

Protocols for determining
Major-Seawater-Ion Toxicity
in
Membrane-Technology
Water-Treatment Concentrate



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Glossary of terms

Concentrate — facility concentrate ('reject water'). Water containing concentrated amounts of dissolved constituents and resulting from the membrane water-purification process. Concentrate is generally discharged as a waste. For the purposes of this study, the concentrate is defined as that which is discharged following any post-treatment and is collected at the NPDES compliance point.

Ion-adjusted concentrate — concentrate adjusted with ACS-grade salts so that the proportions of major seawater ions (Table 1) match that of seawater as closely as possible without individual ions exceeding full seawater (35 ppt) concentrations. With some concentrates, the ion-adjusted concentrate may consist of concentrate with the major ions adjusted to match seawater proportions and then diluted to full-seawater concentrations. In other tests with these concentrates, some of the major seawater ions may remain in excess of the full seawater concentrations.

Major seawater ions—those ions making up greater than 1% of the total molar weight of the ionic constituents of standard seawater (*mer de normal*). These ions are listed in Table 1. Bromide is included in the list even though it constitutes less than 1% because its presence is required for survival of the mysid shrimp used as test organisms.

Major-seawater-ion toxicity — toxicity resulting solely as a result of a surplus or deficit of one or more of the major seawater ions listed in Table 1, or from the relative proportions of those ions.

Mock concentrate — synthesized concentrate created using ACS-grade salts but containing only the major seawater ions (Table 1) in the same concentrations as the facility concentrate.

Mock ion-adjusted concentrate — synthesized concentrate created using ACS-grade salts but containing only the major seawater ions (Table 1) in the same concentrations as the ion-adjusted concentrate. Created by adding to mock concentrate the same salts used to create the ion-adjusted concentrate.

Mock sea-salt slurry — salt slurry (i.e., enough deionized water added to permit good mixing of component salts) created using ACS-grade salts such that, once fully dissolved, the slurry will provide the major seawater ions (Table 1) in the same proportions as seawater.

Mock seawater — synthesized seawater created using ACS-grade salts and deionized water but containing only the major seawater ions (Table 1) in the same concentrations as seawater.

Natural seawater (NSW) — high-quality, filtered seawater from an uncontaminated source.

Raw water — the untreated water that feeds the membrane water-purification process.

Introduction

Drinking water facilities that incorporate membrane technology in the production of drinking water, including reverse-osmosis (RO) and membrane-softening facilities, are found throughout the State of Florida. The concentrate ("reject water") from these facilities is typically characterized by different proportions of the common seawater ions (when compared to natural seawater), and by elevated concentrations of certain elements such as fluoride, strontium, and radium^{226 + 228}. The amounts and types of ions present are dependent upon the source water, the type of membrane system, and the pre- and post-treatment regimes.

When subjected to the aquatic toxicity testing required of most point-sources discharging wastewater to the surface waters of the state of Florida and falling under the NPDES Program (National Pollutant Discharge Elimination System), many of the RO concentrates failed. The amount of toxicity required to constitute a failure depends partly on the characteristics of the receiving water, but in most cases if half or more of the test organisms exposed to the concentrate for 96 hours died, the discharger would be out of compliance with state water quality criteria. More details on the requirements are contained in 62-302.500(1)(d), 62-302.200, 62-302.530(62), and 62-4.244, Florida Administrative Code.

Several membrane-technology water-treatment facilities notified FDEP that the toxicity of their concentrate water was believed to be caused solely by the "imbalance" of the common seawater ions rather than some unidentified toxicant. Some concentrate waters were known to contain toxicants other than major seawater ions. As a result, during December, 1994, through June, 1995, a study was carried out to develop a suite of toxicity tests designed to discriminate between the existence of toxicity resulting from common seawater ions and that from alternate causes.

This study was performed at the FDEP Central Laboratory with general guidance of a Technical Advisory Committee (TAC). The TAC was composed of members with the technical expertise to ensure a thorough, objective study. These members (Appendix 1) were recruited from the ranks of the membrane-technology industry as well as from state and federal government.

The goal of the study was to define the minimum suite of toxicity tests to discriminate between sample toxicity caused solely by a surplus or deficit of the major seawater ions present (or the relative proportions of those ions) and toxicity caused by other unidentified sources. The result is a protocol consisting of from 5 to 9 tests, depending on the ion chemistry of the concentrate.

The protocol can also generally distinguish between those concentrate samples having toxicity only from other causes and those samples with toxicity from both major-seawater-ion imbalance and other causes. This document includes a generalized version of the FDEP Biology Section Standard Operating Procedure for the protocol resulting from this study along with supporting information.

Scope of the Study

As part of this study, the chemical characteristics of the source water and concentrate from 15 membrane-technology water-treatment facilities located within the State of Florida were examined (Appendix 2). By selecting facilities with different major-seawater-ion characteristics in their source water, it was anticipated that the results might be applicable to all facilities. This aspect was, however, affected by differences in membrane technology used at the sites. The facilities use membrane

technologies of different designs and varying pretreatment methods. As a result, the chemical makeup of the raw water itself could not be used as a simple predictor of the chemical or toxicity characteristics of the concentrate.

Since there were too many different treatment technologies in use to have examined them all in this study, the facilities were selected based upon the chemistry of their concentrate. Keeping in mind the goal of examining as wide a variety of water types as practical with available resources, six sites were chosen for inclusion in the study.

For the purposes of the study, the major seawater ions were considered to be those that constitute greater than one percent of the total molar weight of the ionic constituents of standard seawater (oceanographic *mer de normal*). In addition, carbonate was included because of its strong effect on pH and overall chemistry of the solution and the trace seawater ion bromide was included because it was required, in combination with the listed major seawater ions, to form a minimal 'artificial seawater' in which the test organisms could live. These major seawater ions are presented in Table 1.

