup>
ORGANISM BATCH:	KS 2014
HOBO TEMP. NO.:	8377
TEST LOCATION:	Room 2

DILUTION WATER BATCH:	S10 072203	
pH:	DO:	TEMP.:
7.9	94	16.3
REF. TOX.:	LOT NO.:	
C020724.14	13318L0	
48-HR LC50:	TEST DATE:	
G: 146.55 L: 22108	7.23.03	
TEST ACCEPTABILITY:		
~70% GERMINATION IN CONTROL		



**48-HOUR ACUTE TOXICITY DATA SHEET #3  
GERMINATION AND LENGTH**

CLIENT:	Doxidan
PROJECT:	Discharge Samples
CLIENT SAMPLE ID:	Discharge Brine
MEC SAMPLE ID:	0030723.01
STUDY DIRECTOR:	CO

MICROSCOPE:	Olympus
MICROMETER CONVERSION FACTOR:	1.43

Test Container Number	Concentration	Number of Spores Germ.	Number of Spores Not Germ.	LENGTH MEASUREMENTS (in ocular micrometer units)										Tech	Date
				L1	L2	L3	L4	L5	L6	L7	L8	L9	L10		
1	0	81	19	10	10	11	12	14	12	11	10	10	9	MAI	7/25/03
2	↓	70	30	11	8	10	8	10	8	7	11	8	9	Am	8/2/03
3	↓	83	17	10	8	12	9	9	12	9	6	9	10		
4	↓	76	24	9	7	10	9	7	12	12	6	11	7		
5	↓	73	27	9	11	10	8	11	8	11	8	11	12	↓	↓
31	3	72	28	7	13	9	11	10	9	9	7	11	9	8/22/03	am
32	↓	76	24	9	10	11	11	12	7	8	8	12	9	↑	↑
33	↓	82	18	8	10	8	10	11	8	11	15	8	10		
34	↓	81	19	9	10	8	10	11	11	10	9	12	8		
35	↓	86	14	10	6	6	10	10	15	8	10	9	8		
36	6	66	34	10	10	9	14	8	9	10	11	13	8		
37	↓	68	32	11	9	13	10	10	11	13	12	13	7		
38	↓	79	21	12	10	8	11	8	9	7	13	9	9		
39	↓	89	11	9	8	9	13	9	10	12	8	9	10		
40	↓	76	24	10	10	12	10	13	7	11	7	9	10		
41	12	73	27	9	13	7	9	9	11	9	11	7	12		
42	↓	77	23	12	9	8	8	13	9	11	9	13	8		
43	↓	83	17	12	9	11	11	10	16	10	9	8	8		
44	↓	78	22	10	9	8	11	10	10	8	13	10	10		
45	↓	72	28	8	10	9	11	10	9	10	9	10	11		
46	50	81	19	9	9	9	13	10	11	10	14	10	11		
47	↓	68	32	12	10	13	13	8	12	8	10	11	11		
48	↓	76	24	10	12	8	10	12	12	8	11	8	9		
49	↓	88	12	12	8	10	9	10	6	11	11	11	10		
50	↓	65	35	11	11	7	8	13	10	10	9	11	10		
51	100	79	21	16	7	9	10	9	10	12	11	10	13		
52	↓	74	26	14	13	11	10	7	10	8	11	8	13		
53	↓	71	29	9	12	16	7	11	9	8	12	10	10		
54	↓	77	23	14	9	11	11	9	8	11	14	9	13		
55	↓	73	27	12	12	10	7	12	13	10	13	9	11	↓	↓

DWC 8.25.03 BSH

Date: 7.23.03Test: Posed onInvestigator: M41Condition of Majority of Blades Used: Poor Fair GoodNumber of Blades Used: 9 Weight of Blades: 83gVolume of Release Water: 1LTime blades are placed in release beaker: 1505Time blades are removed from release beaker: 1605Temperature of spore solution: 16.0Check for zoospore motility on microscope: ✓

Fix a 9-mL spore sample with 1 mL formalin.

Determine spore density on the hemacytometer.

Determine density with 5 counts.

1. 45
2. 62
3. 67
4. 44
5. 43

Mean 52.2 S.D. \_\_\_\_\_Mean  $\times 10,000 \times 1.11 = 579420$  spores/mL. This is the density of spore release.

1.11 is the dilution factor for 1 mL formalin + 9 mL spore solution.

Volume of test container: 40 mL

The desired final density of zoospore solution is 7,500 spores/mL of test container.

To determine volume of spores to deliver to test containers:

7,500 spores/mL  $\times$  40 mL mL/test container = 300,000 spores/test container300,000 spores/test container / density of spore release 579420 spores/mL = 0.518 mL/test containerTemperature of spore solution: 16.0Temperature of test containers: 16.0

**Macrocyctis Germination and Growth Test-Proportion Germinated**

Start Date: 7/23/2003 16:35 ✓ Test ID: C030723.02 47 ✓ Sample ID: ✓ Poseidon Desal RO Brine/DI ✓  
 End Date: 7/25/2003 18:30 ✓ Lab ID: CAMEC-MEC Analytical Syst Sample Type: EFF3-Power Plant -  
 Sample Date: 7/23/2003 08:00 ✓ Protocol: EPAW 95-EPA West Coast ✓ Test Species: MP-Macrocyctis pyrifera -

Comments:

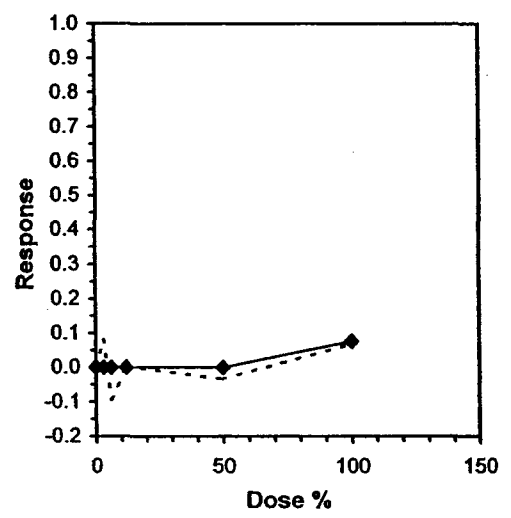
Conc-%	1	2	3	4	5
Control	0.8100	0.7000	0.8300	0.7600	0.7300
3	0.7100	0.6600	0.6400	0.8000	0.7100
6	0.7500	0.8400	0.8000	0.9300	0.8700
12	0.7600	0.6800	0.6900	0.8900	0.8000
50	0.8700	0.7400	0.7800	0.7700	0.8000
100	0.7500	0.6900	0.7300	0.7000	0.7000

Conc-%	Transform: Arcsin Square Root							t-Stat	1-Tailed Critical	MSD	Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	N				Mean	N-Mean
Control	0.7660	1.0000	1.0680	0.9912	1.1458	6.036	5				0.7728	1.0000
3	0.7040	0.9191	0.9974	0.9273	1.1071	6.986	5	1.468	2.360	0.1135	0.7728	1.0000
6	0.8380	1.0940	1.1637	1.0472	1.3030	8.339	5	-1.991	2.360	0.1135	0.7728	1.0000
12	0.7640	0.9974	1.0697	0.9695	1.2327	10.039	5	-0.036	2.360	0.1135	0.7728	1.0000
50	0.7920	1.0339	1.0996	1.0357	1.2019	5.704	5	-0.657	2.360	0.1135	0.7728	1.0000
100	0.7140	0.9321	1.0068	0.9803	1.0472	2.780	5	1.272	2.360	0.1135	0.7140	0.9239

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ( $p > 0.01$ )	0.96212	0.9	0.60532	0.06373
Bartlett's Test indicates equal variances ( $p = 0.28$ )	6.22719	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100	>100		1	0.10187	0.13268	0.01889	0.00578	0.02165	5, 24

Linear Interpolation (200 Resamples)				
Point	%	SD	95% CL(Exp)	Skew
IC05	82.857			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100 ✓			



**Macrocyctis Germination and Growth Test-Growth-Length**

Start Date: 7/23/2003 16:35 ✓ Test ID: C030723.024 ✓ Sample ID: Poseidon Desal RO Brine/DI ✓  
 End Date: 7/25/2003 18:30 ✓ Lab ID: CAMEC-MEC Analytical Syst Sample Type: EFF3-Power Plant ✓  
 Sample Date: 7/23/2003 08:00 ✓ Protocol: EPAW 95-EPA West Coast ✓ Test Species: MP-Macrocyctis pyrifera ✓

Comments:

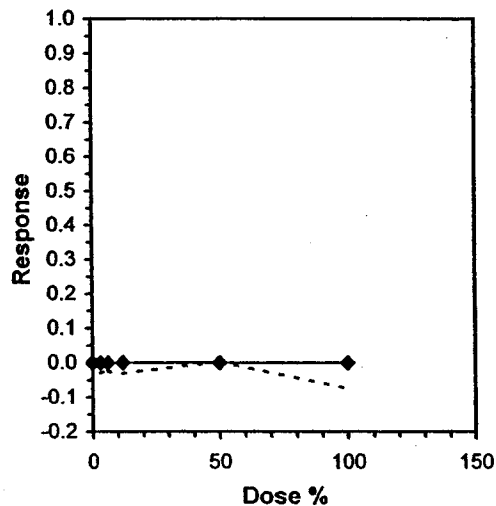
Conc-%	1	2	3	4	5
Control	15.587	12.870	13.442	12.870	14.157
3	14.157	14.443	14.014	13.728	14.586
6	14.443	14.300	13.728	14.300	13.871
12	13.585	13.728	14.300	14.729	14.729
50	12.870	14.443	14.300	13.585	13.585
100	14.586	15.301	14.872	14.443	14.872

