High-Salinity Sensitivity Study:
Short-and Long-Term Exposure Assessments

Prepared For:

West Basin Municipal District
17140 South Avalon Blvd, Ste. 210
Carson, CA 90746-1296

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EXECUTIVE SUMMARY

Overview

The West Basin Municipal Water District (WBMWD) High Salinity Sensitivity Study (HSS Study) comprehensively evaluated the potential short-term and long-term exposure effects of high salinity discharges from the WBMWD ocean water desalination demonstration facility (OWDDF) on aquatic organisms representative of communities indigenous to various near shore environments in Southern California. Study parameters are summarized in Table ES-1. Short-term effects were evaluated using Whole Effluent Toxicity (WET) bioassays developed by the U.S. Environmental Protection Agency (USEPA) to quantify the magnitude and threshold of potential biological effects of discharges (e.g. treated wastewater). Both acute toxicity (mortality effects) and chronic toxicity (mortality + sublethal effects) bioassays were performed by a state accredited bioassay laboratory. Long-term effects were evaluated using mesocosm procedures performed at the OWDDF by exposing multiple organisms for eight weeks to ambient seawater and diluted brine flows from the OWDDF in large aquaria constructed to simulate the OWDDF discharge environment.

Short-Term WET Testing

The short-term WET component of the HSS study (Volume I) consisted of a two-phase screening process involving initial acute and chronic toxicity range-finding bioassays followed by definitive bioassays (i.e. a narrower salinity dilution series). The objective of the WET component of the study was to determine which species and early life-stages among those available under USEPA protocols were the most sensitive to hypersaline conditions, and what salinity levels will elicit adverse effects to those organisms. One objective of the WET testing is to provide toxicity data for the most sensitive species expected to reside in the OWDDF discharge environment. Modeling this data in conjunction with other factors such as water depth and ocean mixing conditions will determine the appropriate salinity thresholds for acute and chronic exposures to OWDDF brine discharges.

WET Phase I (i.e. range-finding) chronic toxicity testing using OWDDF brine discharge samples involved a single test episode and was conducted with the following USEPA authorized test organisms: giant kelp spores (*Macrocystis pyrifera*), purple sea urchin embryos (*Strongylocentrotus purpuratus*), red abalone embryos (*Haliotis rufescens*), larval mysid shrimp (*Americamysis bahia*), and larval topsmelt (*Atherinops affinis*). Each bioassay method evaluated sensitive life stages (e.g. growth or embryo development) over a period of 48-hours to seven days. Since the suite of available USEPA *acute* toxicity test methods (i.e. methods that measure mortality only) is significantly more limited (i.e. only available for fish and shrimp species), Phase I acute toxicity testing was conducted with just three species: larval mysid shrimp, larval topsmelt and juvenile sand dabs (*Citharichthys stigmaeus*). The objective of Phase I testing was to determine relative species sensitivities in order to identify a narrower brine dilution range to be used in the Phase II definitive bioassays.

WET Phase II chronic toxicity testing involved two consecutive test episodes using the three species from each trophic level (plant, invertebrate, and vertebrate) most likely to reside within the soft bottom OWDDF discharge environment: giant kelp (kelp spores), mysid shrimp (larvae) and topsmelt (larvae). A purple urchin bioassay was included in Phase II as urchins were more sensitive in Phase I than abalone, the other hard-bottom habitat species, and because a substantial amount of urchin data has been generated with other high