Water samples were collected and tested sequentially from the six facilities over a period of approximately four months. During this preliminary testing and during the method-development phase of the study, all sampling and analyses followed appropriate EPA and FDEP QA/QC and test methods. The toxicity and chemistry results from testing the six facilities during the method-development study are included in Appendix 3.

While the term 'salinity' is commonly used in this document, it must be understood that it is not always used in a manner reflecting the formal definition of salinity. We have used it in two ways. The most common usage is to describe ion concentrations relative to those of seawater of a certain salinity. The statement that 'the concentrate was ion-adjusted to 25-ppt salinity' means that the concentrations of the major seawater ions were adjusted to match those of 25-ppt seawater. The 'real' salinity and the conductivity of the resulting solution may well be somewhat different than that of 25-ppt seawater. The second usage is to describe solutions having a

Table 1. Major Constituents of 35 ppt Seawater.

	percent	g/kg	millimoles/kg	millimolar
Na ⁺	30.68	10.78	469.0	480.0
K ⁺	1.14	0.399	10.21	10.45
Mg ⁺⁺	3.65	1.284	52.82	54.05
Ca ⁺⁺	1.19	0.4185	10.44	10.68
Cl ⁻	55.07	19.353	545.87	558.62
SO ₄ ⁼	7.72	2.712	28.24	28.90
*HCO ₃ ⁻	0.36	0.126	2.07	2.12
*Br ⁻	<u>0.19</u>	<u>0.0673</u>	<u>0.842</u>	<u>0.862</u>
Total	100.00	35.1398	1,119.492	1,145.682

* The inclusion of bromide, although forming <1% of the ions, is essential for the survival of the mysid shrimp used in the tests. Carbonate is included because of its strong effect on pH and the overall chemistry of the resulting solutions.

conductivity equivalent to seawater of some salinity. The statement, for instance, that 'the concentrate sample received from facility xxx has a salinity of 24 ppt' should not be construed to imply that the concentrate makeup actually resembles seawater at 24 ppt in any aspect except conductivity.

Various methods for converting conductivity to salinity are available, including tables and equations. The FDEP Biology lab used a conversion table (Tiphane and St-Pierre 1966) during this study. Standard Methods also contains an equation for calculating salinity from measurements of conductivity.

Development of Test-Suite Rationale

Figure 1 compares the major-seawater-ion concentrations of 35-ppt seawater and two 'low-salinity' concentrate samples tested during the study. If the major seawater ions in the nominally 4-ppt concentrate sample were in the same proportions as seawater, then we would expect each to be present at about 11% of their 35 ppt concentration (i.e., 4 ppt is 11% of 35 ppt). Similarly, the nominally 3-ppt sample would have each of the ions present at about 8% of full seawater. Figure 2 gives a better picture of how the ions in these particular concentrates compare to their proportions in 35-ppt seawater.

Note that in the 4-ppt sample, all ion concentrations are less than those of 35-ppt seawater. In the 3-ppt sample, however, the concentration of sulfate is equal to that of the full-salinity, 35-ppt seawater, while bicarbonate and calcium concentrations are several times greater than those found in 35-ppt seawater. With the exception of magnesium, the other ions are present in concentrations less than the 8% that would be expected in 3-ppt seawater.

It had been proposed that toxicity resulting from major-seawater-ion 'imbalance' would be detectable by comparing the toxicity of an unaltered concentrate sample to the toxicity of a concentrate sample with the major seawater ions corrected to the proper proportions. If no other causes of toxicity were present (including the final adjusted sample having a salinity too high for the test organism), then the resulting sample should have no toxicity. If toxicity remained, then some other cause or causes were present.

An additional proposition was that an artificial sample with the same amounts of the major seawater ions should exhibit toxicity similar to the concentrate sample if the toxicity is caused by those seawater ions being in incorrect proportions.

These simple ideas harbor several problems. Theoretically, one could 'correct' the major seawater ion proportions by adding those ions that were in deficit and removing those ions that were in surplus as compared to seawater at the same salinity. This raises several difficulties:

- 1) Salts—not individual ions—must be added to the concentrate. This means that both anions and cations must be added at the same time, in whatever proportions that available water-soluble salts allow. This may not provide complete matching of the ions that are in deficit.

- 2) Any mechanism for removing ions that are in surplus in the concentrate (e.g., ion-exchange resins) carries the risk of altering any unknown toxicants that may be present. This could lead to incorrect interpretation of the cause of any changes in toxicity.

