20 July 2006

Technical Memorandum No. 13

To: Joel Faller, Kennedy/Jenks Consultants

From: Cristina Correa, Kennedy/Jenks Consultants
Scott Bodensteiner, MEC/Weston Solutions

Review: Jean Debroux, PhD, Kennedy/Jenks Consultants

Subject: Whole Effluent Biotoxicity Screening Requirements, Protocol and Results
MMWD Seawater Desalination Pilot Program
K/J 0468029

Introduction

The Region 2 Water Quality Control Board (Regional Board), San Francisco Bay Region, issued NPDES Permit No. CA0038814 to MMWD for discharges into San Francisco Bay (the Bay) from its Desalination Pilot Plant operations. In addition, the permit requires a water quality evaluation of expected discharges from a future full-scale desalination plant. The MMWD Desal Pilot Plant NPDES Permit was issued on 16 March 2005 for the pilot plant and will constitute the basis for a future NPDES permit of a full-scale facility. The current permit expires on 31 March 2006 and may be extended if justified to collect additional data toward design or permitting of a full-scale desalination plant.

The MMWD Desal Pilot Plant NPDES permit contains Attachment C, titled “Self-Monitoring Program for Marin Municipal Water District Desalination Pilot Plant” (SMP). This document outlines sampling and testing requirements necessary for permit compliance. In addition to common water quality parameters (temperature, conductivity, TSS, TDS, pH, chlorine concentration, etc.), the schedule of sampling lists requirements for monitoring the toxicity of effluent streams from the pilot plant.

The Regional Board requires acute and chronic biotoxicity testing of the whole effluent to be discharged into the Bay prior to permitting a full-scale desalination facility. Whole effluent (WE) is defined as “discharges that are likely to occur from the permanent desalination plant.” This discharge stream is composed of a blend of Reverse Osmosis (RO) waste brine and Central Marin Sanitation Agency (CMSA) treated effluent.

This memorandum outlines NPDES permit No. CA0038814 Provision D.7 requirements and proposed protocol for WE biotoxicity screening and testing (bioassays) for the MMWD Desalination pilot plant. In addition, the results of the bioassays performed are presented in summary form. As indicated in the permit, if MMWD intends to apply for a permit for a permanent, full-scale desalination facility, it must perform WE screening phase monitoring for acute and chronic toxicity during the pilot study.

The NPDES permit (Provision 7) requires the following WE bioassays:

- acute bioassay screening (two annually (2/Y) at various concentrations as noted in the self-monitoring program)
- chronic bioassay screening (stage 1 and 2)

The listed WE bioassays must be performed to meet the following criteria:

- must follow acute and chronic sensitivity studies
- performed at various brine concentrations (blends)
- performed with 24-hour composite samples

To produce samples of WE, the NPDES permit SMP requires that concentrate (brine) be combined with wastewater effluent from CMSA at various concentrations to evaluate toxicity. Kennedy/Jenks-CH2M Hill proposes the following bioassays to comply with requirements of the NPDES permit:

- High-brine blend
    - acute bioassay
    - chronic bioassay
- Average-brine blend
    - acute bioassay
    - chronic bioassay

Whole Effluent (WE) Blends

The MMWD Desalination Pilot Plant NPDES Permit indicates that RO “concentrate (brine) shall be combined with wastewater effluent from the Central Marin Sanitary Agency at various concentrations to evaluate toxicity.” The ratio of RO brine to CMSA effluent will vary throughout the year and bioassays must evaluate the toxicity of the entire range of blends expected to constitute the WE. Table 1 outlines the possible contributions from the two waste streams that will constitute the WE from a full-scale desalination facility.

Table 1: Contribution from individual effluent streams comprising whole effluent to San Francisco Bay

		RO Brine	CMSA Effluent
Flow Rate (MGD)	Min	1	4
	Avg	4	11
	Max	15	17
Salinity (ppm)		60,000	810

It is important to note that the minimum desalination facility flow rate will not necessarily correspond with the minimum CMSA effluent flow rate. Maximum brine flow rates from the desalination plant are expected during dry months, when CMSA flow rates are lower. On the other hand, maximum flow rates from the CMSA facility are expected during the winter months due to high levels of precipitation when the desalination plant brine flow rates would be low. Therefore, the anticipated range of WE RO brine to CMSA effluent ratios is between 1:17 and 15:4. A 1 MGD RO brine to 17 MGD CMSA effluent blend, however, will not have significantly different water quality to a purely CMSA effluent discharge. Therefore, the proposed blends are a high-brine and an average blend, as indicated in Table 2.

