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Results of Toxicity Evaluation of Cruise Ship Wastewater Whole Effluent

Southeast Alaska

July 2002

Prepared for

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SUMMARY

Acute and chronic toxicity tests were conducted on six effluent samples collected from five commercial cruise ships discharging into Alaskan marine waters. Tests were conducted to measure the toxicity, if any, of commercial passenger vessel effluent to representative marine organisms. Acute toxicity tests were conducted using the test organisms *Mysidopsis bahia* (Mysid shrimp) and *Atherinops affinis* (Topsmelt). Survival was evaluated after 48 hours of exposure using *M. bahia* and 96 hours of exposure using *A. affinis*. Chronic toxicity tests were conducted using the bivalve species *Mytilus galloprovincialis* (formerly *Mytilus edulis*) and the echinoderm species *Strongylocentrotus purpuratus*. Bivalve larval development was evaluated after 48 hours of exposure to effluents. Echinoderm sperm cells were exposed to sample materials for 20 minutes and evaluated for their ability to successfully fertilize egg cells.

Whole effluent toxicity (WET) tests were conducted at the direction of the Alaska Department of Environmental Conservation (ADEC) working in conjunction with a Science Advisory Panel (SAP). Carolyn Morehouse managed the project for ADEC. The sample manager was Jon Lindstrom of Shannon and Wilson, Inc. in Fairbanks, Alaska. Testing was conducted by AMEC Earth & Environmental, Inc. (AMEC) in Fife, Washington. Sampling and WET testing followed procedures specified in the Quality Assurance Project Plan (QAPP) developed by Shannon and Wilson, final revision 27 June 2002.

Samples were tested at concentrations of 50 percent, 5 percent, 0.5 percent, 0.05 percent, 0.005 percent, and 0.0005 percent. Two of the samples (Mercury mixed effluent and Yorktown blackwater) exhibited no effect in any concentration on any of the species tested. One of the samples (Volendam mixed effluent) demonstrated no acute toxicity but produced an effect at 50 percent effluent for both chronic endpoints. Three of the samples (Kennicott mixed effluent, Dawn Princess graywater, and Yorktown graywater) exhibited toxicity at the highest concentration tested for all four species tested.

MATERIALS AND METHODS

Sample Collection and Transport

Effluent samples were collected from five commercial cruise ships on 10, 11, and 13 July 2002. Sheldon Shaw of Shannon and Wilson coordinated sample collection and shipment to the laboratory. Graywater, a combination of laundry water, galley water, and accommodation water, was sampled from two vessels: the Dawn Princess and the

Yorktown. Blackwater, wastewater discharged from the sanitation system, was collected from one vessel: the Yorktown. Mixed effluent containing a combination of graywater and blackwater was collected from three vessels: the Mercury; Kennicott; and Volendam). Detailed sample information for the six samples is shown in Table 1.

Sample containers consisted of 20-liter polyethylene collapsible cubitainers. Immediately upon collection, samples were immersed in an ice bath and cooled to 2 to 4 degrees centigrade (°C). Samples were packed in coolers containing ice, which were then sealed and delivered to the Juneau Airport for transport to the laboratory. All samples were shipped by air express (Alaska Airlines "Goldstreak") and picked up by AMEC personnel at Seattle Tacoma International Airport on 11, 12, and 13 July 2002. Samples arrived within 24 hours of collection in good condition. Appropriate chain-of-custody procedures were employed during collection and transport. Chain-of-custody documentation is contained in Appendix D.

Sample Receipt

Upon arrival at AMEC, the coolers were opened and samples were matched to the chain-of-custody forms. Receipt temperature was measured in a temperature blank contained in each cooler and recorded on the chain-of-custody form. Standard water quality parameters were measured in a subsample taken from each effluent sample and recorded in a bound logbook. Receipt temperatures and initial water quality measurements are included in Table 1. Samples were held in a 4°C cold room until used for testing.

Organism Procurement and Handling

Mysid Shrimp and Topsmelt

Test specimens (*Mysidopsis bahia* and *Atherinops affinis*) were obtained on 9 and 13 July 2002 from Aquatic Biosystems located in Fort Collins, Colorado. The organisms were transported to AMEC in oxygen-saturated water contained in plastic bags. An insulated ice chest containing the bags was shipped by overnight delivery service. Upon arrival at AMEC, organism receipt information including physical parameters and observations regarding organism health was recorded. Test organisms were acclimated to test conditions and held until test initiation.

