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14 June 2006

Mr. Joel Fallor
Kennedy Jenks Consultants
622 Folsom Street
San Francisco, CA 94107

SUBJECT: Results of Marin Municipal Water District Desalinization Pilot Plant Acute Toxicity Screening Study

Dear Mr. Fallor:

Weston Solutions, Inc. (WESTON) is pleased to present the results of the Acute Toxicity Screening Study conducted with water samples prepared with brine collected from the Marin Municipal Water District (MMWD) Desalinization Pilot Plant (DPP) blended with final effluent from the Central Marin Sanitation Agency (CMSA). WESTON performed a series of bioassays to determine the acute toxicity potential of the DPP/CMSA blends, and if toxicity is elicited, which of three aquatic indicator organisms is the most sensitive. Two treatments identified as Average-brine Blend and High-brine Blend were tested for acute toxicity over three test episodes. The species selected for the study represent a wide trophic level range, incorporating a vertebrate, invertebrate, and plant. The sampling and testing dates of each episode are provided in Table 1 and a summary of test species used in the study are found in Table 2.

Table 1: Sampling and Test Dates

| Test Episode | Sampling Date | Sample Receipt | Test Dates |
|--------------|-----------------------|------------------|---------------------|
| Episode 1 | 28 - 29 November 2005 | 29 November 2005 | 30 Nov - 4 Dec 2005 |
| Episode 2 | 30 - 31 January 2006 | 31 January 2006 | 1 - 5 February 2006 |
| Episode 3 | 24 - 25 March 2006 | 25 March 2006 | 23 - 27 March 2006 |

Table 2: Summary of Test Organism Use

| Organism | Taxon | Organism Age* |
|-------------------|---------------------------------|----------------|
| Mysid shrimp | <i>Mysidopsis bahia</i> | 3 – 5 days old |
| Inland silverside | <i>Atherinops affinis</i> | 1- 13 days old |
| Marine diatom | <i>Thalassiosira pseudonana</i> | 6 – 7 days old |

*Age at test initiation



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METHODS

On 29 November 2005, 31 January 2006, and 25 March 2006, samples were received at WESTON's laboratory facility. An Reversis Osmosis (RO) brine grab sample from the DPP and a 24-hour effluent composite from Central Marin Sanitation Agency (CMSA) were collected by personnel from KJC and transported on ice to the WESTON laboratory under chain-of-custody by KJC personnel. Average-brine and High-brine Blend treatments were analyzed for acute toxicity using aquatic bioassays with the mysid shrimp (*Mysidopsis bahia*), the inland silverside (*Menidia beryllina*), and a marine diatom (*Thalassiosira pseudonana*). Bioassay methods for *M. bahia* and *A. affinis* were performed in accordance with *Methods for Measuring Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th Edition* (USEPA 2002). Testing methods with all three species was performed in accordance with the *Annual Book of ASTM Standards* (ASTM 2004a and ASTM 2004b).

Test Solution Preparation. Two test blends were created at the WESTON laboratory on the Day 0 of each testing episode: an Average-brine Blend and a High-brine Blend. Prior to each testing episode, WESTON personnel used the DPP RO brine sample and CMSA effluent composite sample to create the blends using the ratios shown in Table 3.

Table 3: Sampling and Test Dates

| Test Episode | RO Brine / CMSA Effluent Ratio | % Brine | Salinity (ppt) |
|---------------------|--------------------------------|---------|----------------|
| High-brine Blend | 15/4 | 79% | 47.5 |
| Average-brine Blend | 4/11 | 27% | 16.5 |

In order to achieve salinity levels consistent with biologically tolerant and EPA protocol recommended levels, the Average-brine Blend salinity for all tests was raised to 30 ± 2 ppt with CoralSea™ synthetic seasalts, and the High-brine Blend was diluted to 30 ± 2 ppt with spring water (Arrowhead™). Laboratory control water for all tests was 0.2-um filtered, UV-treated San Francisco Bay water with a salinity of 30 ± 2 ppt. A salinity control treatment used for statistical comparisons to Average-brine Blend treatment results was prepared by mixing de-ionized water with CoralSea™ artificial salts to a salinity of 30 ± 2 ppt. The laboratory control treatment results were used for statistical comparison to High-brine Blend treatment results.