Table ES-1. Study Summary

<table>
<tr><th colspan="3">Short-Term WET Testing</th></tr>
<tr><th>Overview</th><th>Chronic Toxicity
Effects on mortality and sub-lethal metrics (e.g. embryo development, growth)</th><th>Acute Toxicity
Evaluation of mortality after acute exposure (typically 96-hours)</th></tr>
<tr><td rowspan="4">• Lab-based biological effects testing (bioassays)
• EPA approved species for monitoring effluents
• Focus on early life-stages (i.e. embryo-larval)
• Mix of species native to both soft-bottom and hard-bottom habitats
• Organisms exposed to multiple brine dilutions
• One range-finder testing episode and two definitive episodes
• Most sensitive species used for definitive episodes
• Statistical analyses performed to identify ‘no observed effect levels’</td><th colspan="2">Range-Finder</th></tr>
<tr><td>• Bioassays
o 7-day mysid shrimp survival & growth
o 96-hr kelp germination & germ-tube growth
o 72-hr purple urchin embryo development
o 48-hr red abalone embryo development
o 7-day topsmelt larval survival & growth
• Dilutions: 33, 42, 51, 60 and 70 ppt</td><td>• Bioassays
o 96-hour mysid shrimp survival
o 96-hour sand dab survival
o 96-hour topsmelt survival
• Dilutions: 33, 42, 51, 60 and 70 ppt</td></tr>
<tr><th colspan="2">Definitive Testing</th></tr>
<tr><td>• Bioassays
o 7-day mysid shrimp survival & growth
o 72-hr purple urchin embryo development
o 7-day topsmelt larval survival & growth
• Dilutions
o Purple urchin: 35, 37, 39, 41, 43 ppt
o Episode 1 Fish: 36.5, 39, 41, 45, 50 ppt
o Episode 1 Fish: 36.5, 39, 41, 45, 60 ppt</td><td>• Bioassays
o 96-hour mysid shrimp survival
o 96-hour topsmelt survival
• Dilutions
o Episode 1: 36.5, 39, 41, 45 & 50 ppt
o Episode 2: 36.5, 39, 41, 45 & 60 ppt</td></tr>
</table>

<table>
<tr><th colspan="4">Long-Term Mesocosm Testing</th></tr>
<tr><th>Overview</th><th>Species</th><th>Exposure Levels</th><th>Parameters Evaluated</th></tr>
<tr><td>• Testing performed on-site
• Expanded variety of Southern California species
• Juvenile & adult life stages
• 1 ambient and 1 elevated salinity test chambers
• Organisms exposed to both salinities under flow through conditions
• Three 8-week trials
• Each trial comprised of 3 successively higher salinity exposure periods in ‘elevated salinity’ chamber
• Mortality, behavior and post-exposure, sub-lethal parameters evaluated</td><td>• Sand dabs
• White sea bass
• Rockfish (multiple species)
• Shiner perch
• 3-spined sticklebacks
• Tube snouts
• Olive snails
• Purple urchins
• Red abalone
• Blue mussels
• Bat stars
• Sand crabs
• Slender crabs
• Kelp crabs</td><td>• Trial 1
o Ambient
o Low salinity: 37 ppt
o Mid salinity: 42.5 ppt
o High salinity: 47 ppt
• Trial 2
o Ambient
o Low salinity: 37 ppt
o Mid salinity: 42.5 ppt
o High salinity: 44.5 ppt
• Trial 3
o Ambient
o Low salinity: 37 ppt
o Mid salinity: 41 ppt
o High salinity: 44.5 ppt</td><td>• Mortality
• Behavior
• Juvenile fish growth after high salinity exposures
o Sand dabs and/or
o White sea bass
• Shellfish embryo development after mid and high salinity exposures
o Blue mussels or
o Purple urchins
• Purple urchin fertilization after mid salinity exposure (Trial 3 only)</td></tr>
</table>

ppt: Parts salt per thousand parts water salinity studies. The Phase II acute toxicity was performed with the two most sensitive species from Phase I: larval mysid shrimp and larval topsmelt.

Results of Phase II chronic toxicity testing performed under the short-term WET component of the HSS Study showed that the most sensitive organism among the three test species most representative of the organisms indigenous to the OWDDF discharge environment was the mysid shrimp. The highest salinity level that resulted in no statistically significant effects to this species was 41 parts salt per thousand parts water (ppt). As expected, purple urchins, the more sensitive hard-bottom habitat species, were somewhat more susceptible to the chronic toxicity effects of high salinity than the mysid shrimp. The average Phase II no effects concentration calculated for the purple urchin was 36 ppt. The chronic toxicity results for the most sensitive hard-bottom and soft-bottom species are presented in Figure ES-1.