Toxicity Testing of Membrane-Technology Concentrate

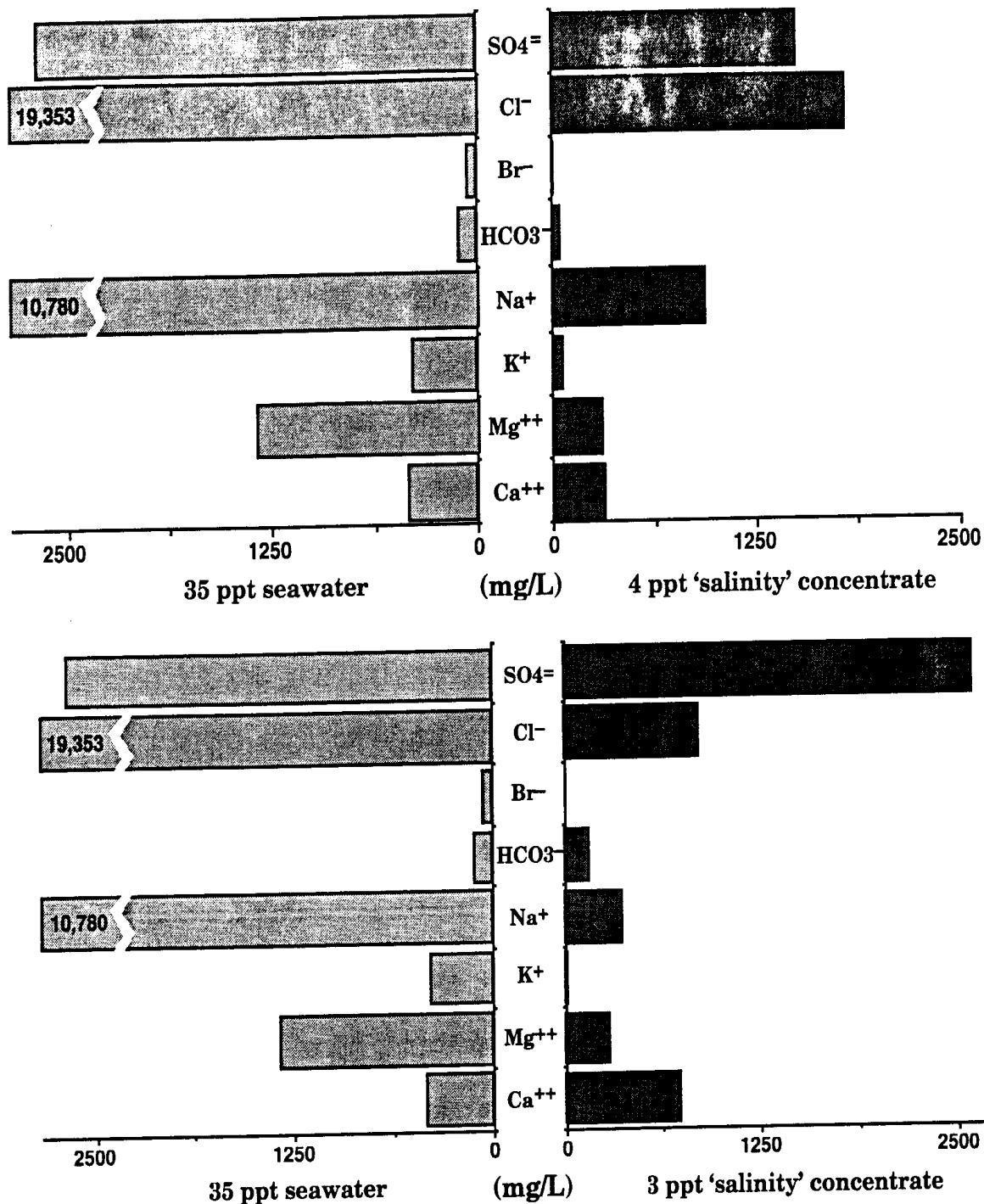


Figure 1. Comparisons of major-ion compositions of seawater and two low-salinity concentrate samples.

3) Creating the 'mock' concentrate could involve the same problem outlined in 1) whereby difficulty may arise in creating the desired mix of ions using available salts.

4) The ions constituting any ion-adjusted or mock solution must, when in correct seawater proportions and adjusted to an appropriate salinity, be able to support the test organisms.

It was decided to address these problems by performing an array of tests that, in combination, would allow the cause of any changes in toxicity to be reliably determined. All tests follow normal EPA protocols (U.S. EPA 1993) for acute toxicity tests of mysid shrimp (the test organism previously identified as most sensitive to this type of toxicity). The test suite evolved with the basic rationale being to compare the results of the following tests.