Table 2: Salinity of potential WE blend discharges to San Francisco Bay

Blend	RO Brine / CMSA Effluent Flow rate ratio (MGD)	Anticipated salinity (ppm)	Percent Brine
High-brine	15/4	47,539	79%
Average	4/11	16,594	27%
Low-brine	1/17	4,098	5.5%

The two blends that are considered to encompass the range of water quality parameters in a future WE discharge are 4:11 and 15:4 (RO brine:CMSA effluent), or 27% brine during the winter and 79% brine during the dry summer months. These blends will have a salinity of approximately 16,500 ppm and 47,500 ppm, respectively.

NPDES Permit Bioassay Requirements

Acute Toxicity Bioassay Requirements

Compliance with acute toxicity, or percent survival, requirements was based on static renewal bioassay techniques using the most sensitive marine test species, and performed according to the most current USEPA approved method in 40 CFR 136. Each bioassay must, at a minimum:

1. have a duration of 96 hours;
2. be performed in undiluted blends of RO brine and CMSA effluent;
3. measure acceptable percent survival of species as follows:

    a. A 3-sample median value of not less than 90 percent survival,
    b. A single-sample maximum of not less than 70 percent survival; and

4. screen three marine species: a fish, an invertebrate, and an aquatic plant. The most sensitive species shall be determined from three sets of concurrent sensitivity screenings performed before the bioassay testing.

Proposed Acute Toxicity Bioassay Protocols

Consultation with MEC/Weston Solutions (formerly MEC Analytical Systems) staff at the Romberg Tiburon Center for Environmental Sudies resulted in the following recommendations for screening species:

- Inland Silverside (*Atherinops affinis*)
- Mysid shrimp (*Mysidopsis bahia)*
- Marine diatom (*Thallasiosira pseudonanna*)

MEC/Weston staff also indicated that there is no EPA-established protocol for bioassays on marine plants. Because NPDES permit requirements include acute bioassays on an aquatic plant, the marine diatom analysis were based on a comparison of the acute biotoxicity endpoint with the chronic biotoxicity endpoint (marine algae growth rate).

For a more detailed description of Whole Effluent acute toxicity screening requirements, refer to Regional Board Permit No. CA0038814 pages 5-8.

High-Brine Blend Acute Bioassay Protocol

WE discharge to San Francisco Bay during the dry season (approximately May through November) will contain a substantial proportion of RO brine from the full-scale desalination plant, in relation to CMSA effluent. The estimated typical maximum ratio is based on the

desalination facility functioning at a design capacity of 15 MGD while CMSA effluent discharge will be at a minimum flow rate of about 4 MGD. The resulting WE is expected to have a salinity of about 47,500 ppm.

The species listed above underwent an initial screening stage to determine the single most sensitive species for dry season (high-brine blend) acute bioassays. The initial screening stage included three rounds of concurrent bioassays.

Average Blend Acute Bioassay Protocol

WE discharge to San Francisco Bay during the wet season (approximately December through April) will consist mainly of CMSA effluent. Therefore, the desalination facility would function at a reduced capacity of about 4 MGD while CMSA effluent discharge will be near an average flow rate of about 11 MGD. The resulting WE is expected to have a salinity of about 16,500 ppm.

The species listed above underwent an initial screening stage to determine the single most sensitive species for wet season (average blend) acute bioassays. The initial screening stage included three rounds of concurrent bioassays.

Chronic Toxicity Bioassay Requirements

The requirements for chronic toxicity screening are outlined in the enclosure to NPDES Permit Attachment C, Self-Monitoring Program (SMP), titled “Chronic Toxicity: Definition of Terms and Screening Phase Requirements”. Design of the chronic toxicity screening phase shall, at a minimum, consist of the following elements:

1. Use of test species specified in Table 1 of the SMP Enclosure to select an appropriate suite of organisms for the screening study, and use of the protocols referenced in that table, or as approved by the Executive Officer (EO).