Results of the Phase II *acute* toxicity testing showed that the most sensitive organism tested was also the mysid shrimp. The highest salinity level that resulted in no statistically significant *acute* toxicity (i.e. mortality after acute exposure) to this species was 45 ppt.

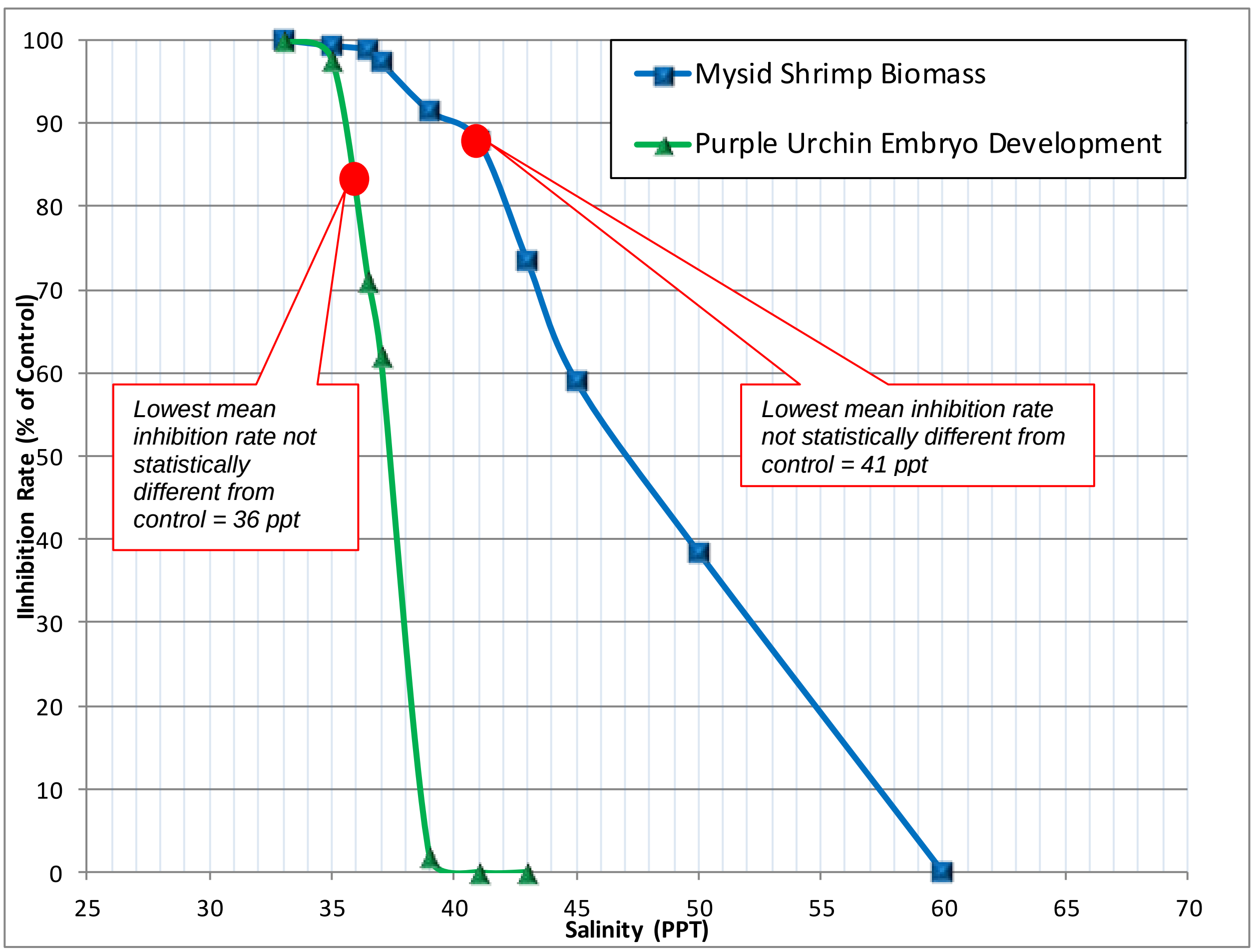

Figure ES-1. Chronic Toxicity Effects of High Salinity on Mysids and Urchins (Phase II Averages)