Conc-%	Transform: Untransformed							t-Stat	1-Tailed Critical	MSD	Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	N				Mean	N-Mean
Control	13.785	1.0000	13.785	12.870	15.587	8.253	5				14.147	1.0000
3	14.186	1.0290	14.186	13.728	14.586	2.407	5	-1.020	2.360	0.926	14.147	1.0000
6	14.128	1.0249	14.128	13.728	14.443	2.194	5	-0.874	2.360	0.926	14.147	1.0000
12	14.214	1.0311	14.214	13.585	14.729	3.804	5	-1.093	2.360	0.926	14.147	1.0000
50	13.757	0.9979	13.757	12.870	14.443	4.614	5	0.073	2.360	0.926	14.147	1.0000
100	14.815	1.0747	14.815	14.443	15.301	2.222	5	-2.623	2.360	0.926	14.147	1.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94038	0.9	0.82253	2.27991						
Bartlett's Test indicates equal variances (p = 0.06)	10.4601	15.0863								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100	>100		1	0.92628	0.06719	0.73562	0.38512	0.12996	5, 24

**Linear Interpolation (200 Resamples)**

Point	%	SD	95% CL(Exp)	Skew
IC05	>100			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			



Test: MC-Macrocytis Germination and Growth Test ✓

Test ID: C030723.0247 ✓

Species: MP-Macrocytis pyrifer ✓

Protocol: EPAW 95-EPA West Coast ✓

Sample ID: Poseldon Desal RO Brine/DI ✓

Sample Type: EFF3-Power Plant ✓

Start Date: 7/23/2003 16:35 ✓

End Date: 7/25/2003 18:30 ✓

Lab ID: CAMEC-MEC Analytical Systems, Inc ✓

Pos	ID	Rep	Group	Total Counted	Number Germ	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	Cal Factor	Notes
1	1	1	Control	100	81	10	10	11	12	14	12	11	10	10	9	1.43	
2	2	2	Control	100	70	11	8	10	8	10	8	7	11	8	9	1.43	
3	3	3	Control	100	83	10	8	12	9	9	12	9	6	9	10	1.43	
4	4	4	Control	100	76	9	7	10	9	7	12	12	6	11	7	1.43	
5	5	5	Control	100	73	9	11	10	8	11	8	11	8	11	12	1.43	
6	1		3.000	100	71	10	10	11	10	8	10	12	10	9	9	1.43	
7	2		3.000	100	66	9	10	13	9	13	9	10	8	10	10	1.43	
8	3		3.000	100	64	8	12	7	15	8	10	12	10	6	10	1.43	
9	4		3.000	100	80	10	9	10	10	11	13	9	11	6	7	1.43	
10	5		3.000	100	71	11	9	11	10	10	10	12	8	10	11	1.43	
11	1		6.000	100	75	8	9	11	9	11	11	9	14	10	9	1.43	
12	2		6.000	100	84	14	8	9	10	10	11	7	13	9	9	1.43	
13	3		6.000	100	80	8	10	10	8	13	8	10	11	9	9	1.43	
14	4		6.000	100	93	9	9	9	11	12	12	11	10	8	9	1.43	
15	5		6.000	100	87	11	12	9	9	9	8	12	9	7	11	1.43	
16	1		12.000	100	76	11	9	8	11	10	10	6	11	12	7	1.43	
17	2		12.000	100	68	9	13	10	9	8	10	9	9	9	10	1.43	
18	3		12.000	100	69	12	8	13	8	7	9	9	14	9	11	1.43	
19	4		12.000	100	89	9	11	8	12	10	10	9	9	15	10	1.43	
20	5		12.000	100	80	13	9	11	10	11	11	7	11	10	10	1.43	
21	1		50.000	100	87	10	10	10	9	8	12	9	6	8	8	1.43	
22	2		50.000	100	74	15	11	6	12	9	8	7	13	10	10	1.43	
23	3		50.000	100	78	9	13	8	12	7	13	8	10	9	11	1.43	
24	4		50.000	100	77	9	9	12	8	11	10	10	6	13	7	1.43	
25	5		50.000	100	80	7	11	12	7	10	10	7	9	13	9	1.43	
26	1		100.000	100	75	12	12	11	8	11	11	7	11	12	7	1.43	
27	2		100.000	100	69	13	9	7	10	12	11	9	16	11	9	1.43	
28	3		100.000	100	73	13	15	7	9	10	10	11	11	7	11	1.43	
29	4		100.000	100	70	10	8	10	12	11	12	8	12	8	10	1.43	
30	5		100.000	100	70	9	7	13	12	8	10	12	10	14	9	1.43	

Comments:

**48-HOUR ACUTE TOXICITY DATA SHEET #3  
GERMINATION AND LENGTH**

CLIENT:	Poseidon
PROJECT:	Discharge Samples
CLIENT SAMPLE ID:	Br. 101
MEC SAMPLE ID:	031723.02
STUDY DIRECTOR:	fo

MICROSCOPE:	Olympus #1
MICROMETER CONVERSION FACTOR:	1.43

Test Container Number	Concentration	Number of Spores Germ.	Number of Spores Not Germ.	LENGTH MEASUREMENTS (in ocular micrometer units)										Tech	Date
				L1	L2	L3	L4	L5	L6	L7	L8	L9	L10		
1	0	81	19	10	10	11	12	14	12	11	10	10	9	MAH	7/25/03
2		70	30	11	8	10	8	10	8	7	11	8	9	AMM	8/22/03
3		83	17	10	8	12	9	9	12	9	6	9	10		
4		76	24	9	7	10	9	7	12	12	6	11	7		
5	↓	73	27	9	11	10	8	11	8	11	8	11	12		
6	3	71	29	10	10	11	10	8	10	12	10	9	9		
7		66	34	9	10	13	9	13	9	10	8	10	10		
8		64	36	8	12	7	15	8	10	12	10	6	10		
9		80	20	10	9	10	10	11	13	9	11	6	7		
10	↓	71	29	11	9	11	10	10	10	12	8	10	11		
11	6	75	25	8	9	11	9	11	11	9	14	10	9		
12		84	16	14	8	9	10	10	11	7	13	9	9		
13		80	20	8	10	10	8	13	8	10	11	9	9		
14		93	7	9	9	9	11	12	12	11	10	8	9		
15	↓	87	13	11	12	9	9	9	8	12	9	7	11		
16	12	76	24	11	9	8	11	10	10	6	11	12	7		
17		68	32	9	13	10	9	8	10	9	9	9	10		
18		69	31	12	8	13	8	7	9	9	14	9	11		
19		89	11	9	11	8	12	10	10	9	9	15	10		
20	↓	80	20	13	9	11	10	11	11	7	11	10	10		
21	50	87	13	10	10	10	9	8	12	9	6	8	8		
22		74	26	15	11	6	12	9	8	7	13	10	10		
23		78	22	9	13	8	12	7	13	8	10	9	11		
24		77	23	9	9	12	8	11	10	10	6	13	7		
25	↓	80	20	7	11	12	7	10	10	7	9	13	9		
26	100	75	25	12	12	11	8	11	11	7	11	12	7		
27		69	31	13	9	7	10	12	11	9	16	11	9		
28		73	27	13	15	7	9	10	10	11	11	7	11		
29		70	30	10	8	10	12	11	12	8	12	8	10		
30	↓	70	30	9	7	13	12	8	10	12	10	14	9	↓	↓

CLIENT:	Posidon
PROJECT:	Discharge Samples
CLIENT SAMPLE ID:	Bone IDI
MEC SAMPLE ID:	CO30723.02
STUDY DIRECTOR:	CO

DATE RECEIVED:	7.23.03
DATE TEST STARTED:	7.23.03
DATE TEST ENDED:	7.25.03
MEC SOP NO.:	B10047
SPECIES:	M. pygmaea

	Concentration	DO (M)	Temp (°C)	Salinity (ppt)	pH
Day 0 (0 Hours) Date: 7.23.03 Time: 1755 Technician: MA1	CONTROL	94	16.3	31.9	7.9
	BRINE CONTROL				8.0 MA1
	3	96	16.4	32.1	8.1
	6	93	16.2	32.2	8.1
	12	98	16.3	32.4	8.1
	50	81	16.3	34.1	8.1
	100	77	16.2	36.1	8.2
48 Hours Date: 7.25.03 Time: Technician: BH/MA1	CONTROL	99	16.7	31.8	8.1
	BRINE CONTROL				
	BH 3	96	16.3	31.9	8.3
	6	93	15.6	31.9	8.3
	12	94	16.0	32.4	8.3
	50	94	16.0	33.8	8.3
	100	92	15.6	35.5	8.4

① WLC 7.23.03 MA1

START TIME:	1625 MA1
END TIME:	1830 BH
ORGANISM BATCH:	KS 2014
HOBO TEMP. NO.:	8371
TEST LOCATION:	Room 2

DWC 7.25.03 WH

DILUTION WATER BATCH:	S10072208
pH:	7.9
DO:	9.4
TEMP.:	16.3
REF. TOX.:	C020724.H
LOT NO.:	B318L0
48-HR LC50:	6: 166.35 L: 224.08
TEST DATE:	7.23.03
TEST ACCEPTABILITY:	

Date: 7.23.03

Test: Poseidon

Investigator: MAI

Condition of Majority of Blades Used: Poor Fair Good

Number of Blades Used: 9 Weight of Blades: 83g

Volume of Release Water: 1L

Time blades are placed in release beaker: 1505

Time blades are removed from release beaker: 1605

Temperature of spore solution: 16.0

Check for zoospore motility on microscope: ✓

Fix a 9-mL spore sample with 1 mL formalin.

Determine spore density on the hemacytometer.

Determine density with 5 counts.

1. 45
2. 62
3. 67
4. 44
5. 43

Mean 52.2 S.D. \_\_\_\_\_

Mean  $\times 10,000 \times 1.11 = 579420$  spores/mL. This is the density of spore release.

1.11 is the dilution factor for 1 mL formalin + 9 mL spore solution.

Volume of test container: 40 mL

The desired final density of zoospore solution is 7,500 spores/mL of test container.