2. Two stages:

   Stage 1 shall consist of one battery of tests conducted concurrently. Selection of the type of test species and minimum number of tests shall be based on Table 1 of the SMP Enclosure; and

   Stage 2 shall consist of a minimum of two test batteries conducted at a monthly frequency using the three most sensitive species based on Stage 1 results and approved by the EO.

3. Appropriate Controls; and

4. Concurrent reference toxicant tests.

Proposed Chronic Bioassay Protocols

Prior to proceeding with screening sampling and bioassays, MMWD submitted a screening phase proposal to the EO for approval. Table 2 lists the species that MEC/Weston suggested for Stage 1 screening, as well as the recommended analysis protocols.

Table 3: Recommendations for Stage 1 Chronic Bioassay Screening

Species	Effect	Test Duration	Test Protocol
Marine diatom (*Thallasiosira pseudonana*)	Growth rate	96 hours	2
Mysid shrimp (*Mysidopsis bahia)*	Survival and Growth	7 days	1
Inland silversides (*Menidia beryllina*)	Survival and Growth	7 days	1
Giant Kelp (*Macrocystis pyrifera*)	Germination and Growth	48 hours	3
Mussel embryos (*Mytilus edulis*)	Larval development	48 hours	3

Test Protocol References:

1. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, 4th Edition. USEPA-821\R-02\013. October 2002.

2. American Society for Testing Materials (ASTM). 2003. Standard Guide for conducting static 96-hour toxicity tests with microalgae. Procedure E 1218-90. ASTM Philadelphia, PA.

3. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. USEPA\600\R-95\136. August 1995.

Following Stage 1 screening, chronic biotoxicity analyses were performed on the three most sensitive species. Both Stage 1 and the subsequent Stage 2 analyses provided the data for compliance with Stage 2 requirements.

High-Brine Blend Chronic Bioassay Protocol

WE discharge to San Francisco Bay during the dry season (approximately May through November) will contain a substantial proportion of RO brine from the full-scale desalination plant, in relation to CMSA effluent. The estimated typical maximum ratio is based on the desalination facility functioning at a design capacity of 15 MGD while CMSA effluent discharge

would be at a minimum flow rate of about 4 MGD. The resulting WE is expected to have a salinity of about 47,500 ppm.

The species listed above underwent an initial (Stage 1) screening to determine the three most sensitive species for dry season (high-brine blend) chronic bioassays. Subsequently, a chronic toxicity bioassay (Stage 2) was performed on those three most sensitive species.

Average Blend Chronic Bioassay Protocol

WE discharge to San Francisco Bay during the wet season (approximately December through April) will consist mainly of CMSA effluent. During this time, the desalination facility may operate at a reduced capacity of 1 to 4 MGD while CMSA effluent discharge will be near an average flow rate of about 11 MGD. The resulting WE is expected to have a salinity of about 16,500 ppm.

The species listed above underwent an initial (Stage 1) screening to determine the three most sensitive species for wet season (average blend) acute bioassays. Subsequently, two chronic toxicity bioassay episodes (Stage 2) were performed on those three most sensitive species. Stage 1 and 2 together determine a single most sensitive species.

General Requirements

- Bioassay samples were collected on days coincident with CMSA effluent composite sampling:
  - Bioassay tests were performed on effluent samples after chlorination-dechlorination.
  - Total ammonia nitrogen was analyzed and un-ionized ammonia calculated whenever fish bioassay test results failed to meet the specified percent survival.

- Composite Sampling: 24-hour composites may be made up of discrete grabs collected over the course of a day and volumetrically or mathematically flow-weighted. Samples for inorganic pollutants may be combined prior to analysis. If only one grab sample was collected, it was collected during periods of maximum peak flows. Samples were taken on random days.

- MEC indicted that the analyses require about 20 liters of total sample for each of 3 samples used over the course of the each bioassay episode (20 liters every other day – 60 liters total). The volume of each WE component required to produce the two WE blends to be analyzed was, therefore, as listed in Table 4.