Long-Term Mesocosm Testing

The objectives for the long-term mesocosm component of the HSS Study were to corroborate the results of the WET component of the study and further inform the overall assessment of potential impacts of the OWDDF brine discharge. The mesocosm component of the study was performed at the OWDDF in Redondo Beach, CA. A mesocosm (i.e. mid-scale habitat simulation) was created with a split-chamber flow-through aquarium of sufficient capacity (300 gallons) to house an assembly of juvenile and adult aquatic organisms representative of the biological community of southern California. Several invertebrate and vertebrate species were acquired from organism providers permitted by the California Department of Fish and Game. Equal numbers of each organism were placed on both sides of the aquarium, and exposed to filtered ambient seawater on one side and high salinity flows on the other. Three 8-week exposure trials were performed. After every two weeks, the salinity level in the high-salinity chamber was reduced to ambient for up to one week and then raised to a higher salinity level. This approach resulted in three different salinity level exposure periods within each trial: low salinity (37 ppt), mid-level salinity (41 or 42.5 ppt), and high salinity (44.5 or 47.5 ppt).

Organisms used in the mesocosm component of the HSS Study in both the ambient and high salinity test chambers were monitored daily throughout each exposure trial for mortalities and variations in behavior. In addition, sub-lethal impacts to select biological endpoints were also assessed at the end of the mid and high salinity exposure periods for each trial. The first post-exposure endpoint measured was mussel (Trial 1) or urchin (Trials 2 and 3) embryo development. Half of the adult invertebrate mussels or urchins were removed after the mid salinity exposure period and used to perform the U.S. EPA chronic toxicity bioassay that assesses embryo fertilization and/or development (fertilization was only measured after the mid-salinity exposure of trial 3). Gametes and/or embryos harvested from adults exposed to both ambient and elevated salinities were placed in test vials containing either ambient seawater or water with an elevated salinity matching that in which the adult organisms had been exposed, and then assessed for fertilization rate and/or embryo development success. This procedure was then repeated after the high salinity exposure period for all three trials. The other sublethal endpoints assessed were weight and length achieved by one or two species of juvenile fish (white sea bass and speckled sanddabs).

Results of the long-term exposure trials (Table ES-2) show that *none* of the vertebrate and invertebrate organisms exposed to the low and mid salinity levels in the elevated salinity chamber exhibited any behavior patterns or mortality rates different from the ambient seawater organisms. The urchins and abalone began showing signs of stress when exposed to the trial 1 high salinity level of 47.5. Most of these urchins and abalone ultimately perished. However, no other species showed any sign of stress throughout the entire 2-week high-salinity exposure period. With the high-salinity level lowered by three ppt in trials 2 and 3, the abalone were not visibly affected, and only three of the 15 urchins suffered mortality. None of the urchins were affected in trial 3.