Bivalve and Echinoderm

Blue mussels, *Mytilus galloprovincialis,* were obtained on 8 July 2002 from Carlsbad Aquafarms, Inc. located in Carlsbad, California. Echinoderms, *Strongylocentrotus*

purpuratus (purple urchin), were collected by AMEC personnel off the Mission Bay Jetty in San Diego, California. Test organisms were transported to the AMEC Aquatic Toxicology Laboratory in San Diego, California where they were held in high volume aquaria of flow-through natural seawater. Mussels and echinoderms were shipped to the Northwest Bioassay Laboratory in three batches. Each shipment was packed in an insulated ice chest and shipped by overnight delivery service. Test organisms were spawned on the day of receipt or held overnight in a room maintained at 12°C for use the next day.

Table 1. Sample Receipt Information

	Sample ID					
	Mercury	**Volendam**	**Kennicott**	**Dawn Princess**	**Yorktown**	**Yorktown**
Sample Type	Mixed Effluent	Mixed Effluent	Mixed Effluent	Graywater	Graywater	Blackwater
AMEC ID	02-347	02-345	02-346	02-349	02-350	02-351
Sample Date	07/10/02	07/10/02	07/10/02	07/11/02	07/13/02	07/13/02
Sample Time	16:25	17:20	13:50	23:30	06:30	08:00
Receipt Date	07/11/02	07/11/02	07/11/02	07/12/02	07/13/02	07/13/02
Receipt Time	08:50	08:50	08:50	17:00	19:15	19:15
Receipt Temp. (°C)	3.5	3.5	1.0	2.5	4.2	5.1
Dissolved Oxygen (mg/L)	8.3	5.0	11.3	0.8	8.8	7.6
pH	7.77	7.42	8.44	6.85	8.55	6.85
Conductivity (µS/cm)	55	655	2010	2690	255	23,600
Salinity (ppt)	-	-	19.7	2.3	-	21.8
Hardness (mg/L $CaCO_3$)	<5	68	240	320	60	>1000
Alkalinity (mg/L $CaCO_3$)	8	220	20	92	56	120
Chlorine (mg/L)	<.03	<.03	30.3	<.03	16.2	<.03
Ammonia (mg/L)	2.7	27.6	0.6	10.0	0.3	7.1

Sample Preparation and Handling

The salinity of each sample was adjusted to 30±2 parts per thousand (ppt) prior to mixing dilutions. Sample salinity for the acute toxicity tests was increased to the prescribed testing range by adding artificial seasalts (40 Fathoms Crystal Sea Marinemix) and mixing the sample on a stir plate for a minimum of 1 hour. Hypersaline brine was used to increase the salinity of samples for the bivalve and echinoderm tests. Hypersaline brine was made by freezing natural seawater to obtain concentrated brine with a final salinity of 60 to 80 ppt.

Test Procedures

Test procedures are summarized in Tables 2 through 5. All tests were initiated within 36 hours of sample collection. Tests conducted with samples collected from the Mercury, Volendam, and Kennicott were initiated on 11 July 2002. Tests conducted with the sample collected from the Dawn Princess were initiated on 13 July 2002. Tests conducted with the graywater and blackwater samples collected from the Yorktown were initiated on 14 July 2002.

Acute Toxicity Tests

The test procedures described follow those referenced in EPA/600/4-90/027F as they pertain to the mysid shrimp *Mysidopsis bahia* and the fish larvae *Atherinops affinis* (topsmelt). Mysids and topsmelt were used as the test organisms to determine the acute toxicity of the effluent samples. Four-day old mysid juveniles were used to initiate tests with samples from the Mercury, Kennicott, Volendam, and Yorktown. Three-day old juveniles were used to initiate tests with sample from the Dawn Princess. The Mysid shrimp were fed daily during the exposure by adding brine shrimp nauplii to each test chamber in the morning prior to solution renewal. An 80 percent solution renewal was conducted 24 hours after test initiation. Temperature, dissolved oxygen (DO), pH, and salinity were monitored and recorded daily. Survival of the mysid juveniles was recorded at 24 and 48 hours of exposure.