To determine volume of spores to deliver to test containers:

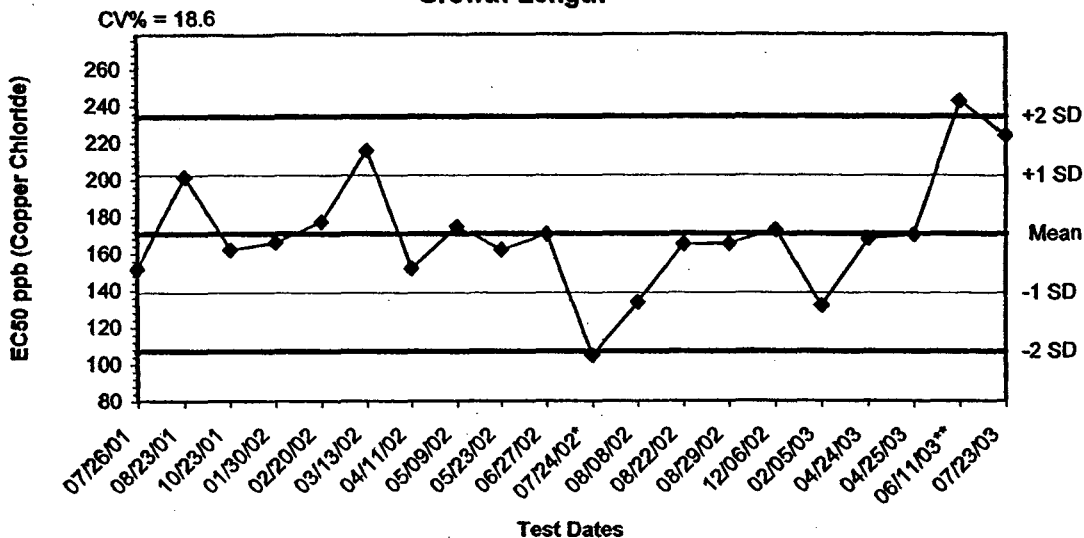
7,500 spores/mL  $\times$  40 mL mL/test container = 300,000 spores/test container

300,000 spores/test container / density of spore release 579420 spores/mL = 0.518 mL/test container

Temperature of spore solution: 16.0 Temperature of test containers: 16.0



## Macrocystis pyrifera Reference Toxicant Control Chart: Growth-Length



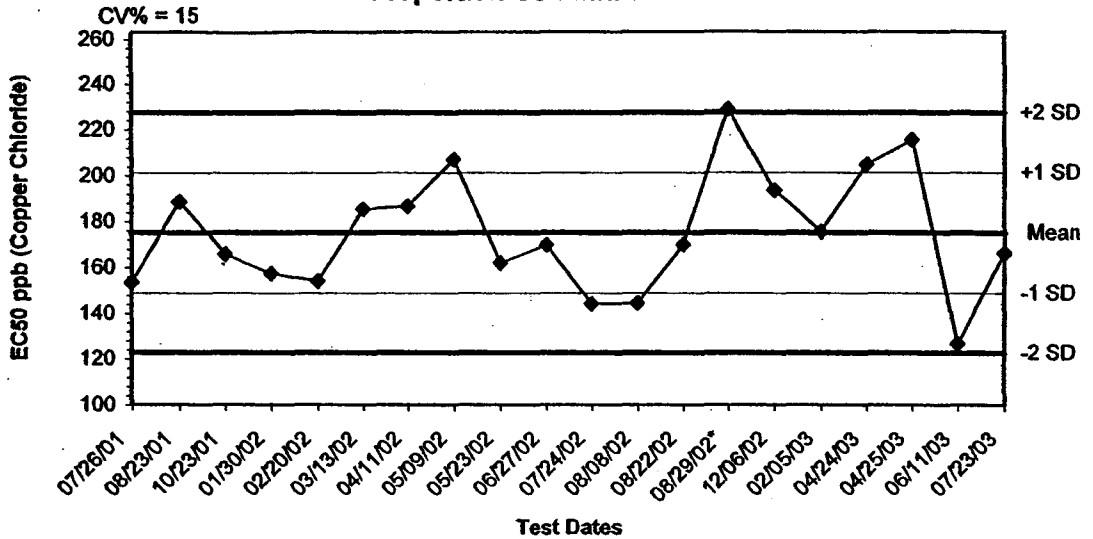
Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
07/26/01	151.7600	170.5685	138.7733	106.9781	202.3637	234.1589
08/23/01	201.3300	170.5685	138.7733	106.9781	202.3637	234.1589
10/23/01	162.0000	170.5685	138.7733	106.9781	202.3637	234.1589
01/30/02	165.9560	170.5685	138.7733	106.9781	202.3637	234.1589
02/20/02	177.2500	170.5685	138.7733	106.9781	202.3637	234.1589
03/13/02	215.7200	170.5685	138.7733	106.9781	202.3637	234.1589
04/11/02	152.0000	170.5685	138.7733	106.9781	202.3637	234.1589
05/09/02	174.5900	170.5685	138.7733	106.9781	202.3637	234.1589
05/23/02	161.9700	170.5685	138.7733	106.9781	202.3637	234.1589
06/27/02	170.4200	170.5685	138.7733	106.9781	202.3637	234.1589
07/24/02*	105.0000	170.5685	138.7733	106.9781	202.3637	234.1589
08/08/02	133.6200	170.5685	138.7733	106.9781	202.3637	234.1589
08/22/02	164.9470	170.5685	138.7733	106.9781	202.3637	234.1589
08/29/02	165.2400	170.5685	138.7733	106.9781	202.3637	234.1589
12/06/02	172.6700	170.5685	138.7733	106.9781	202.3637	234.1589
02/05/03	131.8500	170.5685	138.7733	106.9781	202.3637	234.1589
04/24/03	167.9460	170.5685	138.7733	106.9781	202.3637	234.1589
04/25/03	170.3670	170.5685	138.7733	106.9781	202.3637	234.1589
06/11/03**	242.6580	170.5685	138.7733	106.9781	202.3637	234.1589
07/23/03	224.0760	170.5685	138.7733	106.9781	202.3637	234.1589

\* Value was within 95% CI at time of testing.

\*\* Test of out 95% CI range. This test has met all acceptability criteria outlined in EPA/600/R-95/136.

Updated 8/22/03 AM

**Macrocystis pyrifera Reference Toxicant Control Chart:  
Proportion Germinated**



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
07/26/01	153.4500	174.9777	148.7990	122.6204	201.1563	227.3349
08/23/01	188.4910	174.9777	148.7990	122.6204	201.1563	227.3349
10/23/01	165.6940	174.9777	148.7990	122.6204	201.1563	227.3349
01/30/02	157.4200	174.9777	148.7990	122.6204	201.1563	227.3349
02/20/02	154.0800	174.9777	148.7990	122.6204	201.1563	227.3349
03/13/02	185.3000	174.9777	148.7990	122.6204	201.1563	227.3349
04/11/02	186.6160	174.9777	148.7990	122.6204	201.1563	227.3349
05/09/02	207.0420	174.9777	148.7990	122.6204	201.1563	227.3349
05/23/02	162.0310	174.9777	148.7990	122.6204	201.1563	227.3349
06/27/02	169.7000	174.9777	148.7990	122.6204	201.1563	227.3349
07/24/02	144.2400	174.9777	148.7990	122.6204	201.1563	227.3349
08/08/02	144.5300	174.9777	148.7990	122.6204	201.1563	227.3349
08/22/02	169.8900	174.9777	148.7990	122.6204	201.1563	227.3349
08/29/02*	228.7740	174.9777	148.7990	122.6204	201.1563	227.3349
12/06/02	193.5880	174.9777	148.7990	122.6204	201.1563	227.3349
02/05/03	175.4990	174.9777	148.7990	122.6204	201.1563	227.3349
04/24/03	204.7440	174.9777	148.7990	122.6204	201.1563	227.3349
04/25/03	215.4910	174.9777	148.7990	122.6204	201.1563	227.3349
06/11/03	126.6242	174.9777	148.7990	122.6204	201.1563	227.3349
07/23/03	166.3490	174.9777	148.7990	122.6204	201.1563	227.3349

\* Value was within 95% CI at time of testing.

Updated 8/22/03 AM

**Macrocystis Germination and Growth Test-Growth-Length**

Start Date: 7/23/2003 16:35 · Test ID: C020724.14 · Sample ID: REF-Ref Toxicant ·  
 End Date: 7/25/2003 18:30 · Lab ID: CAMEC-MEC Analytical, Car Sample Type: CUCL-Copper chloride ·  
 Sample Date: Protocol: EPAW 95-EPA West Coast Test Species: MP-Macrocystis pyrifera ·

Comments:

Conc-ppb	1	2	3	4	5
Control	15.587	12.870	13.442	12.870	14.157
5.6	15.158	14.157	13.585	13.156	13.156
10	12.441	13.871	12.727	12.441	12.441
18	11.297	11.011	11.583	11.440	11.583
32	11.297	10.725	10.153	10.296	10.010
100	8.866	9.009	8.723	9.009	8.866
180	7.722	7.150	7.865	7.293	7.722

Conc-ppb	Transform: Untransformed							Rank Sum	1-Tailed Critical	Mean	N-Mean
	Mean	N-Mean	Mean	Min	Max	CV%	N				
Control	13.785	1.0000	13.785	12.870	15.587	8.253	5			13.785	0.0000
5.6	13.842	1.0041	13.842	13.156	15.158	6.085	5	29.50	16.00	13.842	-0.0041
10	12.784	0.9274	12.784	12.441	13.871	4.850	5	18.00	16.00	12.784	0.0726
*18	11.383	0.8257	11.383	11.011	11.583	2.102	5	15.00	16.00	11.383	0.1743
*32	10.496	0.7614	10.496	10.010	11.297	4.969	5	15.00	16.00	10.496	0.2386
*100	8.895	0.6452	8.895	8.723	9.009	1.345	5	15.00	16.00	8.895	0.3548
*180	7.550	0.5477	7.550	7.150	7.865	4.106	5	15.00	16.00	7.550	0.4523