Table 4: Effluent volumes required for bioassay completion

Blend	RO brine (Liters/gallons)	CMSA effluent (Liters/gallons)
High Brine Blend	15.8 / 4.2	4.2 / 1.1
Average Bleld	5.3 / 1.4	14.7 / 3.9

Bioassay Schedule

Table 5 presents a sampling and analysis schedule for fulfilling bioassay requirements of the NPDES permit. The table identifies a screening phase, which entailed determining the most sensitive species for future bioassay testing. Once the most sensitive species were determined for each blend and each type of bioassay, acute and chronic bioassays were conducted on each blend.

Table 5: Bioassay Sampling and Analysis Schedule

	High-Brine blend	Average blend
Acute Bioassay	Screening	Screening
	Test most sensitive species	Test most sensitive species
Chronic Bioassay	Stage 1 Screening	Stage 1 Screening
	Stage 2 testing of three most sensitive species	Stage 2 testing of three most sensitive species

Chronic Bioassay Work Plan

Weston Solutions submitted a Proposed Study Plan for Chronic Toxicity Screening to the Regional Board in January 2006 for review and approval by the EO. Final approval of the plan was obtained on 16 February 2006. The approved plan included a first phase of bioassay screening of the five species listed above, followed by two batteries of tests for completion of a second phase of bioassay screening on the three most sensitive species.

Three blends were used for the bioassays: salinity-adjusted and unadjusted high-brine blends, and an adjusted average blend.

Once prepared, the high-brine blend was handled in two ways: 1) adjusted and 2) unadjusted in regards to salinity. In order to meet bioassay test protocol criteria for salinity ranges (typical maximum = 34 ppT), the adjusted 47.5 ppT blend salinity was reduced to 30 ppT, a level within the range recommended for each species tested. This was accomplished by diluting the blend with commercially available spring water, a practice common in bioassay testing. The

unadjusted 47.5 ppT blend salinity tests were performed without reducing the salinity to within bioassay test protocol criteria.

The minimum salinity requirement for marine bioassays is generally greater than 20 ppT. Therefore, the 16.5 ppT blend salinity was increased to a normal seawater level (30 ppT) using the commercially available synthetic seasalt, Crystal Sea™. This brand has demonstrated consistent performance when tested against natural seawater and is recommended for use by the USEPA when preparing simulated effluents.

In accordance with U.S. EPA and ASTM guidelines, each bioassay was performed by exposing test organisms to several dilutions of the three prepared blend samples. Both of the adjusted blend dilution series (47.5 ppT and 16.5 ppT blends adjusted to 30 ppT) included a salinity-adjusted blend treatment (referred to as 100% blend) and five dilutions using a modified 0.5 dilution factor (i.e. 5, 10, 25, 50, and 75 percent blend). The unadjusted high-brine blend dilution series (47.5 ppT) included five dilutions using a modified 0.5 dilution factor (i.e. 5, 10, 25, 50, and 75 percent blend). In total, six exposure treatments were prepared for the two adjusted blend samples and five exposure treatments were prepared for the unadjusted high-brine blend sample.

For additional details regarding chronic bioassay protocols, refer to the final Proposed Study Plan for Chronic Toxicity Screening at Marin Municipal Water District Desalination Pilot Plant, dated 8 February 2006.

Bioassay Results

Acute Bioassay Results

The final draft report of acute bioassay results was submitted to Kennedy/Jenks on 6 July 2006. The results of all acute bioassays performed by Weston Solutions are summarized On Table 6.