Topsmelt larvae were eight days old at test initiation with samples from the Mercury, Kennicott, and Volendam. Larvae were ten and eleven days old at test initiation with the Dawn Princess and Yorktown samples, respectively. Topsmelt were fed brine shrimp nauplii each morning prior to solution renewal. An 80 percent solution renewal was conducted 48 hours after test initiation. Water quality parameters and organism survival were monitored and recorded daily.

Table 2. *Mysidopsis bahia* 48-hour Acute Survival Test Procedure

Test Organism:	*Mysidopsis bahia*
Test Organism Source:	Aquatic Biosystems; Fort Collins, Colorado
Test Organism Age:	3-5 days post hatch
Test Duration:	48 hours with solution renewal at 24 hours
Feeding:	*Artemia* nauplii during holding time and 2 hours prior to solution renewal
Test Chamber:	1 liter (L) polypropylene beaker
Test Solution Volume:	500 milliliters
Dilution Water:	40 Fathoms Artificial seawater
Test Temperature:	25±1°C
Salinity:	30 ppt
Test Concentrations (% sample):	50, 5, 0.5, 0.05, 0.005, 0.0005, 0 (control)
Number of Organisms/Chamber:	10
Number of Replicates/Concentration:	2
Illumination:	16 hours light/ 8 hours dark
Aeration:	Dawn Princess sample material required aeration prior to preparing dilutions. No other samples were aerated.
Test Protocol:	EPA/600/4-90/027F
Test Acceptability:	≥ 90% control survival
Reference Toxicant:	Copper chloride

Table 3. *Atherinops affinis* 96-hour Acute Survival Test Procedure

Test Organism:	*Atherinops affinis*
Test Organism Source:	Aquatic Biosystems; Fort Collins, Colorado
Test Organism Age:	7-14 days post hatch
Test Duration:	96 hours with solution renewal at 48 hours
Feeding:	*Artemia* nauplii during holding and 2 hours prior to solution renewal
Test Chamber:	1 liter (L) polypropylene beaker
Test Solution Volume:	500 milliliters
Dilution Water:	40 Fathoms Artificial seawater
Test Temperature:	20±1°C
Salinity:	30 ppt
Test Concentrations (% sample):	50, 5, 0.5, 0.05, 0.005, 0.0005, 0 (control)
Number of Organisms/Chamber:	5
Number of Replicates/Concentration:	4
Illumination:	16 hours light/ 8 hours dark
Aeration:	Dawn Princess sample material required aeration prior to preparing dilutions. No other samples were aerated.
Test Protocol:	EPA/600/4-90/027F
Test Acceptability:	≥ 90% control survival
Reference Toxicant:	Copper chloride

Chronic Toxicity Tests

The test procedures described follow those referenced in EPA/600/R-95/136 as they pertain to the bivalve *Mytilus galloprovincialis* and the echinoderm *Strongylocentrotus purpuratus.* The bivalve larval development and echinoderm sperm cell fertilization assays were used to evaluate the chronic toxicity of the commercial cruise ship samples. The bivalve larval development test was initiated with blue mussel larvae less than 4 hours past fertilization. Larvae were exposed to sample for 48 hours, preserved, and evaluated microscopically for survival and normal development. Tests were conducted in 30-milliliter (ml) glass vials each containing 150 to 300 larvae. The percentage of normally-developed embryos of the total number of surviving larvae was calculated. Water quality parameters were monitored daily in surrogate test chambers. Specifications for the bivalve and echinoderm tests are listed in Tables 4 and 5.

Table 4. Bivalve Larval Development Test Procedure

Test Organism:	*Mytilus galloprovincialis*
Test Organism Source:	Carlsbad Aquafarms; Carlsbad, California
Test Organism Age:	<4 hours post-fertilization
Test Duration:	48 hours
Test Chamber:	30-ml glass vials
Test Solution Volume:	10 milliliters
Test Temperature:	15±1°C
Dilution Water:	Seawater collected off Dash Point, Washington and filtered through 5-μm screen
Salinity:	30 ppt
Source of Salinity:	Hypersaline brine made by freezing dilution water to a salinity of 60-80 ppt
Test Concentrations (% sample):	50, 5, 0.5, 0.05, 0.005, 0.0005, 0 (lab and brine controls)
Number of Organisms/Chamber:	150-300
Number of Replicates/Concentration:	5
Illumination:	16 hours light/ 8 hours dark
Aeration:	Dawn Princess sample material required aeration prior to preparing dilutions. No other samples were aerated.
Test Protocol:	EPA/600/R-95/136
Test Acceptability:	≥ 70% normal development in control