**Audliary Tests**

	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.90831	0.91	1.20575	2.23728
Bartlett's Test indicates unequal variances (p = 2.89E-03)	19.8965	16.8119		

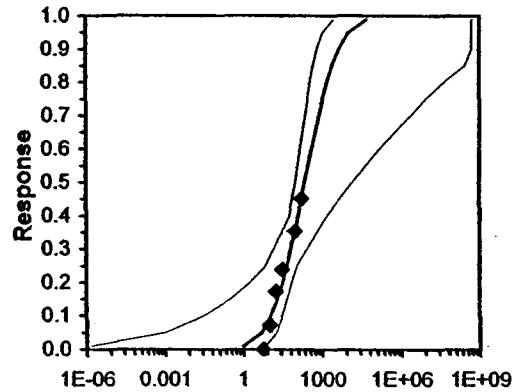
**Hypothesis Test (1-tail, 0.05)**

	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	10 /	18 /	13.4164	

**Maximum Likelihood-Probit**

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	0.98678	0.38277	0.23655	1.73701	0	0.50843	9.48773 /	0.97 /	2.35039	1.0134	3
Intercept	2.68068	0.72079	1.26793	4.09344							

Point	Probits	ppb	95% Fiducial Limits	
EC01	2.674	0.98373	1.3E-06	6.70959
EC05	3.355	4.82505	0.00096	17.4817
EC10	3.718	11.2635	0.03084	30.2162
EC15	3.964	19.956	0.30672	45.7083
EC20	4.158	31.441	1.77502	68.1149
EC25	4.326	46.4373	7.03886	109.068
EC40	4.747	124.066	56.5895	1429.78
EC50	5.000	224.076	98.3416	13555.9
EC60	5.253	404.703	151.122	145345
EC75	5.674	1081.24	284.078	8148831
EC80	5.842	1596.96	360.565	4.1E+07
EC85	6.036	2516.03	473.956	2.7E+08
EC90	6.282	4457.77	665.526	4.9E+08
EC95	6.645	10406.1	1094.15	4.9E+08
EC99	7.326	51040.4	2750.88	4.9E+08



Dose ppb

**Macrocystis Germination and Growth Test-Growth-Length**

Start Date: 7/23/2003 16:35 · Test ID: C020724.14 · Sample ID: REF-Ref Toxicant ·  
 End Date: 7/25/2003 18:30 · Lab ID: CAMEC-MEC Analytical Syst-Sample Type: CUCL-Copper chloride ·  
 Sample Date: 7/23/2003 08:00 · Protocol: EPAW 95-EPA West Coast · Test Species: MP-Macrocystis pyrifera ·  
 Comments:

Conc-ppb	1	2	3	4	5
Control	15.587	12.870	13.442	12.870	14.157
5.6	15.158	14.157	13.585	13.156	13.156
10	12.441	13.871	12.727	12.441	12.441
18	11.297	11.011	11.583	11.440	11.583
32	11.297	10.725	10.153	10.296	10.010
100	8.866	9.009	8.723	9.009	8.866
180	7.722	7.150	7.865	7.293	7.722

Conc-ppb	Transform: Untransformed							1-Tailed		
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control	13.785	1.0000	13.785	12.870	15.587	8.253	5			
5.6	13.842	1.0041	13.842	13.156	15.158	6.085	5	-0.142	2.409	0.968
*10	12.784	0.9274	12.784	12.441	13.871	4.850	5	2.490	2.409	0.968
*18	11.383	0.8257	11.383	11.011	11.583	2.102	5	5.976	2.409	0.968
*32	10.496	0.7614	10.496	10.010	11.297	4.969	5	8.182	2.409	0.968
*100	8.895	0.6452	8.895	8.723	9.009	1.345	5	12.166	2.409	0.968
*180	7.550	0.5477	7.550	7.150	7.865	4.106	5	15.509	2.409	0.968

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.90831	0.91	1.20575	2.23728						
Bartlett's Test indicates unequal variances (p = 2.89E-03)	19.8965	16.8119								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	5.6	10	7.48331		0.96825	0.07024	29.4353	0.40401	8.8E-16	6, 28

Use for  
MSDp

**Macrocystis Germination and Growth Test-Proportion Germinated**

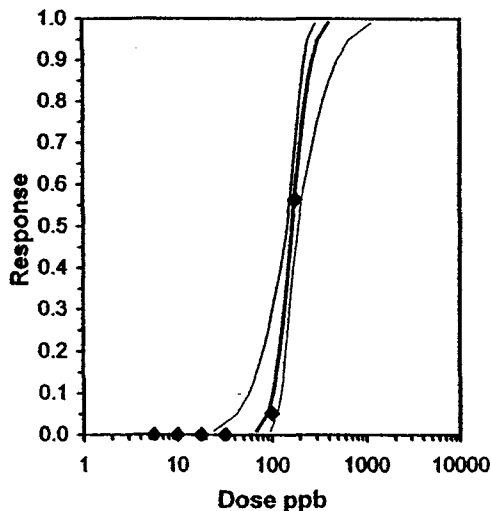
Start Date: 7/23/2003 16:35 Test ID: C020724.14 Sample ID: REF-Ref Toxicant  
 End Date: 7/25/2003 18:30 Lab ID: CAMEC-MEC Analytical, Car-Sample Type: CUCL-Copper chloride  
 Sample Date: Protocol: EPAW 95-EPA West Coast Test Species: MP-Macrocystis pyrifera  
 Comments:

Conc-ppb	1	2	3	4	5
Control	0.8100	0.7000	0.8300	0.7600	0.7300
5.6	0.8300	0.6800	0.8200	0.8300	0.7600
10	0.8800	0.8100	0.8500	0.8900	0.8500
18	0.7400	0.8200	0.8700	0.7500	0.8500
32	0.8000	0.7900	0.8600	0.7400	0.7200
100	0.7000	0.7900	0.7700	0.7200	0.6500
180	0.3700	0.2700	0.3400	0.3500	0.3400 ✓

Conc-ppb	Transform: Arcsin Square Root							t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N					
Control	0.7660	1.0000	1.0680	0.9912	1.1458	6.036	5				117	500
5.6	0.7840	1.0235	1.0905	0.9695	1.1458	7.033	5	-0.566	2.409	0.0960	108	500
10	0.8560	1.1175	1.1831	1.1198	1.2327	3.739	5	-2.891	2.409	0.0960	72	500
18	0.8060	1.0522	1.1181	1.0357	1.2019	6.644	5	-1.258	2.409	0.0960	97	500
32	0.7820	1.0209	1.0876	1.0132	1.1873	6.270	5	-0.493	2.409	0.0960	109	500
100	0.7260	0.9478	1.0215	0.9377	1.0948	6.150	5	1.167	2.409	0.0960	137	500
*180	0.3340	0.4360	0.6157	0.5464	0.6539	6.625	5	11.353	2.409	0.0960	333	500

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95752	0.91	-0.2506	-0.9188						
Bartlett's Test indicates equal variances (p = 0.88)	2.38376	16.8119								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100 ✓	180 ✓	134.164		0.08545	0.1113	0.17607	0.00397	5.0E-13	6, 28

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	6.03412	1.16617	2.79631	9.27193	0.234	11.8984	-9.48773	2.0E-02	2.22102	0.16572	4
Intercept	-8.4019	2.58341	-15.575	-1.2292							
TSCR	0.2012	0.01383	0.1628	0.2396							
Point	Probits	ppb	95% Fiducial Limits								
EC01	2.674	68.4681	24.1973	94.6392							
EC05	3.355	88.8032	42.1681	112.737							
EC10	3.718	102.009	56.5678	124.047							
EC15	3.964	112.01	68.8379	132.563							
EC20	4.158	120.655	80.3067	140.016							
EC25	4.326	128.6	91.4511	147.074							
EC40	4.747	151.02	124.296	169.94							
EC50	5.000	166.349	144.853	191.315							
EC60	5.253	183.234	162.867	223.239							
EC75	5.674	215.179	188.009	303.704							
EC80	5.842	229.35	197.457	345.901							
EC85	6.036	247.049	208.536	403.573							
EC90	6.282	271.272	222.834	491.151							
EC95	6.645	311.611	245.174	658.914							
EC99	7.326	404.16	292.043	1148.33							



Significant heterogeneity detected (p = 2.00E-02)