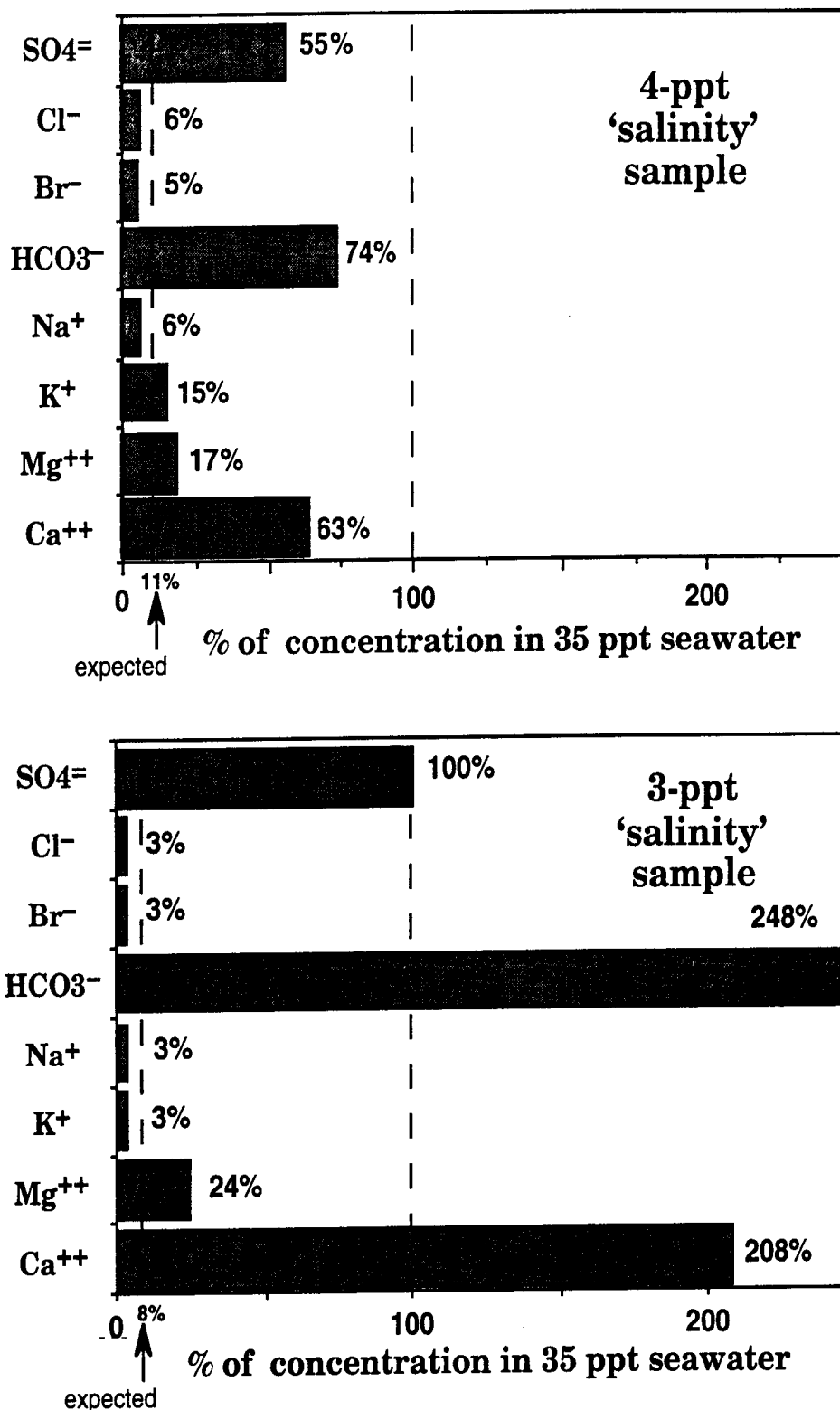


Figure 2. Comparison of proportions of the major-ion compositions of seawater and two low-salinity concentrate samples.

Test 1—Initial test on unaltered concentrate.

This test is a normal biomonitoring test, meeting the 36-hr holding time required by EPA methods (U.S. EPA 1993). Natural seawater (NSW) diluted to the concentrate's salinity is used as the control and diluent.

If the concentrate in Test 1 is not toxic, no additional tests in this suite are performed on this sample. This might occur if sufficient variability in the concentrate exists that not all samples collected are toxic.

Figures 1 and 2 show ion distributions in one of the low-salinity concentrate samples tested.

Test 2—Baseline test on unaltered concentrate.

This test is identical to Test 1, but is performed at the same time as the tests of ion-manipulated concentrate. The time lag between Test 1 and the remaining tests is caused by the necessity of awaiting the results of chemical analyses of the concentrate before performing any ion manipulations, and the time required to perform the manipulations themselves. In our study, the time between Test 1 and the remaining tests was typically 24 hours. Comparing Test 2 to Test 1 indicates changes in toxicity with time and Test 2 provides the measure of toxicity against which the results of the remaining tests are compared. NSW diluted to the concentrate's salinity is used as the control and diluent.

If the concentrate in Test 2 is not toxic, no additional tests in this suite are performed on this sample. This might occur if the cause of toxicity in Test 1 was a degradable toxicant that had been rendered non-toxic during the period between setting up Test 1 and setting up Test 2. This result would indicate that the cause of toxicity in Test 1 was not major-seawater-ion toxicity.

Test 3—Mock concentrate.

This test uses a synthetic concentrate created by mixing ACS-grade chemical salts in deionized water. NSW diluted to the concentrate's salinity is used as the control and diluent.

Test 4—Ion-adjusted concentrate.

This test uses a concentrate sample to which ACS-grade chemical salts have been added to balance the major seawater ions to the proportions present in seawater. This balancing is controlled by the concentrate ion that is in the highest proportion relative to seawater. This ion remains unchanged and the remaining ions are adjusted to achieve balance. NSW diluted to the ion-adjusted-concentrate salinity is used as the control and diluent.

Figures 3 and 4 illustrate ion-adjusting the 4-ppt sample shown in Figures 1 and 2 in an attempt to "correct" the ion concentrations to match those of seawater.

We assume Tests 1 and 2 show toxicity or this suite of tests wouldn't have been completed.

If the solution in Test 3 was toxic and that in Test 4 was not toxic, one would nominally conclude that the toxicity seen in the concentrate was a result of the major seawater ions being out of proportion.

If the solution in Test 3 was not toxic and that in Test 4 was toxic, one would nominally conclude that the toxicity seen in the concentrate was a result of some unidentified toxicant(s), not from the major seawater ions.

If the solution in Test 3 was toxic and that in Test 4 was toxic (but both possibly less toxic than Test 2), one would nominally conclude that the toxicity seen in the concentrate was a result of both the major seawater ions being out of proportion and the presence of some 'other' toxicant(s).

One wouldn't expect to see results where neither Test 3 nor Test 4 was toxic. If this result is found, the implication would be that the toxicity was not caused by