The echinoderm fertilization test assesses the viability of sperm cells after 20 minutes of exposure to sample materials. Purple sea urchins were spawned and sperm cells were distributed to 30-ml glass vials containing concentrations of each sample. After 20 minutes, eggs were added to the vials and allowed an additional 20 minutes for

fertilization to occur. The embryos were then preserved for future examination with a microscope to assess the percentage of successful fertilization. Water quality parameters were evaluated in surrogate test chambers.

Table 5. Echinoderm Fertilization Test Procedure

Test Organism:	*Strongylocentrotus purpuratus*
Test Organism Source:	Collected by AMEC (San Diego Lab), Mission Bay Jetty, CA
Test Organism Age:	<4 hours post gamete collection
Test Duration:	40 minute total (20 min. sperm exposure; 20 min. fertilization)
Test Chamber:	30-ml glass vials
Test Solution Volume:	10 milliliters
Test Temperature:	12±1°C
Dilution Water:	Seawater collected off Dash Point, Washington and filtered through 5-μm screen
Salinity:	30 ppt
Source of Salinity:	Hypersaline brine made by freezing dilution water to a salinity of 60-80 ppt
Test Concentrations (% sample):	50, 5, 0.5, 0.05, 0.005, 0.0005, 0 (laboratory and brine controls)
Number of organisms/chamber:	1500-2000 eggs
Sperm:Egg Ratio:	200:1
Number of Replicates/Concentration:	5
Aeration:	Dawn Princess sample material required aeration prior to preparing dilutions. No other samples were aerated.
Test Protocol:	EPA/600/R-95/136
Test Acceptability:	≥ 70% control fertilization

RESULTS

Acute and chronic toxicity test results for the six samples collected from the commercial cruise ships are summarized in Table 6. Tables detailing individual replicate results are contained in Appendix A. Two of the samples (Mercury mixed effluent and Yorktown blackwater) exhibited no effect in any concentration for any of the four species tested. One of the samples (Volendam mixed effluent) demonstrated no acute toxicity but produced an observable effect at 50 percent material for both chronic endpoints. Three of the samples (Kennicott mixed effluent, Dawn Princess graywater, and Yorktown graywater) exhibited toxicity at the highest concentration in all four species tested.

Table 6. Whole Effluent Toxicity Test Results – NOEC and LC/EC_{50} in % Effluent

Sample ID	Mysid Acute Survival		Topsmelt Acute Survival		Bivalve Larvae Normal Development		Echinoderm Sperm Cell Fertilization	
	NOEC [a]	LC_{50}	NOEC	LC_{50}	NOEC	EC_{50}	NOEC	EC_{50}
Dawn Princess	5	15.8	5	15.8	0.5	1.5	0.5	1.5
Kennicott	5	19.4	5	15.8	5	14.7	0.5	1.60
Mercury	50	>50	50	>50	50	>50	50	>50
Volendam	50	>50	5	>50	5	12.7	5	>50
Yorktown Graywater	0.5	4.0	0.5	1.5	0.5	1.6	0.05	0.25
Yorktown Blackwater	50	>50	50	>50	50	>50	50	>50

[a]No Observed Effect Concentration

REFERENCE TOXICANT TESTS

Reference toxicant tests were conducted in conjunction with the samples to assess the health of test organisms and soundness of procedures. Bivalve and echinoderm reference toxicant tests were conducted concurrent to each sample assay. Mysid and Topsmelt acute reference toxicant tests were conducted within one week of the sample tests. Results are summarized in Table 7. Results for all tests were within internal control chart limits of ± two standard deviations (Appendix B).