Test: MC-Macrocyctis Germination and Growth Test

Test ID: C020724.14

Species: MP-Macrocyctis pyrifer

Protocol: EPAW 95-EPA West Coast

Sample ID: REF-Ref Toxicant

Sample Type: CUCL-Copper chloride

Start Date: 7/23/2003 16:35

End Date: 7/25/2003 18:30

Lab ID: CAMEC-MEC Analytical, Carlsbad

Pos	ID	Rep	Group	Total Counted	Number Germ	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	Cal Factor	Notes
	1	1	Control	100	81	10	10	11	12	14	12	11	10	10	9	1.43	
	2	2	Control	100	70	11	8	10	8	10	8	7	11	8	9	1.43	
	3	3	Control	100	83	10	8	12	9	9	12	9	6	9	10	1.43	
	4	4	Control	100	76	9	7	10	9	7	12	12	6	11	7	1.43	
	5	5	Control	100	73	9	11	10	8	11	8	11	8	11	12	1.43	
	6	1	5.600	100	83	10	7	13	8	14	9	11	10	12	12	1.43	
	7	2	5.600	100	68	8	15	10	11	7	9	8	10	13	8	1.43	
	8	3	5.600	100	82	11	10	9	7	14	7	9	8	11	9	1.43	
	9	4	5.600	100	83	11	11	8	9	7	10	9	10	10	7	1.43	
	10	5	5.600	100	76	9	9	7	10	10	9	11	9	8	10	1.43	
	11	1	10.000	100	88	12	7	8	8	8	8	10	8	9	9	1.43	
	12	2	10.000	100	81	9	8	9	12	11	12	11	8	8	9	1.43	
	13	3	10.000	100	85	8	11	7	8	10	9	11	10	8	7	1.43	
	14	4	10.000	100	89	8	8	9	9	8	8	10	8	12	7	1.43	
	15	5	10.000	100	85	9	10	9	10	11	6	9	7	7	9	1.43	
	16	1	18.000	100	74	6	7	7	9	7	10	10	6	7	10	1.43	
	17	2	18.000	100	82	7	10	7	8	7	9	6	9	7	7	1.43	
	18	3	18.000	100	87	5	9	6	7	10	9	10	10	6	9	1.43	
	19	4	18.000	100	75	11	8	6	10	9	9	7	6	7	7	1.43	
	20	5	18.000	100	85	7	9	10	9	8	9	5	7	9	8	1.43	
	21	1	32.000	100	80	10	8	6	10	5	8	6	10	7	9	1.43	
	22	2	32.000	100	79	10	6	7	6	7	7	6	9	8	9	1.43	
	23	3	32.000	100	86	7	8	6	6	6	11	7	6	5	9	1.43	
	24	4	32.000	100	74	5	9	9	7	7	6	8	8	6	7	1.43	
	25	5	32.000	100	72	5	6	6	9	8	9	6	7	7	7	1.43	
	26	1	100.000	100	70	7	6	6	7	7	7	7	5	5	5	1.43	
	27	2	100.000	100	79	6	6	6	6	7	8	5	6	7	6	1.43	
	28	3	100.000	100	77	6	6	7	5	6	6	7	6	6	6	1.43	
	29	4	100.000	100	72	5	9	6	9	6	5	6	6	5	6	1.43	
	30	5	100.000	100	65	7	6	7	7	6	5	5	7	5	7	1.43	
	31	1	180.000	100	37	6	5	6	6	5	5	4	4	7	6	1.43	
	32	2	180.000	100	27	4	4	4	5	6	5	6	7	4	5	1.43	
	33	3	180.000	100	34	7	6	5	5	5	5	6	6	5	5	1.43	
	34	4	180.000	100	35	7	6	5	4	5	4	5	6	4	5	1.43	
	35	5	180.000	100	34	4	5	6	7	5	5	7	4	5	6	1.43	

Comments:

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### 48 Hour *Macrocystis pyrifer* Reference Toxicant Test

REF TOX ID:	C020724.14
LOT NUMBER:	13318L0
ASSOCIATED TEST:	Poseidon

MICROSCOPE:	Olympus I
MICROMETER CONVERSION FACTOR:	1.43
STUDY DIRECTOR:	<i>[Signature]</i>

Test Container Number	Concentration	Number of Spores Germ.	Number of Spores Not Germ.	LENGTH MEASUREMENTS (in ocular micrometer units)										Tech	Date
				L1	L2	L3	L4	L5	L6	L7	L8	L9	L10		
1	0	81	19	10	10	11	11	14	12	11	10	10	9	Cmm	8/22/03
2	0	70	30	11	8	10	8	10	8	7	11	8	9		
3	0	83	17	10	8	12	9	9	12	9	6	9	10		
4	0	76	24	9	7	10	9	7	12	12	6	11	7		
5	↓	73	27	9	11	10	8	11	8	11	8	11	12	↓	↓
55	5.6	83	17	10	7	13	8	14	9	11	10	12	12		
56	5.6	68	32	8	15	10	11	7	9	8	10	13	8	↓	↓
57	5.6	82	18	11	10	9	7	14	7	9	8	11	9		
58	5.6	83	17	11	11	8	9	7	10	9	10	10	7		
60	↓	76	24	9	9	7	10	10	9	11	9	8	10		
61	10	88	12	12	7	8	8	8	8	10	8	9	9		
62	10	81	19	9	8	9	12	11	12	11	8	8	9		
63	↓	85	15	8	11	7	8	10	9	11	10	8	7		
64	↓	89	11	8	8	9	9	8	8	10	8	12	7		
65	↓	85	15	9	10	9	10	11	6	9	7	7	9		
66	18	74	26	6	7	7	9	7	10	10	6	7	10		
67	18	82	18	7	10	7	8	7	9	6	9	7	7		
68	18	87	13	5	9	6	7	10	9	10	10	6	9		
69	↓	75	25	11	8	6	10	9	9	7	6	7	7		
70	↓	85	15	7	9	10	9	8	9	5	7	9	8		
71	32	80	20	10	8	6	10	5	8	6	10	7	9		
72	↓	79	21	10	6	7	6	7	7	6	9	8	9		
73	↓	86	14	7	8	6	6	6	11	7	6	5	9		
74	↓	74	26	5	9	9	7	7	6	8	8	6	7		
75	↓	72	28	5	6	6	9	8	9	6	8	7	7		
76	100	70	30	7	6	6	7	7	7	7	5	5	5		
77	↓	79	21	6	6	6	6	7	8	5	6	5	6		
78	↓	77	23	6	6	7	5	6	6	7	6	6	6		
79	↓	72	28	5	9	6	9	6	5	6	6	5	6		
80	↓	65	35	7	6	7	7	6	5	5	7	5	7		
81	180	37	63	6	5	6	6	5	5	5	4	4	5		
82	↓	27	73	4	4	4	5	6	5	6	7	4	5		
83	↓	34	66	7	6	5	5	5	5	5	6	5	5		
84	↓	35	65	7	6	5	4	5	4	5	6	4	5		
85	↓	34	66	4	5	6	7	5	5	7	4	5	6		

Date: 7.23.03Test: Posed wInvestigator: MAI

Condition of Majority of Blades Used:    Poor                      Fair

GoodNumber of Blades Used: 9                      Weight of Blades: 83gVolume of Release Water: 1LTime blades are placed in release beaker: 1505Time blades are removed from release beaker: 1605Temperature of spore solution: 16.0Check for zoospore motility on microscope: ✓

Fix a 9-mL spore sample with 1 mL formalin.

Determine spore density on the hemacytometer.

Determine density with 5 counts.

1. 452. 623. 674. 445. 43Mean 52.2    S.D. \_\_\_\_\_Mean x 10,000 x 1.11 = 579420 spores/mL. This is the density of spore release.

1.11 is the dilution factor for 1 mL formalin + 9 mL spore solution.

Volume of test container: 40mL

The desired final density of zoospore solution is 7,500 spores/mL of test container.

To determine volume of spores to deliver to test containers:

7,500 spores/mL x 40mL mL/test container = 300,000 spores/test container300,000 spores/test container/density of spore release 579420 spores/mL = 0.518 mL/test containerTemperature of spore solution: 16.0Temperature of test containers: 16.0





## 48 Hour *Macrocystis pyrifera* Reference Toxicant Test

Test ID: <b>C020724.14</b>	Replicates: 5	Study Director: <b>JW</b>	Location: <b>Room 2</b>
Dilution Water Batch: <b>S10072203</b>	Organism Batch: <b>KS 2014</b>	Associated Test(s): <b>Poseidon</b>	Organism: <b>M. pyrifera</b>
Lot Number: <b>B318L0</b>	Date Prepared: <b>7.23.03</b>	Initials: <b>J</b> <b>MAI</b>	

Target Concentration	Quantity of Stock Target	Actual	Quantity of Diluent Target	Actual
5.6 ppb	0.280 mL	0.270	500 mL	500
10 ppb	0.500 mL	0.500	500 mL	500
18 ppb	0.900 mL	0.902	500 mL	500
32 ppb	1.60 mL	1.60	500 mL	500
100 ppb	5.00 mL	5.01	500 mL	500
180 ppb	9.00 mL	9.01	500 mL	500

**0 Hours**    Date: **7.23.03**    WQ Time: **1740**    Start Time: **1635<sup>PM</sup>**    Initials: **MAI**  
**STOCK**

	Control	5.6	10	18	32	100	180
Survival	94	94	94	96	97	97	99
Chlorophyll	16.3	16.0	16.0	16.4	16.2	16.2	16.2
Protein	31.9	31.9	31.9	32.0	31.9	31.6	31.4
Chlorophyll	7.9	8.0	8.0	8.0	8.0	8.0	8.1

**48 Hours**    Date: **7.25.03**    WQ Time: **1815**    End Time: **1830<sup>BH</sup>**    Initials: **BH/MAI**  
**STOCK**

	Control	5.6	10	18	32	100	180
Survival	99	94	92	91	88	88	86
Chlorophyll	16.7	15.9	15.6	16.3	<del>17</del> 16.8	16.2	<del>16</del> 15.7
Protein	31.8	31.9	31.9	31.8	31.8	31.6	31.3
Chlorophyll	8.1	8.3	7.9	7.9	7.9	7.9	7.9

Pass

Fail

ONE 7.25.03 BH

MEC Analytical Systems, Inc.  
Analytical Report

Client: Poseidon Date Received: 06 Aug 03  
Project: Poseidon 1-Sample Date Test Started: 06 Aug 03  
Client Sample ID: Encina Cooling Water Discharge/Desal Date Test Ended: 08 Aug 03  
RO Brine Matrix: Liquid  
MEC Test ID: C030806.0144

Abalone Larval Development  
MEC Testing Protocol No.: BIO044  
EPA/600/R-95/136

Test Organism: *Haliotis rufescens*

Treatments: 100, 50, 12, 6, 3 percent test substance, Seawater Control

Results

Concentration (%)	Average Proportion Normal
Seawater Control	0.8760
3	0.8780
6	0.9160
12	0.8780
50	0.9100
100	0.8680

**Chronic Toxicity Statement:** Development data meet the assumptions of normality (Shapiro-Wilk's Test,  $p > 0.01$ ). Equality of variance was confirmed (Bartlett's Test,  $p = 0.20$ ). Dunnett's Multiple-Comparison Test provided a NOEC (No-Observed-Effect-Concentration) of 100 percent and a LOEC (Lowest-Observed-Effect-Concentration) of greater than 100 percent test substance.