***Mysidopsis bahia* Test.** A single 100% concentration of each blend treatment, a laboratory control, and a salinity control were tested with each episode. Seven-day old mysids were obtained from Aquatic Biosystems, Fort Collins, CO. Ten organisms were exposed to 500 mLs in each replicate. The test was conducted with two replicates for



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each treatment along with the laboratory and salinity controls at $20 \pm 1^\circ\text{C}$ under a 16 hour light : 8 hour dark photoperiod. Temperature, pH, dissolved oxygen, and salinity were measured daily. Mortality and behavior observations were recorded and dead organisms were removed on a daily basis. A water renewal using the blended samples created prior to the test was performed on each replicate at 48 hours. A reference toxicant test was conducted using copper (as copper chloride) as a positive control with concentrations of 50, 100, 200, 400, and 800 $\mu\text{g Cu/L}$. The test conditions and acceptability criteria for the *Mysidopsis bahia* bioassay are summarized in a table presented in Attachment A.

***Atherinops affinis* Test.** A single 100% concentration of each sample, a laboratory control, and a salinity control were tested for each episode. 11-day old *Atherinops affinis* were obtained from Aquatic Biosystems of Fort Collins, CO. Ten organisms were exposed to 250mLs in each replicate. The test was conducted using two replicates for each treatment along with the laboratory and salinity controls at $21 \pm 1^\circ\text{C}$ under a 16 hour light : 8 hour dark photoperiod. Temperature, pH, dissolved oxygen, and salinity were measured daily. Mortality and behavior observations were recorded and dead organisms were removed on a daily basis. A water renewal was performed on each replicate at 48 hours. A reference toxicant test was conducted using copper as copper chloride as a positive control with concentrations of 50, 100, 200, 400 and 800 $\mu\text{g Cu/L}$. The test conditions and acceptability criteria for the *Atherinops affinis* acute toxicity test are summarized in a table presented in Attachment A.

***Thalassiosira pseudonana* Test.** A single 100% concentration of each sample, a laboratory control, and a salinity control were tested for each episode. 7-day old *Thalassiosira pseudonana* were used for each test from WESTON's in-house culture. Four 250 mL flasks containing 100 mL of test solution were inoculated for each treatment along with the laboratory and salinity controls using a log-phase in-house culture to a density of approximately 20,000 cells/mL of phytoplankton. Test treatments were fed 25 mL *Thalassiosira* Alga-Gro™ seawater prior to initiation. An uninoculated fifth flask was used as a water quality surrogate. The test was run at $20 \pm 1^\circ\text{C}$ under continuous light. Test containers were randomized and shaken gently twice daily. Temperature, salinity and pH were recorded in each concentration upon initiation and once daily. At the conclusion of the 96-hour exposure period, duplicate turbidity readings were taken on each replicate using a Hach DR2000 spectrophotometer. A calibration curve was then constructed to establish the relationship between turbidity and cell density. A reference toxicant test was conducted using chromium as potassium chromate as a positive control with concentrations of 0.54, 1.07, 2.14, 4.29 and 8.58 mg Cr/L. The test conditions and acceptability criteria for the *Thalassiosira pseudonana* acute toxicity test are summarized in a table presented in Attachment A.



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RESULTS

Bioassay Results. Results of all bioassays are tabulated in Attachment B. In summary, no significant effects on survival were observed among bioassays conducted with shrimp, topsmelt or marine algae during any of the three episodes of testing. These results were consistent between both DPP/CMSA blend preparations. Consequently, no distinction in species sensitivity to the DPP/CMSA discharge was detected.

QA/QC Results. With the exception of a minor exceedance observed with the marine algae tested in episode 2, all concurrent reference toxicant test (positive controls) results indicate normal organism sensitivity for acute survival. The LC50 achieved with the marine algae reference toxicant test performed concurrently with the episode 2 bioassay fell below the normal range by 0.05 mg/L, which is considered negligible relative to the degree by which the test treatments outperformed the control treatments. In general, quality control parameters evaluated with the bioassay procedure were within acceptable ranges for all protocols. Water quality parameter measurements and minor exceedances are tabulated in Attachment A. All laboratory benchsheets, statistical summaries, and sample control forms are available upon request.

REFERENCES

ASTM 2004a. Standard Guide for Conducting Acute Toxicity Tests on Aqueous Ambient Samples with Fishes, Macroinvertebrates and Amphibians. Method E 1192-97. American Society for Testing and Materials. Philadelphia, PA.

ASTM 2004b. Standard Guide for Conducting Static 96-h Toxicity Tests with Microalgae. Method E 1218-97a. American Society for Testing and Materials. Philadelphia, PA.

USEPA 2002. Methods for measuring acute toxicity of effluents and receiving waters to freshwater and marine organisms, 5th edition. EPA 821/R-02/012. October 2002.