Table 7. Reference Toxicant Results

Test	Date Initiated	LC_{50} (µg/L $CuCl_2$)	CV (%)
Mysidopsis bahia 48-H Survival	07/11/02	312	30.2
Atherinops affinis 96-H Survival	07/11/02	297	30.4
Bivalve Normal Development	07/11/02	14.1	
	07/13/02	13.5	
	07/14/02	16.2	18.0
Echinoderm Fertilization	07/11/02	17.4	
	07/13/02	11.2	
	07/14/02	33.9	34.7

DISCUSSION

All testing was conducted in accordance with the protocols specified in the QAPP and referenced herein. No procedural deviations from protocol were implemented, however, one acceptability criterion for one assay was not met and resulted in an internal quality control review. For the echinoderm test conducted on the Dawn Princess sample on 13 July 2002, the mean control fertilization was 43.4%, well below the acceptability criterion of 70%. A quality control review consisted of blind recounting of the vials in question by a second technician and review of the data set, bench sheets, and procedures. These data were deemed acceptable and usable based on the following: 1) brine control results of 93.4% fertilization (all concentrations contained brine therefore the brine control was used for all statistical comparisons); 2) a full range of responses demonstrated by the population (0% to 96.8%); 3) a clear sample dose response; 4) evidence of crystallization in only the suspect vials indicating an isolated problem; and 5) results of all other chronic laboratory controls (using the same water) during the study were valid and incomparable to these anomalous results. Our review did not confirm a specific cause for the low mean fertilization, although the vials have been saved for additional evaluation as deemed appropriate.

Two of the samples (Mercury mixed effluent and Yorktown blackwater) exhibited no effect in any concentration on any of the species tested. One of the samples (Volendam mixed effluent) demonstrated no acute toxicity but produced an effect at 50 percent effluent for both chronic endpoints. Three of the samples (Kennicott mixed effluent, Dawn Princess graywater, and Yorktown graywater) exhibited toxicity at the highest concentration tested for all four species tested.

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Tidepool Scientific Software. 1992-1994. TOXCALC Comprehensive Toxicity Data Analysis and Database Software, Version 5.0.

WADOE. 1998. Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria. Washington State Department of Ecology. Water Quality Program. Publication number: WQ-R-95-80, Revised December 2001.

Appendix Table A-1. Acute Toxicity Tests
Mercury
July 2002

Concentration		*Mysidopsis bahia* Survival				*Atherinops affinis* Survival			
%	Replicate	# Survivors	% Survival	Mean % Survival	LC_{50} (% effluent)	# Survivors	% Survival	Mean % Survival	LC_{50} (% effluent)
Control	1	10	100	100	>50	5	100	100	>50
	2	10	100			5	100		
	3					5	100		
	4					5	100		
0.0005	1	10	100	100		5	100	95	
	2	10	100			4	80		
	3					5	100		
	4					5	100		
0.005	1	10	100	100		5	100	90	
	2	10	100			3	60		
	3					5	100		
	4					5	100		
0.05	1	10	100	100		4	80	95	
	2	10	100			5	100		
	3					5	100		
	4					5	100		
0.5	1	10	100	95		5	100	100	
	2	9	90			5	100		
	3					5	100		
	4					5	100		
5	1	10	100	100		5	100	95	
	2	10	100			5	100		
	3					4	80		
	4					5	100		
50	1	10	100	100		5	100	95	
	2	10	100			4	80		
	3					5	100		
	4					5	100		

Appendix Table A-2. Chronic Toxicity Tests
Mercury
July 2002

Concentration		Bivalve Larval Development			Echinoderm Sperm Cell Fertilization		
%	Replicate	% Normal	Mean % Normal	EC_{50} (% effluent)	% Fertilized	Mean % Fertilized	EC_{50} (% effluent)
Control	1	75	77.5	>50	100	97.8	>50
	2	74			97		
	3	79			96		
	4	80			98		
	5	79			98		
Brine Control	1	81	81.3		99	98.6	
	2	79			100		
	3	85			97		
	4	81			99		
	5	79			98		
0.0005	1	78	77.7		98	96.8	
	2	77			95		
	3	75			93		
	4	81			99		
	5	78			99		
0.005	1	77	80.8		99	98.6	
	2	81			100		
	3	80			97		
	4	82			98		
	5	85			99		
0.05	1	81	77.9		98	98.8	
	2	71			99		
	3	73			98		
	4	80			99		
	5	84			100		
0.5	1	77	78.6		98	98.0	
	2	83			97		
	3	80			98		
	4	80			99		
	5	73			98		
5	1	81	80.3		100	99.0	
	2	78			100		
	3	85			97		
	4	78			98		
	5	80			100		
50	1	71	79.8		98	97.6	
	2	81			97		
	3	82			98		
	4	85			95		
	5	80			100		