Table ES-2. Long-Term Mesocosm Results Summary

Salinity Exposure Level	Trial (Salinity)	Post-Exposure Parameters: Urchin Fertilization	Post-Exposure Parameters: Urchin/Mussel Embryo Development	Post-Exposure Parameters: Fish Growth	Mortality
Low	Trial 3 (37 ppt)	Not Measured	Not Measured	Not Measured	No significant mortality among 10 different species
	Trial 2 (37 ppt)	Not Measured	Not Measured	Not Measured	No significant mortality among 12 different species
	Trial 1 (37 ppt)	Not Measured	Not Measured	Not Measured	No significant mortality among 9 different species
Mid	Trial 3 (41 ppt)	No significant inhibition for urchin gametes exposed to 41 ppt solution or ambient solutions	Significant inhibition in **urchin** embryos exposed to 41 ppt solution ***but not*** ambient solution	Not Measured	No significant mortality among 10 different species
	Trial 2 (42.5 ppt)	Not Measured	Significant inhibition in **urchin** embryos exposed to ambient and 42.5 ppt solutions	Not Measured	No significant mortality among 12 different species
	Trial 1 (42.5 ppt)	Not Measured	Not Measured	Not Measured	No significant mortality among 9 different species
High	Trial 3 (44.5 ppt)	Not Measured	Significant inhibition in **urchin** embryos exposed to ambient and 44.5 ppt solutions	No significant effect on length or weight measured for 1 fish species: white sea bass	No significant mortality among 10 different species
	Trial 2 (44.5 ppt)	Not Measured	Significant inhibition in **urchin** embryos exposed to ambient and 44.5 ppt solutions	No significant effect on length or weight measured for 2 fish species: sand dabs & white sea bass	Slightly significant mortality among 1 out of 12 different species: 88.5% urchin survival
	Trial 1 (47.5 ppt)	Not Measured	Significant inhibition in **mussel** embryos exposed to ambient and 47.5 ppt solutions	No significant effect on length or weight measured for 1 fish species: sand dabs	Significant mortality among 2 out of 9 different species: 16.7% urchin survival 57.2% abalone survival

No significant effects

Slightly significant effects

Significant effects

Results of the mesocosm sub-lethal endpoint evaluations show that there were no significant differences in weight gain or length between fish exposed to high salinity flows and those exposed to ambient seawater throughout all three exposure periods for all three trials. The post-exposure invertebrate bioassays showed that exposure of adult shellfish to mid or high salinity levels did not result in an increased tolerance of their embryos in elevated salinities. However, embryos from adult urchins exposed at 41 ppt did develop normally in ambient seawater. Additionally, the urchin fertilization bioassay performed after the trial 3 mid-salinity exposure period showed that adults first exposed to 41 ppt resulted in normal fertilization rates for urchin gametes exposed to both ambient *and* 41 ppt salinities.

Summary

The results of the HSS Study are summarized in Table 3-1. In general, the mesocosm component of the study demonstrated that most organisms that have matured past larval stages of development, especially those indigenous to the DDF discharge environment, are tolerant of long-term exposure to salinities at least as high as 47.5 ppt. The only exceptions were purple urchins and red abalone.that showed tolerance of long-term exposures as high as 42.5 ppt. The long-term mesocosm tolerances were greater than those observed with the short-term WET component of the study. The ‘no effects levels’ established by the WET chronic toxicity bioassays were 41 ppt for the most sensitive soft-bottom organism (mysid shrimp), and 36 ppt for the most sensitive hard-bottom organism (purple urchin).

Long-term exposure of adult purple urchins and mussels above 41 ppt did not result in improved embryo-development sensitivity in elevated *or* ambient salinities. However, embryos developed normally in *ambient* seawater after the adults were exposed at the 41 ppt salinity level. Additionally, the single post-exposure gamete fertilization bioassay performed, showed that purple urchin fertilization rates were not affected for gametes (i.e. pre-embryo eggs and sperm) from adults exposed to ambient or mid-salinity (41 ppt) waters when the gametes were subsequently exposed to ambient *or* 41 ppt salinities.

Table ES-3. High Salinity Sensitivity Study Summary

<table>
<tr><th rowspan="2">Study Component</th><th colspan="2">Observed Salinity Thresholds</th><th rowspan="2">Other Significant Findings</th></tr>
<tr><th>Soft-Bottom Organisms</th><th>Hard-Bottom Organisms</th></tr>
<tr><td>Short-Term WET (chronic toxicity)</td><td>41 ppt</td><td>36 ppt</td><td rowspan="2">• No significant effect on urchin embryo development rates in ambient seawater when adults are first exposed to 41 ppt salinities.
• Fertilization rate not effected at 41 ppt regardless of whether adults are previously exposed to this salinity.
• Acute toxicity threshold observed with the WET study component = 45 ppt, which was observed with the mysid shrimp.</td></tr>
<tr><td>Long-Term Mesocosm</td><td>47.5 ppt</td><td>42.5 ppt*</td></tr>
</table>

* Mortality effect