**Toxicity Statement:** Test substance "Encina Cooling Water Discharge/Desal RO Brine" produced an estimated  $IC_{50}$  of greater than 100 percent test substance after 48 hours, using the red abalone, *Haliotis rufescens*.

**Toxicity Units Chronic (TUC):**1

**Protocol Deviations:** To ensure there were embryos for all test chambers, 700 rather than 1000 embryos were added to each chamber.

  
QA Officer

8/26/03  
Date

  
Approved

9/17/03  
Date

**MEC Analytical Systems, Inc.**  
**Analytical Report**

Client:	Poseidon	Date Received:	06 Aug 03
Project:	Poseidon 1-Sample	Date Test Started:	06 Aug 03
Client Sample ID:	Encina Cooling Water Discharge/Desal RO Brine	Date Test Ended:	08 Aug 03
MEC Test ID:	C030806.0144	Matrix:	Liquid

**Abalone Larval Development**  
MEC Testing Protocol No.: BIO044  
EPA/600/R-95/136

Test Organism: *Haliotis rufescens*

**Test Solution Physical and Chemical Data**

<b>Analyte:</b>	<b>Dissolved Oxygen</b>	<b>pH</b>
<b>EPA Method:</b>	360.1	150.1
<b>Method Reporting Limit:</b>	1% Sat.	---

Concentration (%)	D.O. % Saturation	Temp. (°C)	Salinity (ppt)	pH
<b>Initial (Day 0)</b>				
Seawater Control	94	15.6	32.4	7.7
3	95	15.1	32.8	7.8
6	96	15.0	32.4	7.8
12	95	15.0	32.8	7.8
50	95	15.3	33.9	7.9
100	95	15.3	35.3	7.9
<b>Final (Day 2)</b>				
Seawater Control	93	15.5	32.4	8.0
3	95	15.5	32.5	8.0
6	95	15.4	32.7	8.0
12	96	15.4	32.8	8.0
50	97	15.6	33.9	8.0
100	94	15.6	35.3	8.0

\*Water quality measured in surrogate chambers.

**MEC Analytical Systems, Inc.**  
**Analytical Report**

Client:	Poseidon	Date Received:	06 Aug 03
Project:	Poseidon 1-Sample	Date Test Started:	06 Aug 03
Client Sample ID:	Encina Cooling Water Discharge/Desal RO Brine	Date Test Ended:	08 Aug 03
MEC Test ID:	C030806.0144	Matrix:	Liquid

**APPENDIX**  
Pertinent Test Data

**TEST:** Abalone Larval Development, MEC Testing Protocol B10044, EPA/600/R-95/136

**TEST SUBSTANCE:** Initial Salinity (100%) 35.3 ppt  
**DILUTION WATER:** Treated Sea Water.  
Salinity 32.4 ppt  
pH 7.7  
Dissolved Oxygen 94% saturation  
Temperature 15.6 °C

**TEST ORGANISM:** Red Abalone, *Haliotis rufescens*, received from The Cultured Abalone, Goleta, CA. 6 males and 10 females were used (1 male and 4 females spawned).

**TEST CHAMBER:** 600 mL sterile polystyrene flasks, five replicates for each of five concentrations of effluent, and a seawater control. Test chambers contained 200mL test volume each.

**EXPERIMENTAL DESIGN:**

1. Encina Power Station (E.P.S.) personnel collected a 24-hour composite sample of Encina Cooling Water Discharge at 1000 hours on Aug 6, 2003. E.P.S. personnel prepared a mixture of the Encina Cooling Water Discharge with a grab sample of the Desal RO Brine in a 10 to 1 ratio, respectively. MEC personnel received approximately 10 liters of sample in 1 container at 1110 hours on the same day. The temperature of the sample was 9.5°C and the dissolved oxygen level was 78% saturation upon arrival at the MEC laboratory. Proper Chain of Custody procedures were implemented.
2. The temperature of the effluent was adjusted to 15 ± 1°C.
3. Approximately 700 embryos were inoculated into each flask.
4. Test chambers were held at 15 ± 1 °C during testing.
5. Light cycle was 16 hours light to 8 dark.

**REFERENCE TOXICITY:** (Control Chart Attached)

1. ZnSO<sub>4</sub> Lot No. 15105L1, received 2/22/02, opened 12/3/02, expires 2/22/04.
2. LC<sub>50</sub>: 13.75 ppb
3. Laboratory Mean: 29.21 ppb
4. Test Date: 08/06/03 Within 95% Confidence Limits

**EFFECT CRITERIA:** Development

**MORTALITY CRITERIA:** ≥ 80% normal development in the control. MSD < 20%.

**STUDY DIRECTOR:** A. Margolis  
**INVESTIGATORS:** B. Hester, M.A. Irwin, R. Dias, A. Margolis



Test: AB-Abalone Larval Development Test ,

Test ID: C030806.0144

Species: HR-Haliotis rufescens

Protocol: EPAW 95-EPA West Coast

Sample ID: Encina Discharge/Desal RO Brine

Sample Type: EFF3-Power Plant .

Start Date: 8/6/2003 23:30

End Date: 8/8/2003 00:24

Lab ID: CAMEC-MEC Carlsbad .

Pos	ID	Rep	Group	Total Counted	Number Normal	Notes
	1	1	Control	100	91	
	2	2	Control	100	81	
	3	3	Control	100	91	
	4	4	Control	100	86	
	5	5	Control	100	89	
	6	1	3.000	100	87	
	7	2	3.000	100	85	
	8	3	3.000	100	87	
	9	4	3.000	100	85	
	10	5	3.000	100	95	
	11	1	6.000	100	93	
	12	2	6.000	100	93	
	13	3	6.000	100	91	
	14	4	6.000	100	92	
	15	5	6.000	100	89	
	16	1	12.000	100	90	
	17	2	12.000	100	84	
	18	3	12.000	100	93	
	19	4	12.000	100	80	
	20	5	12.000	100	92	
	21	1	50.000	100	92	
	22	2	50.000	100	96	
	23	3	50.000	100	91	
	24	4	50.000	100	88	
	25	5	50.000	100	88	
	26	1	100.000	100	88	
	27	2	100.000	100	86	
	28	3	100.000	100	89	
	29	4	100.000	100	86	
	30	5	100.000	100	85	

Comments:

CLIENT: Poseidon  
 PROJECT: Poseidon 1-Sample  
 CLIENT SAMPLE ID: 7 C030806.0144  
 MEC SAMPLE ID: Erma Lockmiller Discharge / Desal 20 Brine  
 STUDY DIRECTOR: MAH

DATE RECEIVED: 8.6.03  
 DATE TEST STARTED: 8.6.03  
 DATE TEST ENDED: 8.9.03  
 MEC SOP NO.: B10044  
 SPECIES: H. rufescens

	Concentration	DO (%)	Temp (°C)	Salinity (ppt)	pH
Day 0 (0 Hours) Date: <u>8/26/03</u> Time: <u>1610</u> Technician: <u>19</u>	Control	94	<u>15.6</u>	32.4	7.7
	Brine-Control				
	3	95	15.1	32.8	7.8
	6	96	15.0	32.4	7.8
	12	95	15.0	32.8	7.8
	50	95	15.3	33.9	7.9
	100	95	15.3	35.3	7.9
48 Hours Date: <u>8/18/03</u> Time: <u>1405</u> Technician: <u>AMM</u>	Control	93	15.5	32.4	8.0
	Brine-Control				
	3	95	15.5	32.5	8.0
	6	95	15.4	32.7	8.0
	12	96	15.4	32.8	8.0
	50	97	15.6	33.9	8.0
	100	94	15.6	35.3	8.0

**SPAWNING DATA**

Initial Spawning Time: <u>2125</u>	Final Spawning Time: <u>2250</u>	Fertilization Time: <u>2250</u>	No. Of Females: <u>4</u>	No. of Males: <u>1</u>
Count/mL of Egg Dilution:	1. <u>215</u>	2. <u>192</u>	3. <u>194</u>	Mean: <u>200.3</u>
Dilution Factor: <u>3.5 mL</u>		<u>700 eggs/chamber</u>		

**LARVAL COUNT DATA**

Conc.	Rep 1		Rep 2		Rep 3		Rep 4		Rep 5		Initials
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
Control	91	9	81	19	91	9	86	14	89	11	MAH
Brine-											
3	87	13	85	15	87	13	85	15	95	5	MAH
6	43	7	93	7	91	9	92	8	89	11	MAH
12	90	10	84	16	93	7	80	20	92	8	MAH
50	92	8	90	4	91	9	88	12	88	12	MAH
100	88	12	86	14	89	11	86	14	85	15	MAH

QA counts: 3 89/11 63 92/8 505 90/10 ✓ 590 difference 137

START TIME: 2330  
 END TIME: 0040  
 ORGANISM BATCH: TCA 71166 + 7121  
 HOBO TEMP. NO.: 8316  
 TEST LOCATION: Room 2

DILUTION WATER BATCH: S10080403  
 pH: 7.7 DO: 94 TEMP: 15.6  
 REF TOX: C02022.08 LOT NO.: 15105L1  
 48-HR LC50: 13.75 TEST DATE: 8.6.03  
 TEST ACCEPTABILITY:  
 80% NORMAL SHELL DEVELOPMENT IN SURVIVING CONTROLS  
 MSD < 20%

WE 8/26/03 19  
 IE 8/25/03 19

Analytical Report

Client: Poseidon Date Received: 21, 23, 25 Jul 03  
 Project: Poseidon 1-Discharge Samples Date Test Started: 22 Jul 03  
 Client Sample ID: Enc. Cooling Water Discharge/Brine RO Desal Date Test Ended: 29 Jul 03  
 MEC Sample ID: C030721.0863, C030723.0163, C030725.1763 Matrix: Liquid

Chronic 7-Day Survival/Growth Bioassay

MEC Testing Protocol: BIO063  
 EPA/600/R-95/136

Test Organism: *Atherinops affinis*  
 Age: 10 days old

Percent Test Solution	Number of Test Organisms at Start of Test	Number of Test Organisms at End of Test	Percent Survival	Mean Dry Weight (mg) After 7 Days	Change in Weight (mg) After 7 Days
Control	25	23	92	1.227	1.110
3	25	23	92	1.326	1.106
6	25	25	100	1.197	1.197
12	25	23	92	1.359	1.250
50	25	25	100	1.367	1.367
100	25	24	96	1.162	1.119

Survival Endpoint Summary

Distribution Method	Result	Variance Method	Result
Shapiro-Wilk's Test	Non-Normal; $p \leq 0.01$	Cannot Be Confirmed	N/A

Hypothesis Method	NOEC	LOEC	TUc	Point Estimation Method	LOD
Steel's Many-One Rank Test	100%	>100%	1	Linear Interpolation	>100%

**Chronic Toxicity Statement - Survival:** Test substance Enc. Cooling Water Discharge/Brine RO Desal and renewals produced 96 percent survival at the 100 percent concentration at the end of 7 days. The LC<sub>50</sub> at 7 days was calculated to be greater than 100 percent test substance.