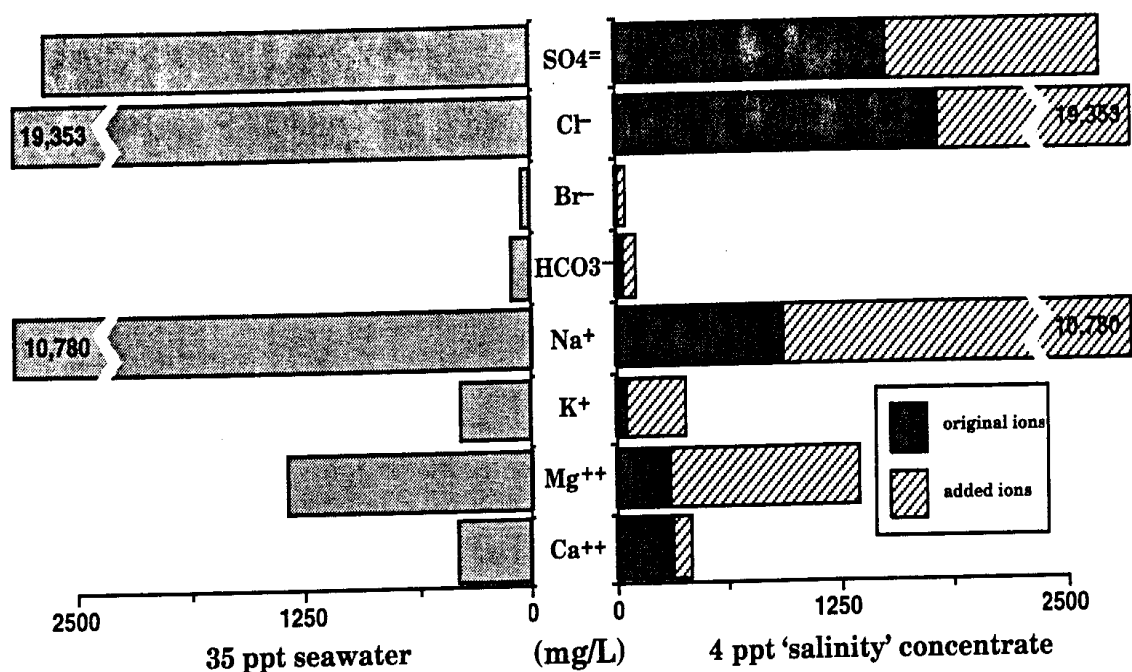


Figure 3. Major-seawater-ion correction of a 'low-salinity' concentrate sample.

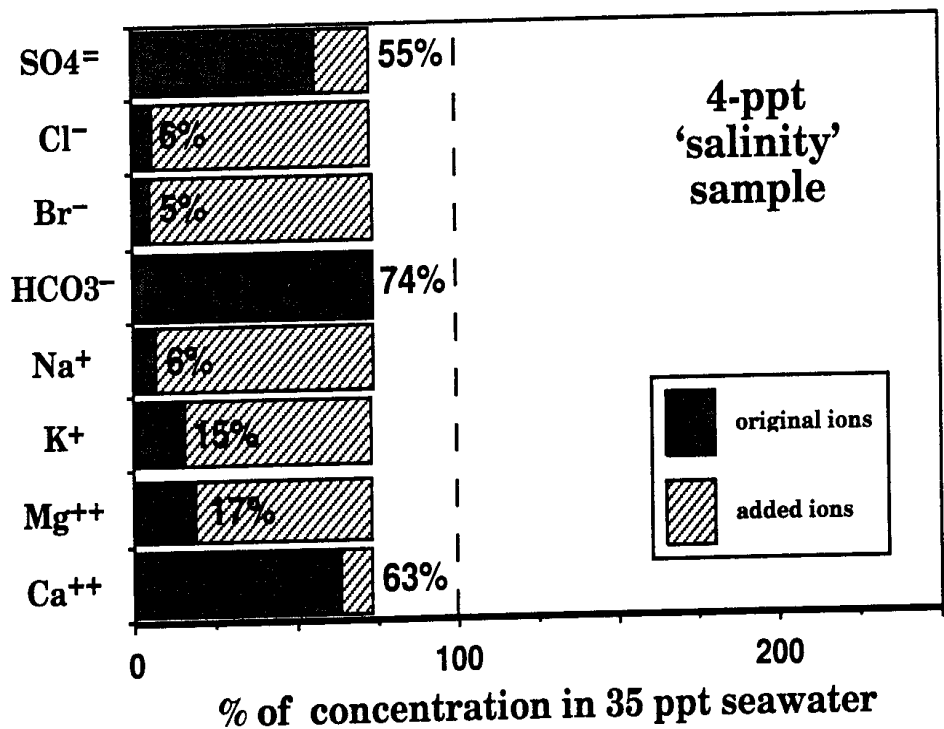


Figure 4. Relative proportions of major-seawater-ion correction of a 'low-salinity' concentrate sample.

major seawater ion imbalance and the toxicant was rendered nontoxic by the steps used in adding the ions needed to balance the seawater ions. This might be through reactions with the added ions themselves or through effects on the toxicants of the sample manipulations during the ion additions.

The problem with this testing is in creating the ion-adjusted concentrate for Test 4. After considering various ion-exchange resins, precipitation techniques, and gasification methods, we decided to avoid them because each offered the prospect of inadvertently altering any unidentified toxicants in the concentrate. This left adding ACS-grade salts as the means of achieving seawater proportions. This method has two drawbacks of its own.

- a) Adding salts to balance the major seawater-ion proportions increases the total dissolved solids ("salinity") of the concentrate.
- b) In some concentrates tested, achieving seawater proportions for all major ions resulted in a concentrate salinity of as much as 219 ppt—well above the salinity range which mysid shrimp can tolerate.

This increased salinity also offered the possibility of toxicity changes in any non-major-seawater-ion toxicants present in the concentrate. The toxicity of some toxicants (e.g., ammonia) is altered by changes in salinity.

To address these complications, in cases where the ion-adjusted concentrate salinity would be greater than 35 ppt, Test 4 was split into two tests, Test 4a and 4b.

Test 4a—Ion-adjusted concentrate, major seawater ions incompletely adjusted.

This test uses a concentrate sample in which the major seawater ions present in concentrations below that of 35 ppt seawater are adjusted up to the 35 ppt-seawater concentrations. Any of the major seawater ions present in concentrations greater than that of 35 ppt seawater are left unaltered. NSW at 35 ppt is used as the control and diluent.

Figures 5 and 6 show the ion distribution for a 'moderate-salinity' concentrate having bicarbonate concentrations greater than those of 35-ppt seawater. Salts were added to bring any concentrate ions that were below 35-ppt seawater concentrations up to that level. This produced a solution with the major seawater ions equivalent to 35-ppt seawater except for a higher concentration of bicarbonate.

Test 4b—Ion-adjusted concentrate, major seawater ions completely adjusted then diluted to 35 ppt.