Appendix Table A-3. Acute Toxicity Tests
Kennicott
July 2002

Concentration		*Mysidopsis bahia* Survival				*Atherinops affinis* Survival			
%	Replicate	# Survivors	% Survival	Mean % Survival	LC_{50} (% effluent)	# Survivors	% Survival	Mean % Survival	LC_{50} (% effluent)
Control	1	10	100	100	19.4	5	100	95	15.8
	2	10	100			4	80		
	3					5	100		
	4					5	100		
0.0005	1	10	100	100		4	80	95	
	2	10	100			5	100		
	3					5	100		
	4					5	100		
0.005	1	10	100	100		5	100	95	
	2	10	100			5	100		
	3					4	80		
	4					5	100		
0.05	1	10	100	100		5	100	100	
	2	10	100			5	100		
	3					5	100		
	4					5	100		
0.5	1	10	100	100		5	100	100	
	2	10	100			5	100		
	3					5	100		
	4					5	100		
5	1	10	100	100		5	100	100	
	2	10	100			5	100		
	3					5	100		
	4					5	100		
50	1	3	30	15		0	0	0	
	2	0	0			0	0		
	3					0	0		
	4					0	0		

Appendix Table A-4. Chronic Toxicity Tests
Kennicott
July 2002

Concentration		Bivalve Larval Development			Echinoderm Sperm Cell Fertilization		
%	Replicate	% Normal	Mean % Normal	EC_{50} (% effluent)	% Fertilized	Mean % Fertilized	EC_{50} (% effluent)
Control	1	89	80.4	14.7	96	92.0	1.6
	2	85			78		
	3	78			96		
	4	77			97		
	5	74			93		
Brine Control	1	82	79.8		96	94.0	
	2	82			96		
	3	74			94		
	4	76			89		
	5	85			95		
0.0005	1	80	77.5		97	90.0	
	2	78			94		
	3	77			76		
	4	76			90		
	5	77			93		
0.005	1	83	78.8		93	94.6	
	2	80			94		
	3	80			93		
	4	74			98		
	5	77			95		
0.05	1	74	76.4		84	88.0	
	2	83			88		
	3	72			94		
	4	82			98		
	5	72			76		
0.5	1	84	81.4		95	88.4	
	2	81			94		
	3	83			93		
	4	80			69		
	5	80			91		
5	1	78	76.5		4	1.6	
	2	69			1		
	3	83			1		
	4	81			2		
	5	73			0		
50	1	0	0.0		7	2.8	
	2	0			1		
	3	0			1		
	4	0			1		
	5	0			4		

Appendix Table A-5. Acute Toxicity Tests
Volendam
July 2002

Concentration		*Mysidopsis bahia* Survival				*Atherinops affinis* Survival			
%	Replicate	# Survivors	% Survival	Mean % Survival	LC_{50} (% effluent)	# Survivors	% Survival	Mean % Survival	LC_{50} (% effluent)
Control	1	10	100	100	>50	5	100	95	>50
	2	10	100			5	100		
	3					4	80		
	4					5	100		
0.0005	1	9	90	95		5	100	85	
	2	10	100			3	60		
	3					5	100		
	4					4	80		
0.005	1	10	100	100		5	100	100	
	2	10	100			5	100		
	3					5	100		
	4					5	100		
0.05	1	10	100	100		5	100	80	
	2	10	100			2	40		
	3					4	80		
	4					5	100		
0.5	1	10	100	100		5	100	95	
	2	10	100			5	100		
	3					5	100		
	4					4	80		
5	1	9	90	95		5	100	85	
	2	10	100			5	100		
	3					4	80		
	4					3	60		
50	1	10	100	100		2	40	50	
	2	10	100			3	60		
	3					3	60		
	4					2	40		