If you have any questions or comments following your review of this report summary, please do not hesitate to contact me at via phone or e-mail:

scott.bodensteiner@westonsolutions.com.

Sincerely,

Scott Bodensteiner
Program Manager

Attachments

- A: Bioassay Procedure and Organism Data
- B: Acute Toxicity Results Summary

TABLE A-1

Procedure and Organism Data for *Mysidopsis bahia* Acute Toxicity Bioassays

| PARAMETER | TEST EPISODES | | |
|--------------------------------------|---|------------------------------|------------------------------|
| | Episode 1 | Episode 2 | Episode 3 |
| SAMPLE PROPERTIES | | | |
| Dates Sampled | 28 -29 November 2005 | 30 - 31 January 2006 | 24 - 25 March 2006 |
| Dates Received | 29 November 2005 | 31 January 2006 | 25 March 2006 |
| Sample Names | RO Brine, CMSA 24-hr | RO Brine, CMSA 24-hr | RO Brine, CMSA 24-hr |
| Volume Received | 20L per sample | 20L per sample | 20L per sample |
| Storage Conditions | 4 Degrees °C - dark | 4 Degrees °C - dark | 4 Degrees °C - dark |
| TEST SPECIES INFORMATION | | | |
| Supplier | Aquatic BioSystems | Aquatic BioSystems | Aquatic BioSystems |
| Date Acquired | 29 November 2005 | 27 January 2006 | 21 March 2006 |
| Acclimation Time | 2 days | 5 days | 2 days |
| Organism Age (Day 0) | 3-days-old | 5-days-old | 4-days-old |
| TEST PROCEDURES | | | |
| Type/Duration | Acute; 96-hours | Acute; 96-hours | Acute; 96-hours |
| Test Dates | 30 Nov - 4 Dec 2005 | 1 - 5 February 2006 | 23 - 27 March 2006 |
| Control Water | Lab Control: 0.2-um, UV-treated filtered San Francisco Bay water at 30 ppt Salinity Control: deionized water mixed with CoralSea™ artificial salts to 30 ppt | | |
| Temp (recommended/actual) | 19.0-21.0°C / 19.0-20.3°C | 19.0-21.0°C / 20.5-21.0°C | 19.0-21.0°C / 19.0-21.0°C |
| Photoperiod | 16 hours light: 8 hours dark | 16 hours light: 8 hours dark | 16 hours light: 8 hours dark |
| Salinity (recommended/actual) | 28 - 32 ppt / 30 - 32 ppt | 28 - 32 ppt / 29 - 31 ppt | 28 - 32 ppt / 29 - 32 ppt |
| Test Chamber | 1L containers | 1L containers | 1L containers |
| Exposure Volume | 500 mL | 500 mL | 500 mL |
| Organisms/Replicate | 10 | 10 | 10 |
| Replicates/Treatment | 2 | 2 | 2 |
| Feeding | None | None | None |
| DEVIATIONS FROM PROTOCOL | None | None | None |

TABLE A-2

Procedure and Organism Data for *Atherinops affinis* Acute Toxicity Bioassays

| PARAMETER | TEST EPISODES | | |
|--------------------------------------|---|------------------------------|------------------------------|
| | Episode 1 | Episode 2 | Episode 3 |
| SAMPLE PROPERTIES | | | |
| Dates Sampled | 28 -29 November 2005 | 30 - 31 January 2006 | 24 - 25 March 2006 |
| Dates Received | 29 November 2005 | 31 January 2006 | 25 March 2006 |
| Sample Names | RO Brine, CMSA 24-hr | RO Brine, CMSA 24-hr | RO Brine, CMSA 24-hr |
| Volume Received | 20L per sample | 20L per sample | 20L per sample |
| Storage Conditions | 4 Degrees °C - dark | 4 Degrees °C - dark | 4 Degrees °C - dark |
| TEST SPECIES INFORMATION | | | |
| Supplier | Aquatic BioSystems | Aquatic BioSystems | Aquatic BioSystems |
| Date Acquired | 29 November 2005 | 31 January 2006 | 21 March 2006 |
| Acclimation Time | 2 days | 1 day | 2 days |
| Organism Age (Day 0) | 13-days-old | 10-days-old | 12-days-old |
| TEST PROCEDURES | | | |
| Type/Duration | Acute; 96-hours | Acute; 96-hours | Acute; 96-hours |
| Test Dates | 30 Nov - 4 Dec 2005 | 1 - 5 February 2006 | 23 - 27 March 2006 |
| Control Water | Lab Control: 0.2-um, UV-treated filtered San Francisco Bay water at 30 ppt Salinity Control: deionized water mixed with CoralSea™ artificial salts to 30 ppt | | |
| Temp (recommended/actual) | 20.0-22.0°C / 20.7-21.0°C | 20.0-22.0°C / 20.0-22.0°C | 20.0-22.0°C / 20.0-20.1°C |
| Photoperiod | 16 hours light: 8 hours dark | 16 hours light: 8 hours dark | 16 hours light: 8 hours dark |
| Salinity (recommended/actual) | 28 - 32 ppt / 30-31 ppt | 28 - 32 ppt / 29 - 31 ppt | 28 - 32 ppt / 29 - 32 ppt |
| Test Chamber | 500 mL containers | 500 mL containers | 500 mL containers |
| Exposure Volume | 250 mL | 250 mL | 250 mL |
| Organisms/Replicate | 10 | 10 | 10 |
| Replicates/Treatment | 2 | 2 | 2 |
| Feeding | None | None | None |
| DEVIATIONS FROM PROTOCOL | None | No significant deviations | None |