This test uses a concentrate sample in which ACS-grade chemical salts have been added to balance the major seawater ions to the proportions present in seawater. This balancing is controlled by the concentrate ion that is in the highest proportion relative to seawater. The resulting sample is then diluted with deionized water to 35 ppt salinity. NSW at 35 ppt is used as the control and diluent. In actual practice, the sample manipulations are conducted such that any dilution is performed first then the necessary salts added. This helps minimize problems with salts reaching saturation and precipitating.

Figures 7 and 8 show the ion distribution for a 'moderate-salinity' concentrate having bicarbonate concentrations greater than those of 35-ppt seawater. Salts were added to bring all ions to the same proportions present in 35-ppt seawater. As a result, the ion concentrations are much greater than those in seawater and dilution with deionized water is necessary to reduce the salinity to that of full seawater. Performing these two tests keeps the salinity of the test solutions in the range

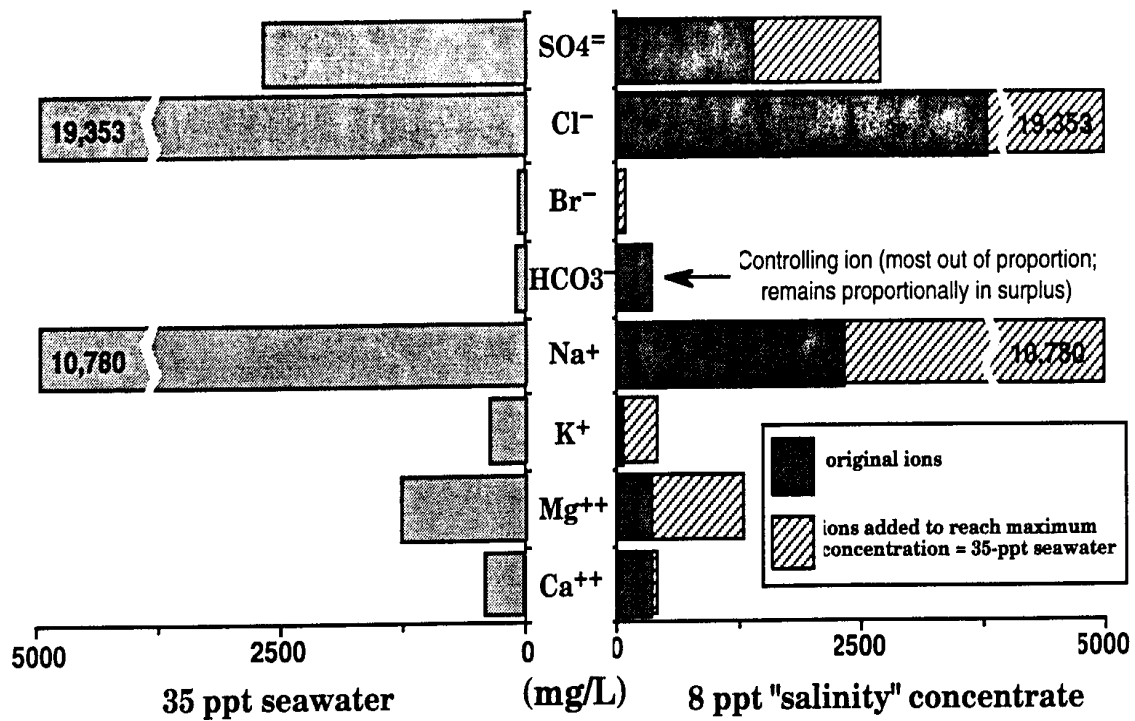


Figure 5. Example of ion correcting a moderate-'salinity' concentrate sample having one ion present at a concentration greater than that in 35-ppt seawater. This ion remains proportionally in surplus, with all other ions made proportional at 35-ppt seawater concentrations (per Tests 4A and 5A).

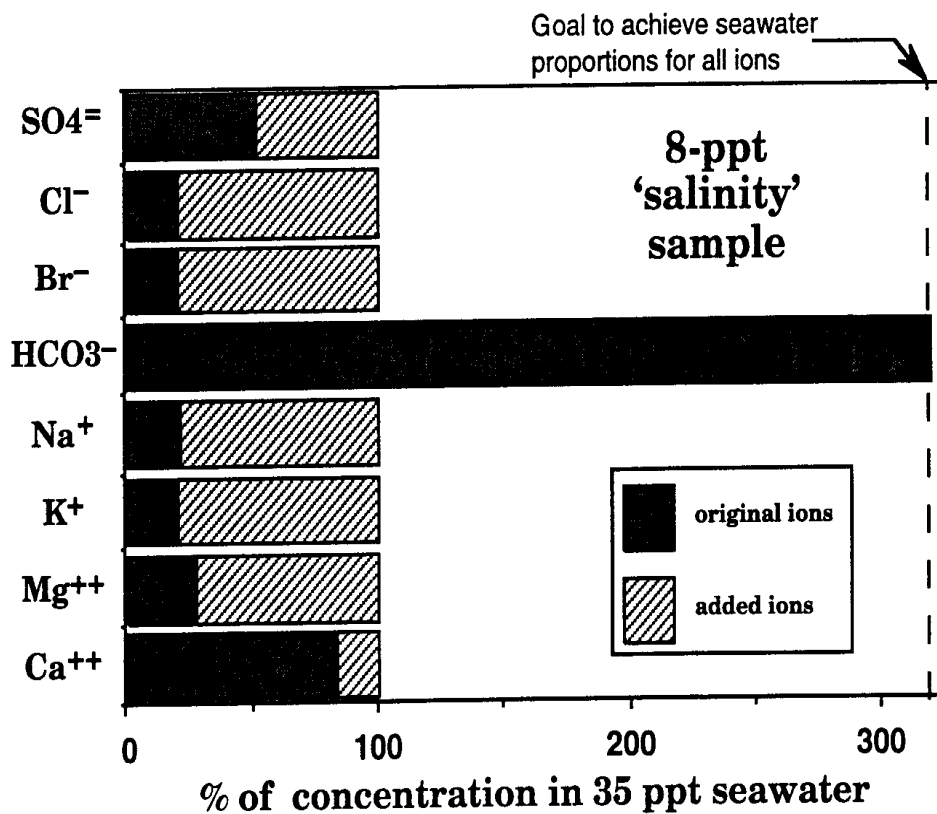


Figure 6. Proportion example of ion correction of a moderate-salinity concentrate sample, leaving one ion proportionally in surplus, with all other ions made proportional at 35-ppt seawater concentrations (per Tests 4A and 5A).