Appendix Table A-6. Chronic Toxicity Tests
Volendam
July 2002

Concentration		Bivalve Larval Development			Echinoderm Sperm Cell Fertilization		
%	Replicate	% Normal	Mean % Normal	EC_{50} (% effluent)	% Fertilized	Mean % Fertilized	EC_{50} (% effluent)
Control	1	81	79.1	12.7	99	98.4	>50
	2	72			98		
	3	79			99		
	4	82			99		
	5	81			97		
Brine Control	1	81	82.8		99	98.4	
	2	83			99		
	3	84			99		
	4	84			97		
	5	81			98		
0.0005	1	82	81.8		96	98.0	
	2	84			97		
	3	81			100		
	4	80			98		
	5	82			99		
0.005	1	85	80.6		98	99.2	
	2	80			100		
	3	77			99		
	4	79			99		
	5	82			100		
0.05	1	80	80.5		100	98.6	
	2	80			99		
	3	81			96		
	4	83			99		
	5	78			99		
0.5	1	81	79.9		96	98.0	
	2	83			98		
	3	81			99		
	4	81			97		
	5	75			100		
5	1	82	78.6		100	98.0	
	2	79			98		
	3	81			94		
	4	74			98		
	5	78			100		
50	1	0	0.0		67	63.8	
	2	0			72		
	3	0			50		
	4	0			75		
	5	0			55		

Appendix Table A-7. Acute Toxicity Tests
Dawn Princess
July 2002

Concentration %	Replicate	*Mysidopsis bahia* Survival # Survivors	% Survival	Mean % Survival	LC_{50} (% effluent)	*Atherinops affinis* Survival # Survivors	% Survival	Mean % Survival	LC_{50} (% effluent)
Control	1	10	100	100	15.8	5	100	100	15.8
	2	10	100			5	100		
	3					5	100		
	4					5	100		
0.0005	1	10	100	100		5	100	100	
	2	10	100			5	100		
	3					5	100		
	4					5	100		
0.005	1	10	100	100		5	100	100	
	2	10	100			5	100		
	3					5	100		
	4					5	100		
0.05	1	10	100	100		5	100	100	
	2	10	100			5	100		
	3					5	100		
	4					5	100		
0.5	1	10	100	100		5	100	100	
	2	10	100			5	100		
	3					5	100		
	4					5	100		
5	1	10	100	100		5	100	100	
	2	10	100			5	100		
	3					5	100		
	4					5	100		
50	1	0	0	0		0	0	0	
	2	0	0			0	0		
	3					0	0		
	4					0	0		

Appendix Table A-8. Chronic Toxicity Tests
Dawn Princess
July 2002

Concentration		Bivalve Larval Development			Echinoderm Sperm Cell Fertilization		
%	Replicate	% Normal	Mean % Normal	EC_{50} (% effluent)	% Fertilized	Mean % Fertilized	EC_{50} (% effluent)
Control	1	94	92.2	1.5	33	43.4	1.5
	2	94			43		
	3	87			51		
	4	93			45		
	5	93			45		
Brine Control	1	91	90.4		98	93.4	
	2	88			87		
	3	90			88		
	4	91			97		
	5	92			97		
0.0005	1	94	90.5		87	83.8	
	2	91			90		
	3	89			78		
	4	91			86		
	5	88			78		
0.005	1	91	92.3		84	81.2	
	2	93			79		
	3	92			74		
	4	90			83		
	5	95			86		
0.05	1	93	93.4		91	92.2	
	2	95			89		
	3	94			90		
	4	93			98		
	5	93			93		
0.5	1	91	89.4		97	96.8	
	2	89			96		
	3	90			98		
	4	92			96		
	5	85			97		
5	1	0	0.0		0	0.0	
	2	0			0		
	3	0			0		
	4	0			0		
	5	0			0		
50	1	0	0.0		0	0.0	
	2	0			0		
	3	0			0		
	4	0			0		
	5	0			0		

Appendix Table A-9. Acute Toxicity Tests
Yorktown Graywater
July 2002

Concentration		*Mysidopsis bahia* Survival				*Atherinops affinis* Survival			
%	Replicate	# Survivors	% Survival	Mean % Survival	LC_{50} (% effluent)	# Survivors	% Survival	Mean % Survival	LC_{50} (% effluent)
Control	1	10	100	100	4.0	5	100	100	1.5
	2	10	100			5	100		
	3					5	100		
	4					5	100		
0.0005	1	10	100	100		5	100	95	
	2	10	100			4	80		
	3					5	100		
	4					5	100		
0.005	1	10	100	100		5	100	100	
	2	10	100			5	100		
	3					5	100		
	4					5	100		
0.05	1	10	100	100		5	100	95	
	2	10	100			5	100		
	3					5	100		
	4					4	80		
0.5	1	10	100	100		5	100	100	
	2	10	100			5	100		
	3					5	100		
	4					5	100		
5	1	4	40	40		0	0	0	
	2	4	40			0	0		
	3					0	0		
	4					0	0		
50	1	0	0	0		0	0	0	
	2	0	0			0	0		
	3					0	0		
	4					0	0		