Table 6: Results of Acute Toxicity Bioassays on Whole Effluent Blends

Species	Test Episode	Sample	Mean Survival (%)	Reference Toxicant LC50 (Positive Control)	LC50 Control Limits
Mysid Shrimp (*Mysidopsis bahia*)	Episode 1	Lab Control	95	236.11 µg/L Cu	98.9 - 370.9
		Salinity Control	100		
		Average Blend	95		
		High Blend	100		
	Episode 2	Lab Control	100	175.0 µg/L Cu	100.6 - 366.2
		Salinity Control	100		
		Average Blend	95		
		High Blend	100		
	Episode 3	Lab Control	100	183.3 µg/L Cu	124.6- 336.2
		Salinity Control	100		
		Average Blend	95		
		High Blend	95		
Species	**Test Episode**	**Sample**	**Mean Survival (%)**	**Reference Toxicant LC50 (Positive Control)**	**LC50 Control Limits**
Topsmelt (*Atherinops affinis*)	Episode 1	Lab Control	90	133.3 µg/L Cu	69.2 - 220.4
		Salinity Control	75		
		Average Blend	65		
		High Blend	95		
	Episode 2	Lab Control	100	138.5 µg/L Cu	67.0 - 216.2
		Salinity Control	100		
		Average Blend	95		
		High Blend	100		
	Episode 3	Lab Control	95	146.2 µg/L Cu	64.9 - 214.5
		Salinity Control	100		
		Average Blend	85		
		High Blend	100		
Species	**Test Episode**	**Sample**	**Cell Density (10^5 cells/mL)**	**Reference Toxicant LC50 (Positive Control)**	**LC50 Control Limits**
Marine algae (*Thalassiosira pseudonana*)	Episode 1	Lab Control	1.47	5.77 mg/L Cr	1.09 - 8.61
		Salinity Control	1.02		
		Average Blend	2.64		
		High Blend	1.82		
	Episode 2	Lab Control	1.20	0.46 mg/L Cr	0.51 - 8.91
		Salinity Control	0.93		
		Average Blend	1.52		
		High Blend	2.40		
	Episode 3	Lab Control	4.59	0.75 mg/L Cr	0.06 - 9.10
		Salinity Control	6.40		
		Average Blend	8.42		
		High Blend	13.6		

Chronic Bioassay Results

Weston Solutions, Inc. submitted a Chronic Toxicity Draft Report on 8 June 2006. The results of all chronic bioassays performed are summarized on Table 7. The table also includes, for comparison, the results of chronic toxicity bioassays performed by CMSA for compliance with that facility's NPDES permit requirements. Note that, overall, the brine/effluent blends elicited less toxic effects than the CMSA effluent alone. This indicates that the anticipated effluent blends pose no additional threat to the health of San Francisco Bay than existing effluent discharges.

To aid in the interpretation of bioassay results, the following definitions are used:

- **NOEC**: "No Observable Effect Concentration" is the highest concentration of toxicant (CMSA effluent or Brine/Effluent blend) to which organisms are exposed in a full life-cycle or partial life-cycle (short-term) test, that causes no observable adverse effects on the test organisms (ie, the highest concentration of toxicant concentration of toxicant in which the values for the observed responses are not statistically significantly different from the controls).
- **EC25**: "Effect Concentration 25%" is the concentration of toxicant having an effect on 25% of the population, compared to the control.
- **IC25**: "Inhibition Concentration 25%" is a calculated percentage of toxicant at which the test organism exhibits a 25% reduction in a biological function such as reproduction or growth.
- **TUc**: Chronic toxicity unit. USEPA recommends using a Tuc of 1.0 as the limit to indicate that no toxics are present in toxic amounts.
- **NA**: Not tested / Not applicable.