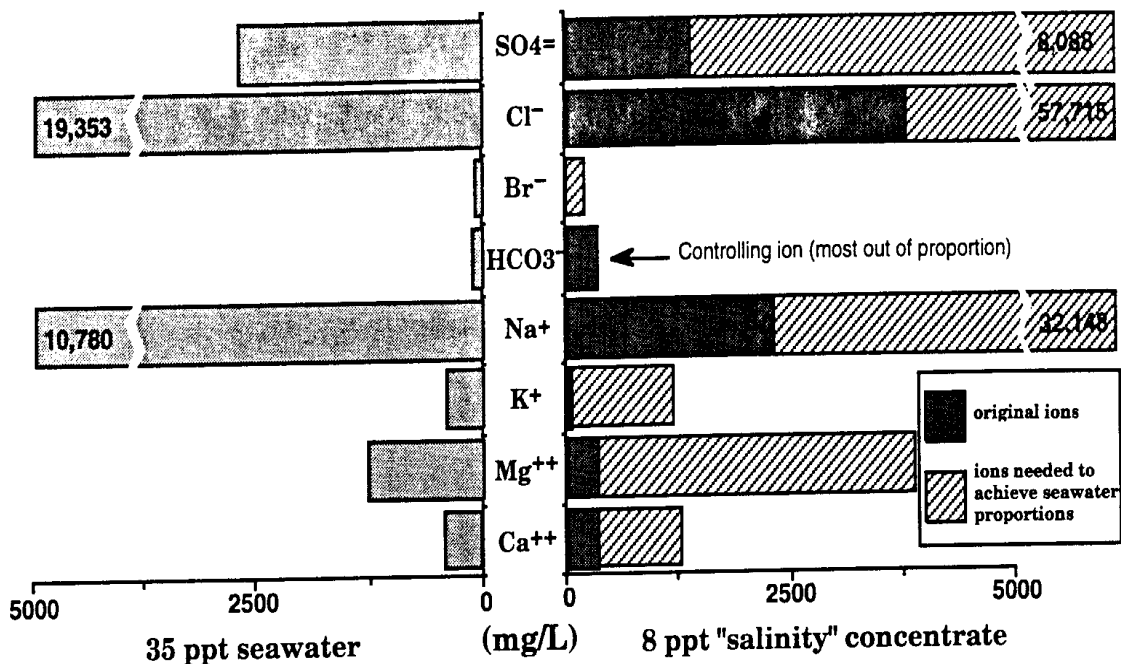


Figure 7. Ion correction of a moderate-salinity concentrate sample, requiring dilution (per Test 4B and 5B).

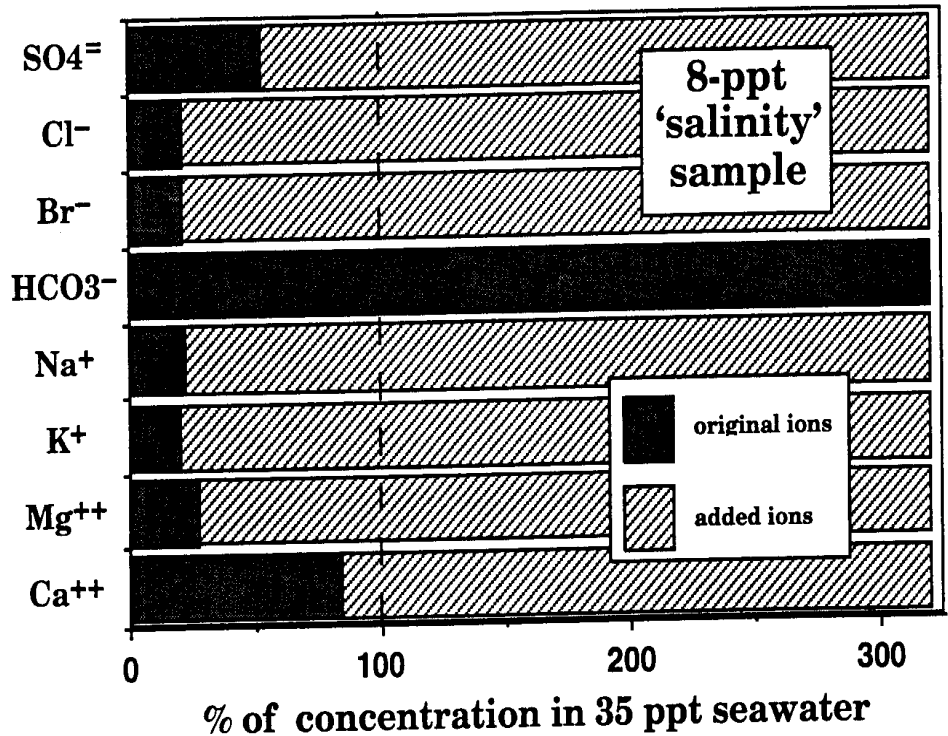


Figure 8. Proportion example of ion correction of a moderate-salinity concentrate sample, requiring dilution (per Test 4B and 5B).

appropriate for the mysids, but Test 4a could incorrectly indicate toxicity from the presence of unidentified toxicants when it actually results from the seawater ions that remain out-of-proportion. Test 4b could incorrectly indicate that the ion-adjusting had removed major seawater-ion toxicity when the toxicity removal actually was a result of diluting unidentified toxicants to non-toxic concentrations as a side effect of diluting the salinity to 35 ppt.