Appendix Table A-10. Chronic Toxicity Tests
Yorktown Graywater
July 2002

Concentration		Bivalve Larval Development			Echinoderm Sperm Cell Fertilization		
%	Replicate	% Normal	Mean % Normal	EC_{50} (% effluent)	% Fertilized	Mean % Fertilized	EC_{50} (% effluent)
Control	1	98	96.4	1.6	100	97.6	0.25
	2	98			99		
	3	96			89		
	4	96			100		
	5	93			100		
Brine Control	1	97	94.6		100	99.8	
	2	92			100		
	3	93			100		
	4	95			100		
	5	96			99		
0.0005	1	96	96.3		99	97.2	
	2	98			99		
	3	95			96		
	4	95			98		
	5	97			94		
0.005	1	98	97.0		96	98.6	
	2	99			100		
	3	95			99		
	4	97			100		
	5	97			98		
0.05	1	93	96.5		99	98.8	
	2	97			96		
	3	98			100		
	4	97			99		
	5	98			100		
0.5	1	94	94.5		27	21.8	
	2	93			26		
	3	95			25		
	4	95			17		
	5	96			14		
5	1	4	2.7		0	0.0	
	2	4			0		
	3	3			0		
	4	2			0		
	5	1			0		
50	1	0	0.0		0	0.0	
	2	0			0		
	3	0			0		
	4	0			0		
	5	0			0		

Appendix Table A-11. Acute Toxicity Tests
Yorktown Blackwater
July 2002

Concentration		*Mysidopsis bahia* Survival				*Atherinops affinis* Survival			
%	Replicate	# Survivors	% Survival	Mean % Survival	LC_{50} (% effluent)	# Survivors	% Survival	Mean % Survival	LC_{50} (% effluent)
Control	1	10	100	100	>50	5	100	100	>50
	2	10	100			5	100		
	3					5	100		
	4					5	100		
0.0005	1	9	90	95		5	100	100	
	2	10	100			5	100		
	3					5	100		
	4					5	100		
0.005	1	10	100	100		5	100	95	
	2	10	100			5	100		
	3					5	100		
	4					4	80		
0.05	1	10	100	95		5	100	100	
	2	9	90			5	100		
	3					5	100		
	4					5	100		
0.5	1	9	90	95		5	100	95	
	2	10	100			5	100		
	3					5	100		
	4					4	80		
5	1	10	100	100		5	100	100	
	2	10	100			5	100		
	3					5	100		
	4					5	100		
50	1	10	100	95		5	100	90	
	2	9	90			5	100		
	3					5	100		
	4					3	60		

Appendix Table A-12. Chronic Toxicity Tests
Yorktown Blackwater
July 2002

Concentration		Bivalve Larval Development			Echinoderm Sperm Cell Fertilization		
%	Replicate	% Normal	Mean % Normal	EC_{50} (% effluent)	% Fertilized	Mean % Fertilized	EC_{50} (% effluent)
Control	1	89	82.3	>50	99	98.4	>50
	2	84			98		
	3	87			97		
	4	78			99		
	5	74			99		
Brine Control	1	96	91.4		98	95.4	
	2	94			100		
	3	89			98		
	4	88			89		
	5	90			92		
0.0005	1	95	90.6		98	97.2	
	2	91			98		
	3	89			95		
	4	95			95		
	5	84			100		
0.005	1	91	93.0		93	97.6	
	2	93			97		
	3	89			99		
	4	96			100		
	5	96			99		
0.05	1	92	91.8		83	91.0	
	2	86			92		
	3	96			82		
	4	94			100		
	5	91			98		
0.5	1	90	92.6		94	96.0	
	2	93			96		
	3	96			99		
	4	89			98		
	5	96			93		
5	1	93	91.9		98	97.4	
	2	88			98		
	3	91			93		
	4	93			99		
	5	95			99		
50	1	90	91.4		100	97.4	
	2	91			97		
	3	94			98		
	4	89			97		
	5	93			95		