Toxicity, expressed as toxic units chronic (TUc), was calculated to be 1.

**Protocol Deviations:** The temperature of replicate 2 of the 6 percent concentration went above protocol limits on Day 2 and replicate 3 of the 100 percent concentration did the same, on Day 3. The test chambers were moved to a different shelf and temperatures were within range for the remainder of the test. Since there was no significant mortality seen in the test, this deviation is not expected to impact the significance of the test results.

  
 QA Officer

9/10/03  
 Date

  
 Approved

9/17/03  
 Date





# MEC Analytical Systems, Inc.

## Analytical Report

**Client:** Poseidon  
**Project:** Poseidon 1-Discharge Samples  
**Client Sample ID:** Enc. Cooling Water Discharge/Brine  
 RO Desal  
**MEC Sample ID:** C030721.0863, C030723.0163,  
 C030725.1763

**Date Received:** 21, 23, 25 Jul 03  
**Date Test Started:** 22 Jul 03  
**Date Test Ended:** 29 Jul 03  
**Matrix:** Liquid

**Chronic 7-Day Survival/Growth Bioassay**  
 MEC Testing Protocol: BIO063  
 EPA/600/R-95/136

Test Organism: *Atherinops affinis*

### Test Solution Physical and Chemical Data

Concn	Parameter	Temp (°C)	pH	DO (ppm)	Salinity (ppt)	Hardness (ppm)
Control	Mean	82	20.7	8.0	90	31.9
	Minimum	79	20.3	7.9	85	31.8
	Maximum	85	21.2	8.1	97	32.1
3	Mean	84	20.8	8.0	91	32.0
	Minimum	83	20.3	7.9	86	31.9
	Maximum	86	21.3	8.1	97	32.2
6	Mean	84	20.8	8.0	91	32.2
	Minimum	82	20.2	7.9	85	31.9
	Maximum	86	21.5	8.2	98	32.3
12	Mean	83	20.7	8.0	93	32.4
	Minimum	80	20.1	7.9	89	32.3
	Maximum	85	21.3	8.1	99	32.5
50	Mean	83	20.6	8.1	99	33.7
	Minimum	81	19.6	7.9	97	33.4
	Maximum	86	21.3	8.1	102	33.8
100	Mean	83	20.6	8.1	107	35.5
	Minimum	81	19.1	7.9	102	35.4
	Maximum	85	21.6	8.2	112	35.6

MEC Analytical Systems, Inc.

Analytical Report

**Client:** Poseidon  
**Project:** Poseidon 1-Discharge Samples  
**Client Sample ID:** Enc. Cooling Water Discharge/Brine  
RO Desal  
**MEC Sample ID:** C030721.0863, C030723.0163,  
C030725.1763

**Date Received:** 21, 23, 25 Jul 03  
**Date Test Started:** 22 Jul 03  
**Date Test Ended:** 29 Jul 03  
**Sample Matrix:** Liquid

**APPENDIX**  
Pertinent Test Data

**TEST:** Chronic 7-Day Survival / Growth Bioassay, MEC Testing Protocol BIO063, EPA/600/R-95/136

**LAB CONTROL WATER:** Sea Water from Scripps Institute of Oceanography  
Dissolved Oxygen 97 % Saturation  
Temperature 20.3°C  
pH 7.9  
Salinity 31.9 ppt

**TEST ORGANISM:** Topsmelt, *Atherinops affinis* Age: 10 days old  
Supplier: Aquatic Biosystems, Fort Collins, CO.  
Organisms were acclimated to test salinity and fed newly hatched *Artemia ad libitum* prior to test initiation

**TEST CHAMBER:** 500 mL plastic containers, 5 concentrations, 5 replicates per concentration, and 5 replicate controls, brought to a final volume of 250 mL.

**EXPERIMENTAL DESIGN:**

1. Encina Power Station personnel collected the test substance on July 21, 23, and 25, 2003 at 0900, 0800, and 0800 hours, respectively. MEC personnel received 10 liters the same days as the collections at 1230, 0925, and 1140 hours, respectively. Temperatures ranged from 5.6 to 8.7°C upon receipt.
2. The salinity of the samples was adjusted prior to receipt by MEC personnel. Encina Power Station personnel diluted the Poseidon brine sample by using 10 parts discharge water of Encina Power Station to every 1 part Poseidon brine sample with resulting salinities ranging from 35.1 to 35.2 ppt.
3. The temperature of the test substance was adjusted to 20±1°C.
4. Five test organisms were placed into each chamber.
5. Test chambers were randomized and held at 20±1°C for 7 days with a photoperiod of 16 hours light, 8 hours darkness.

**MORTALITY CRITERIA:** Lack of respiratory movement and lack of reaction to gentle prodding.

**ACCEPTIBILITY CRITERIA:** ≥ 80% survival in controls; ≥ 0.85 mg mean dry weight per surviving organism in control.  
Evaluation of the concentration-response relationship indicated that the data represented in this report are reliable.

**REFERENCE TOXICITY:** Toxicant: CuSO<sub>4</sub>, Lot No.: 2351-09, Received: 3/14/03, Opened: 3/25/03, (Control Charts Included) Expires: 12/14/04.  
7 Day LC50: 158.74 ppb Survival / 166.47 ppb Combined  
Laboratory Mean: 160.93 ppb Survival / 168.34 ppb Combined  
Test Date: 7/22/2003 Within 95 % Confidence Limits

**STUDY DIRECTOR:** T. Staker  
**INVESTIGATORS:** T. Staker, R. Marshall, C. Osuch, D. Sowersby, J. Word, M.A. Irwin, A. Margolis, D. McCoy, R. Dias, L. Sequeira

**Larval Fish Growth and Survival Test-7 Day Survival**

Start Date: 7/22/03 16:45 ✓ Test ID: C030721.0863 ✓ Sample ID: Brine/Discharge ✓  
 End Date: 7/29/03 14:50 ✓ Lab ID: CAMEC-MEC Analytical, Car Sample Type: EFF3-Power Plant ✓  
 Sample Date: 7/21/03 09:00 ✓ Protocol: EPAM 91-EPA Marine ✓ Test Species: AA-Atherinops affinis ✓  
 Comments:

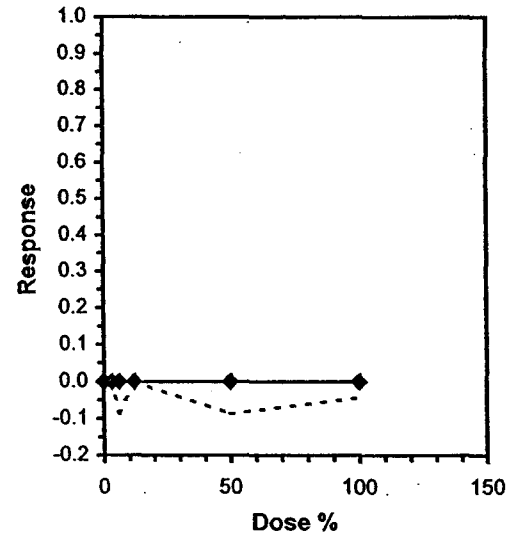
Conc-%	1	2	3	4	5
Control	0.8000	1.0000	1.0000	1.0000	0.8000
3	1.0000	1.0000	0.8000	1.0000	0.8000
6	1.0000	1.0000	1.0000	1.0000	1.0000
12	0.8000	1.0000	1.0000	1.0000	0.8000
50	1.0000	1.0000	1.0000	1.0000	1.0000
100	1.0000	0.8000	1.0000	1.0000	1.0000

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root				Rank Sum	1-Tailed Critical	Isotonic	
			Mean	Min	Max	CV%			Mean	N-Mean
Control	0.9200	1.0000	1.2500	1.1071	1.3453	10.434	5	0.9533	1.0000	
3	0.9200	1.0000	1.2500	1.1071	1.3453	10.434	5	0.9533	1.0000	
6	1.0000	1.0870	1.3453	1.3453	1.3453	0.000	5	0.9533	1.0000	
12	0.9200	1.0000	1.2500	1.1071	1.3453	10.434	5	0.9533	1.0000	
50	1.0000	1.0870	1.3453	1.3453	1.3453	0.000	5	0.9533	1.0000	
100	0.9600	1.0435	1.2977	1.1071	1.3453	8.207	5	0.9533	1.0000	

Audliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.82013	0.9	-0.7502	-0.7133
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1

Linear Interpolation (200 Resamples)				
Point	%	SD	95% CL(Exp)	Skew
IC05	>100			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			



**Larval Fish Growth and Survival Test-7 Day Growth**

Start Date: 7/22/2003 16:45 Test ID: C030721.0863 Sample ID: Brine/Discharge  
 End Date: 7/29/2003 14:50 Lab ID: CAMEC-MEC Carlsbad Sample Type: EFF3-Power Plant  
 Sample Date: 7/21/2003 09:00 Protocol: EPAM 91-EPA Marine Test Species: AA-Atherinops affinis

