Review of the Report on the West Basin Municipal District by Weston Solutions

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The first part of this document reports on results of some standard toxicity testing of elevated salinity from the West Basin Desalination Demonstration Facility on a number of local California organisms. It is good to use local species to ascertain effects of stressors in California. The short-term WET component involved initial acute and chronic toxicity range-finding bioassays followed by definitive bioassays – focusing on mortality as the endpoint. The objective was to determine which species and early life-stages were the most sensitive, and what salinity will elicit adverse effects. WET Phase I chronic tests involved 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 test evaluated sensitive life stages over four to seven days. Acute tests were done with larval mysids and topsmelt and juvenile sand dabs (*Citharichthys stigmaeus*) to determine relative sensitivities.

WET Phase II chronic tests involved two consecutive tests using species from each trophic level considered likely to reside in the soft bottom environment: kelp spores, mysid larvae and topsmelt larvae. A purple urchin bioassay was also included. Phase II acute toxicity was performed with larval mysids and larval topsmelt. Results showed that the most sensitive organism was the mysid. The highest salinity level that resulted in no statistically significant mortality to this species was 45 ppt. The work appears to have been done well, with proper controls, protocols and statistical analysis. The various tests performed were all short-term, with exposures up to one week. Abalone and sea urchin embryos/larvae were sensitive, being affected at salinities >33 and 36 ppt, respectively. Since effects were seen at a few units above ambient, this raises concerns that longer exposures would result in more severe effects.

However, short term tests are of limited usefulness is assessing impacts of elevated salinity that will persist in the environment as long as a desalination plant is operating. Long-term tests on sublethal effects such as growth, development, and reproduction are far more relevant and important. There is an extensive literature (references will be provided if requested) showing that exposures to various substances during embryonic stages can produce effects that are not exhibited for a long time, in some cases not until the animal has become mature, at which time reproduction or behavior is impaired. This is one reason for concerns about relying on short-term tests - using mortality or embryo/larval development and terminating shortly thereafter - to conclude that an environmental stressor is not a problem. Long term studies are far more preferable, therefore I will devote more of this review to this aspect of the report.

In the second, long term phase of the project, groups of varying numbers of different species were placed in 150 gallon tanks with ambient and elevated (>40) salinity for three successive 2-k wk periods. There is no explanation as to why such high salinities were chosen rather than 2-4 psu above ambient. Standard protocols were generally not used. This is not necessarily a problem, however, as long as the study is performed well with proper controls and statistics. However, that was not the case. Having one tank for control and one tank for treatment leads to an N of 1, even if many individuals are in that tank – this is called "pseudoreplication." To avoid pseudoreplication, a separate container is needed for each replicate.

Repeated trials were done with varying salinity and apparently the same individual organisms were re-used – which is not advisable since conditions may change and older organisms and/or those that have previously been exposed to elevated salinity are likely to respond differently. The number of individuals placed in each test chamber differed among test species; "the number of individuals was dependent upon the size and type of species selected." In many cases, the number of animals of a particular species added was less than 10. This makes any statistical analysis of questionable validity – but such analysis was not presented in any case, and if it had been, it would not have been valid because of pseudoreplication. The data (only mortality data provided) on all the species other then the mussels and sea urchins (for fertilization and embryo tests) and the fishes (growth studies) are therefore not of any use.

The report claims that health and behavior were monitored, but it does not include any protocols. Merely looking at an organism and seeing it move in a tank doesn't make it healthy or behaving normally. Just like one would not evaluate growth by eyeballing the sizes of the animals, but rather by actually measuring them, behavior should also be measured and quantified to evaluate whether or not it has been altered. Behavioral toxicology is a well-developed field of research. Gross problems such as those described and illustrated for sea urchins in high salinity - shedding spines, etc., are interesting, but it is likely that more subtle effects occurred at lower salinities that were not measured. Stating: "The urchins, abalone and bat stars on the ambient side of the aquarium during the high salinity exposure periods were able to better adhere to the aquarium walls than the same animals exposed to the high salinity," and "The crabs and other invertebrates appeared and behaved normally in both the ambient and high salinity chambers throughout all three exposure periods of the three trials" may be interesting, but is not data. Measurements are needed for any valid scientific conclusions on the strength of adherence to aquarium walls or on aspects of the animals' behavior.