cc: Todd Reynolds, K/J SFO
Jean Debroux, K/J SFO

Table 7: Results of Chronic Toxicity Bioassays on Whole Effluent Blends

Bioassay Phase and Test Dates	Bioassay Endpoint		Giant Kelp (*Macrocystis pyrifera*) Sporophyte Germination / Gametophyte Growth[c]				Mussel (*Mytilus edulis*) Larval Development[c]				Opossum Shrimp (*Mysidopsis bahia*) Survival / Growth[c]				Inland Silverside (*Menidia beryllina*) Survival / Growth[c]				Marine diatom (*Thallasiosira pseudonana*) Growth			Fathead Minnow (*Pimephales promelas*) Survival / Growth
			CMSA eff.	Avg. Blend	High Blend	Unadj. High B.	CMSA eff.	Avg. Blend	High Blend	Unadj. High B.	CMSA eff.	Avg. Blend	High Blend	Unadj. High B.	CMSA eff.	Avg. Blend	High Blend	Unadj. High B.	Avg. Blend	High Blend	Unadj. High B.	CMSA eff.
Phase I (20-28 Feb 2006)	NOEC	Germination/ Survival	75%	100%	100%	75%	--	100%	100%	100%	75%	100%	100%	100%	75%	100%	100%	10%	-	-	-	50%
		Growth/ Development	<12.5%	5	50	50	<12.5%	25%	100%	100%	50%	100%	100%	100%	75%	100%	100%	25%	100%	100%	100%	25%
	LC_{25} or EC_{25} or IC_{25}	Germination/ Survival	>100%	>100%	>100%	>100%	--	>100%	>100%	>100%	80.6%	>100%	>100%	96.8%	84.2%	>100%	>100%	17.5%	-	-	-	52.0%
		Growth/ Development	64.9%	73	>100	96.4	15.5%	56.7%	>100%	>100%	54.9%	>100%	>100%	89.2%	77.8%	>100%	>100%	23.2%	>100%	>100%	>100%	32.8%
	TUc (100 / NOEC)	Germination/ Survival	<1.0	1.0	1.0	1.3	--	1.0[a]	1.0[a]	1.0[a]	1.2	1.0[b]	1.0[b]	1.0[b]	1.2	1.0[a]	1.0[a]	5.7[a]	-	-	-	1.9
		Growth/ Development	1.5	1.4	2.0	1.04	6.4	1.8[a]	1.0	1.0	1.8	1.0	1.0	1.1[b]	1.3	1.0	1.0	4.3[a]	1.0	1.0	1.0	3.0
Phase II, Episode 1 (21-28 Mar 2006)	NOEC	Germination/ Survival	--	100%	100%	100%	--	100%	100%	100%	75%	--	--	--	--	100%	100%	25%	--	--	--	100%
		Growth/ Development	--	25%	100%	75%	12.5%	<5%	25%	<5%	75%	--	--	--	--	100%	100%	75%	--	--	--	75%
	LC_{25} or EC_{25} or IC_{25}	Germination/ Survival	--	>100%	>100%	>100%	--	>100%	>100%	>100%	97.7%	--	--	--	--	>100%	>100%	43.6%	--	--	--	>100%
		Growth/ Development	--	>100%	>100%	>100%	30.5%	20.9%	>100%	49.4%	79.6%	--	--	--	--	>100%	>100%	40.2%	--	--	--	>100%
	TUc (100 / NOEC)	Germination/ Survival	--	1.0	1.0	1.0	--	1.0	1.0	1.0	1.0	--	--	--	--	1.0	1.0	2.3[b]	--	--	--	<1.0
		Growth/ Development	--	4.0	1.0	1.3	3.3	4.8[a]	4.0	2.0[a]	1.2	--	--	--	--	1.0	1.0	2.5[b]	--	--	--	<1.0
Phase II, Episode 2 (5-14 April 2006)	NOEC	Germination/ Survival	--	100%	100%	75%	--	100%	100%	100%	100%	--	--	--	--	100%	100%	100%	--	--	--	75%
		Growth/ Development	--	25%	75%	50%	<12.5%	50%	100%	50%	75%	--	--	--	--	50%	100%	100%	--	--	--	50%
	LC_{25} or EC_{25} or IC_{25}	Germination/ Survival	--	>100%	>100%	>100%	--	>100%	>100%	>100%	>100%	--	--	--	--	>100%	>100%	>100%	--	--	--	56.6%
		Growth/ Development	--	83.0%	>100%	92.8%	28.4%	85.7%	>100%	>100%	80.4%	--	--	--	--	>100%	>100%	>100%	--	--	--	72.3%
	TUc (100 / NOEC)	Germination/ Survival	--	1.0	1.0	1.3	--	1.0	1.0	1.0	<1.0	--	--	--	--	1.0	1.0	1.0	--	--	--	1.8
		Growth/ Development	--	1.2[b]	1.3	1.1[b]	3.5	1.2[b]	1.0	2.0	1.2	--	--	--	--	2.0	1.0	1.0	--	--	--	1.4

(a) TU value calculated as 100 / IC25
(b) TU value calculated as 100 / EC25
(c) Species are listed as identified by Weston Solutions. When a species within the same genus was used in a CMSA effluent bioassay, results are shown under the species in the same genus, even though the specific species may be different.