TABLE A-3

Procedure and Organism Data for *Thalassiosira pseudonana* Acute Toxicity Bioassays

| PARAMETER | TEST EPISODES | | |
|--------------------------------------|---|--|--|
| | Episode 1 | Episode 2 | Episode 3 |
| SAMPLE PROPERTIES | | | |
| Dates Sampled | 28 -29 November 2005 | 30 - 31 January 2006 | 24 - 25 March 2006 |
| Dates Received | 29 November 2005 | 31 January 2006 | 25 March 2006 |
| Sample Names | RO Brine, CMSA 24-hr | RO Brine, CMSA 24-hr | RO Brine, CMSA 24-hr |
| Volume Received | 20L per sample | 20L per sample | 20L per sample |
| Storage Conditions | 4 Degrees °C - dark | 4 Degrees °C - dark | 4 Degrees °C - dark |
| TEST SPECIES INFORMATION | | | |
| Supplier | In-house culture | In-house culture | In-house culture |
| Organism Age (Day 0) | 7-days old | 6-days-old | 7-days-old |
| TEST PROCEDURES | | | |
| Type/Duration | Acute; 96-hours | Acute; 96-hours | Acute; 96-hours |
| Test Dates | 30 Nov - 4 Dec 2005 | 1 - 5 February 2006 | 23 - 27 March 2006 |
| Control Water | Lab Control: 0.2-um, UV-treated filtered San Francisco Bay water at 30 ppt Salinity Control: deionized water mixed with CoralSea™ artificial salts to 30 ppt | | |
| Temp (recommended/actual) | 19.0-21.0°C / 19.0-21.0°C | 19.0-21.0°C / 19.2-21.0°C | 19.0-21.0°C / 20.3-21.0°C |
| Photoperiod | Continuous light | Continuous light | Continuous light |
| Salinity (recommended/actual) | 28 - 32 ppt / 30 - 34 ppt | 28 - 32 ppt / 29 - 39 ppt | 28 - 32 ppt / 29 - 31 ppt |
| Test Chamber | 250 mL flasks | 1 L containers | 1 L containers |
| Exposure Volume | 100 mL | 500 mL | 500 mL |
| Organisms/Replicate | 10,000 cells/mL | 10,000 cells/mL | 10,000 cells/mL |
| Replicates/Treatment | 4 | 4 | 4 |
| Feeding | 25 ml/L Thalassiosira Alga-Gro ^M seawater | 25 ml/L Thalassiosira Alga-Gro ^M seawater | 25 ml/L Thalassiosira Alga-Gro ^M seawater |
| DEVIATIONS FROM PROTOCOL | No significant deviations | Salinity levels exceeded by 1-3 ppt on Days 2-3 of test. | None |

Table B-1: Acute Toxicity Results Summary

| Species | Test Episode | Sample | Mean Survival* (%) | Reference Toxicant LC50 (Positive Control) | LC50 Control Limits |
|---|--------------|------------------|---|--|---------------------|
| <i>Mysidopsis bahia</i> (Mysid shrimp) | 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 |
| <i>Atherinops affinis</i> (Topsmelt) | 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 |
| <i>Thalassiosira pseudonana</i> (Marine algae) | 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 | | |

* Average Blend treatment results statistically compared to Lab Control results ($\alpha = 0.05$). High Blend treatment results statistically compared to Salinity Control results ($\alpha = 0.05$).