Scientific endpoints examined were fertilization and embryo development for mussels and sea urchins, and growth of juvenile sand dabs and sea bass. The fertilization and development tests appear to have been done with standard protocols and showed deleterious effects of elevated salinity. The experimental design – using both animals that had been living in, and perhaps acclimated to high salinity and those that hadn't acclimated as the source of gametes - to see the sensitivity of the gametes/embryos is a good design. This enables the evaluation of potential effects on gametogenesis in the adults as well as early life stages of the offspring. However, while in the test "Unfertilized eggs were separated from debris by filtering the suspension through an 80 µm screen" there was no enumeration or analysis to see if there were differences in gametogenesis or fertilization success in the differentially exposed groups to investigate effects of the adults' exposure to elevated salinity. This was a lost opportunity.

The tables refer to these tests as "Long Term High Salinity Fertilization Tests" yet they are not long term – the adults may have been in elevated sea water for a longer time, but the sensitive early life stages were exposed for the normal short period of time. It is not clear if results were analyzed statistically, but it appears that salinities over 40 totally (or severely) inhibit sea urchin and mussel embryo development, regardless of whether the adults had been pre-exposed to the high salinity or not. It is unfortunate these studies were not done with less of a salinity increase. It also appears in some cases that adults that had been living in high salinity produced embryos that couldn't develop normally in either ambient or normal salinity (Tables 2-8 and 2-9, 2-10, 2-12). It also appears that sea urchin fertilization alone is not a sensitive parameter (Table 2-13). As brief exposures during fertilization alone are much less sensitive than longer exposures during embryonic development, so too, short-term exposures during embryonic development are less sensitive then longer exposures that would follow the organisms through larval life and metamorphosis, and ideally maturation, and reproduction in a full life cycle.

It is clear that the early life stages of these invertebrates are affected by the high salinity, but this effect was demonstrated previously with sea urchins embryos at lower, more realistic salinities (~36-37) in another report dealing with effects of brine discharge from "Granite Canyon."

Growth is an excellent parameter of sublethal effects of stressors, and there are data provided on growth of sand dabs and sea bass in the mesocosms. However, there is no information about the food provided – type and/or quantity – which is certainly important in any growth study. For a proper growth study, food intake of experimental animals is critical. It should be quantified and controlled. One approach is to feed a constant amount of food per fish per day. Another way is to feed the group to satiation each day. This latter approach can give information about potential loss of appetite in stressed fish if they require less food over time to reach satiation. In either case, it is critical to control and know about food intake. There is no mention of feeding in the report, so any growth information is not useful. Even if a constant food quantity was being provided, the fish were sharing the tank with many other species, so there is no way to know how much food they consumed. In one trial with the fish, the sea bass ate all the sand dabs so growth data were not available for them – this also suggests that perhaps the fish were not being fed enough. The authors of the report say the growth differences in ambient vs elevated salinity are not significant, but do not specify if the variance numbers in the table are standard deviations or standard errors. The high salinity fish seem to be always a bit smaller than the controls. If the variances are standard deviations, it is possible that with a higher "n" the differences might have been statistically significant. If the variances are standard errors, the means are indeed not different. But in the absence of standard feeding protocols and knowledge of food intake, any growth data, differences or lack thereof, is meaningless.

In conclusion, while the short-term tests reflect standard protocols and results, short-term testing, lethal or sublethal, is of dubious relevance to potential environmental effects of a functioning desalination plant. Long term studies are much more important, but some of the results and conclusions in this part of the report have major scientific flaws. This report does not provide scientific evidence that would cause me to wish to modify the recommendations (e.g., that salinity should not be raised > 5 ppt above ambient) of our earlier report on desalination.