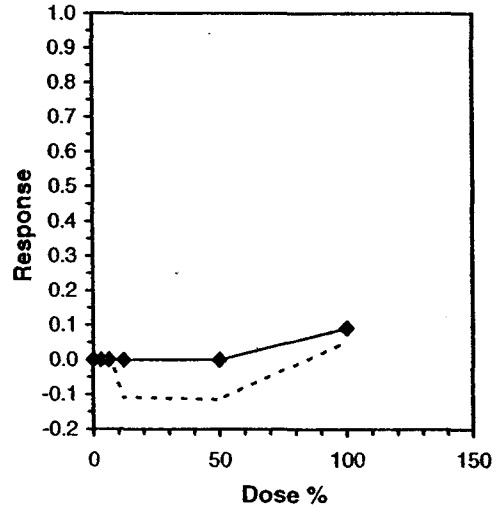
Comments:

Conc-%	1	2	3	4	5
Control	1.2950	0.9840	1.5040	0.7420	1.6075
3	0.9000	0.8840	1.6550	1.1400	1.6050
6	1.1580	1.2100	1.3060	1.1720	1.1400
12	1.1150	1.2040	1.8540	0.9940	1.6300
50	1.0540	1.3420	1.3820	1.4360	1.6220
100	1.2580	1.0825	0.5920	1.4360	1.4420 ✓

Conc-%	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%					Mean	N-Mean
Control	1.2265	1.0000	1.2265	0.7420	1.6075	29.409	5				1.2774	1.0000
3	1.2368	1.0084	1.2368	0.8840	1.6550	30.191	5	-0.053	2.360	0.4614	1.2774	1.0000
6	1.1972	0.9761	1.1972	1.1400	1.3060	5.516	5	0.150	2.360	0.4614	1.2774	1.0000
12	1.3594	1.1084	1.3594	0.9940	1.8540	26.909	5	-0.680	2.360	0.4614	1.2774	1.0000
50	1.3672	1.1147	1.3672	1.0540	1.6220	15.017	5	-0.720	2.360	0.4614	1.2774	1.0000
100	1.1621	0.9475	1.1621	0.5920	1.4420	30.238	5	0.329	2.360	0.4614	1.1621	0.9097

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ( $p > 0.01$ )	0.96961	0.9	-0.1538	-0.7481
Bartlett's Test indicates equal variances ( $p = 0.09$ )	9.57283	15.0863		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Dunnett's Test	MSDu	MSDp	MSB	MSE
	100 ✓	>100 ✓	1 ✓	0.46139 0.37619 0.03654 0.09556 0.85584 5, 24

Linear Interpolation (200 Resamples)				
Point	%	SD	95% CL(Exp)	Skew
IC05	77.693			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100 ✓			



**Larval Fish Growth and Survival Test-7 Day Biomass**

Start Date: 7/22/03 16:45 ✓ Test ID: C030721.0863 ✓ Sample ID: Brine/Discharge ✓  
 End Date: 7/29/03 14:50 ✓ Lab ID: CAMEC-MEC Analytical, Car Sample Type: EFF3-Power Plant ✓  
 Sample Date: 7/21/03 09:00 ✓ Protocol: EPAM 87-EPA Marine ✓ Test Species: AA-Atherinops affinis ✓

Comments:

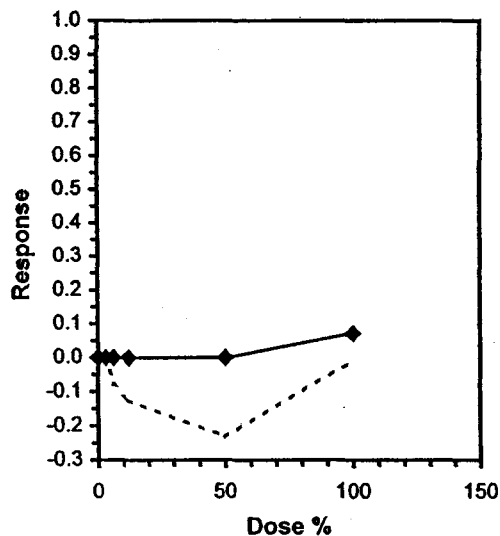
Conc-%	1	2	3	4	5
Control	1.0360	0.9840	1.5040	0.7420	1.2860
3	0.9000	0.8840	1.3240	1.1400	1.2840
6	1.1580	1.2100	1.3060	1.1720	1.1400
12	0.8920	1.2040	1.8540	0.9940	1.3040
50	1.0540	1.3420	1.3820	1.4360	1.6220
100	1.2580	0.8660	0.5920	1.4360	1.4420

Conc-%	Mean	N-Mean	Transform: Untransformed					N	t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	Mean					N-Mean	
Control	1.1104	1.0000	1.1104	0.7420	1.5040	26.371	5				1.2062	1.0000	
3	1.1064	0.9964	1.1064	0.8840	1.3240	18.747	5	0.023	2.360	0.4122	1.2062	1.0000	
6	1.1972	1.0782	1.1972	1.1400	1.3060	5.516	5	-0.497	2.360	0.4122	1.2062	1.0000	
12	1.2496	1.1254	1.2496	0.8920	1.8540	30.038	5	-0.797	2.360	0.4122	1.2062	1.0000	
50	1.3672	1.2313	1.3672	1.0540	1.6220	15.017	5	-1.470	2.360	0.4122	1.2062	1.0000	
100	1.1188	1.0076	1.1188	0.5920	1.4420	33.619	5	-0.048	2.360	0.4122	1.1188	0.9276	

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.99117	0.9	0.14378	0.08824
Bartlett's Test indicates equal variances (p = 0.08)	9.76559	15.0863		
<b>Hypothesis Test (1-tail, 0.05)</b>	<b>NOEC</b>	<b>LOEC</b>	<b>ChV</b>	<b>TU</b>
Dunnett's Test	100	>100		1
				MSDu
				MSDp
				MSB
				MSE
				F-Prob
				df
				0.62886
				5, 24

**Linear Interpolation (200 Resamples)**

Point	%	SD	95% CL(Exp)	Skew
IC05	84.517			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100 ✓			



Test: LF-Larval Fish Growth and Survival Test

Test ID: C030721.0863

Species: AA-Atherinops affinis

Protocol: EPAM 91-EPA Marine

Sample ID: Brine/Discharge

Sample Type: EFF3-Power Plant

Start Date: 7/22/2003 16:45

End Date: 7/29/2003 14:50

Lab ID: CAMEC-MEC Carlsbad

Pos	ID	Rep	Group	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Total Wgt(mg)	Tare Wgt(mg)	Wgt Count
	1	1	Control	5							4	52.66	47.48	4
	2	2	Control	5							5	54.39	49.47	5
	3	3	Control	5							5	55.61	48.09	5
	4	4	Control	5							5	53.02	49.31	5
	5	5	Control	5							4	53.8	47.37	4
	6	1	3.000	5							5	53.63	49.13	5
	7	2	3.000	5							5	52.41	47.99	5
	8	3	3.000	5							4	56.29	49.67	4
	9	4	3.000	5							5	53.07	47.37	5
	10	5	3.000	5							4	55.3	48.88	4
	11	1	6.000	5							5	52.68	46.89	5
	12	2	6.000	5							5	54.55	48.5	5
	13	3	6.000	5							5	54.61	48.08	5
	14	4	6.000	5							5	55.29	49.43	5
	15	5	6.000	5							5	53.54	47.84	5
	16	1	12.000	5							4	53.96	49.5	4
	17	2	12.000	5							5	46.6	40.58	5
	18	3	12.000	5							5	50.35	41.08	5
	19	4	12.000	5							5	45.96	40.99	5
	20	5	12.000	5							4	47.49	40.97	4
	21	1	50.000	5							5	49.88	44.61	5
	22	2	50.000	5							5	52.71	46	5
	23	3	50.000	5							5	53.35	46.44	5
	24	4	50.000	5							5	56.26	49.08	5
	25	5	50.000	5							5	50.88	42.77	5
	26	1	100.000	5							5	50.5	44.21	5
	27	2	100.000	5							4	48.32	43.99	4
	28	3	100.000	5							5	46.05	43.09	5
	29	4	100.000	5							5	50.12	42.94	5
	30	5	100.000	5							5	54.92	47.71	5

Comments:

7-DAY CHRONIC BIOASSAY DATA SHEET #3  
ORGANISM WEIGHT DATA FOR TOPSMELT — Definitive

BRINE / DISCH.  
BIO063

Concentration (%)	Replicate	Boat Number	Weight Empty Boat (mg)	Weight Boat & Animals (mg)
CONTROL	1	78	47.48	52.66
	2	79	49.47	54.39
	3	80	48.09	55.61
	4	81	49.31	53.02
	5	82	47.37	53.80
3	1	83	49.13	53.63
	2	84	47.99	52.41
	3	85	49.67	56.29
	4	86	47.37	53.07
	5	87	48.88	55.30
6	1	88	46.89	52.68
	2	89	48.50	54.55
	3	90	49.08	54.61
	4	91	49.43	55.29
	5	92	47.84	53.54
12	1	93	49.50	53.96
	2	94	40.58	46.60
	3	95	41.08	50.35
	4	96	40.99	45.96
	5	97	40.97	47.49
50	1	98	44.61	49.88
	2	99	46.00	52.71
	3	100	46.44	53.35
	4	101	49.08	56.26
	5	102	42.77	50.88
100	1	103	44.21	50.50
	2	104	43.99	48.32
	3	105	43.09	45.08A 46.05
	4	106	42.94	50.12
	5	107	47.71	54.92
	1			
	2			
	3			
	4			
	5			
	1			
	2			
	3			
	4			
	5			

① M.L. 7/30/03 B.A.

7/29/03  
AM

7/30/03  
B.A.  
1420