To address these concerns, an additional test was added to determine the result when the concentration of major seawater ions remains constant while the

concentration of any unidentified toxicants is diluted across the test concentration series.

Test 5—Ion-adjusted concentrate

This test uses a concentrate sample to which ACS-grade chemical salts have been added to balance the major seawater ions to the proportions present in seawater. This balancing is controlled by the concentrate ion that is in the highest proportion relative to seawater. This is the same as in Test 4. However, the control and diluent for the toxicity test is composed of mock ion-adjusted concentrate.

The mock ion-adjusted concentrate is composed of an aliquot of the mock concentrate prepared for Test 3 that has been ion adjusted using the same amounts of the same salts used to ion adjust the concentrate in Test 4 and 5. By virtue of the dilution water having the same amount of the major seawater ions as are present in the ion-adjusted concentrate, this test results in a concentration series in which the major seawater ions are the same in all test concentrations, but where any unidentified toxicants are diluted along that series. Thus any differences in toxicity along the test concentration gradient should indicate toxicity from causes other than major seawater ions.

Of course, this test suffers from the same problem as Test 4 in that the composition of some concentrates can result in salinities that are too high for mysid survival. Therefore, this test is also split into two tests for this type concentrate.

Test 5a—Ion-adjusted concentrate, major seawater ions incompletely adjusted, using mock ion-adjusted concentrate for diluent.

This test uses a concentrate sample in which the major seawater ions present in concentrations below that of 35 ppt seawater are adjusted up to the 35 ppt-seawater concentrations. Any of the major seawater ions present in concentrations greater than that of 35 ppt seawater are left unaltered. This is the same as Test 4A. However, the control and diluent for the toxicity test is composed of a mock ion-adjusted concentrate made to match the major-seawater-ion concentrations of the ion-adjusted concentrate used in this test.

Test 5b— Ion-adjusted concentrate, major seawater ions completely adjusted then diluted to 35 ppt, using mock ion-adjusted concentrate for diluent.

This test uses a concentrate sample in which ACS-grade chemical salts have been added to balance the major seawater ions to the proportions present in seawater. This balancing is controlled by the concentrate ion that is in the highest proportion relative to seawater. The resulting sample is then diluted with deionized water to 35 ppt salinity. This is the same as Test 4B. However, the control and diluent for the toxicity test is composed of a mock ion-adjusted concentrate made to match the major-seawater-ion concentrations of the ion-adjusted concentrate used in this test after dilution to 35 ppt.

It is very important to note that the two mock ion-adjusted concentrates used as diluent in 5a and 5b are *not* the same. In each test, the mock concentrate is ion-adjusted in the same manner as the concentrate in that test is adjusted. As a result, the mock ion-adjusted concentrate in 5a has the major seawater ions incompletely adjusted while that created for 5b has the major seawater ions completely adjusted to 35 ppt proportions.

Also as a result, the control for test 5a tells us whether the toxicity seen in the ion-adjusted concentrate in Test 4a and 5a results from some of the major seawater ions remaining out of proportion, or from the presence of 'other' toxicants.

Interpreting Test-Suite Results

After running a concentrate sample through this test suite, the results are interpreted as follows:

Toxicity of the sample due solely to an "imbalance" of the major seawater-ion proportions:

The unaltered-concentrate in Test 2 and the mock-concentrate in Test 3 should show similar results with the toxicity reducing with dilution. The ion-adjusted concentrate in Tests 4 and 5 should not exhibit toxicity. Should it be impossible to adjust the major seawater ions in Test 4 to seawater proportions without the ion concentrations exceeding those in 35-ppt seawater, then Tests 4a and 5a should either show no toxicity or, if toxicity is present, the toxicity of all the test concentrations of Test 5a should be similar, including the control. Tests 4b and 5b should show no toxicity.

Part of the toxicity due to major seawater-ion "imbalance" and the rest from other toxicants:

The mock concentrate in Test 3 and the ion-adjusted concentrate in Tests 4 and 5 (or all of 4a & 4b and 5a & 5b) should be less toxic than the unaltered concentrate in Test 2.

All of the toxicity caused by toxicants other than the major seawater ions:

The unaltered concentrate in Test 2, the ion-adjusted concentrate in Tests 4 and 5 (or all of 4a & 4b and 4a & 5b) should show similar toxicity decreasing with dilution. The mock concentrate in Test 3 should show no toxicity.

Some areas of uncertainty arose in interpreting the test-suite results during the study. The situation has been identified in those concentrates where correcting the major seawater ions results in ion-adjusted concentrates with some major seawater ions present in much greater concentrations than seawater and where very high dilutions are needed to create 35 ppt concentrate samples for Tests 4b and 5b. In this situation, it is possible to have a set of data where one cannot determine whether a concentrate has unidentified causes of toxicity only or has both major seawater ion plus unidentified causes of toxicity.

This study encountered no situations where there was difficulty in distinguishing concentrates toxic solely because of major-seawater-ion proportions from concentrates possessing unidentified toxicants, whether solo or in conjunction with major-seawater-ion toxicity.

Note: this suite of tests is not designed to identify the actual toxicant(s). It is designed to determine whether the observed toxicity results from the proportions of major seawater ions or if it results from other unidentified toxicants. If other ions that can be manipulated in a manner similar to the major seawater ions are strongly suspected, then this protocol could be readily modified to confirm or deny the suspected toxicant. If desired, Toxicity Identification Evaluation (TIE) protocols (U.S. EPA 1988, 1989a, 1989b) could be used to attempt to identify the specific cause(s) of any toxicity not caused by major-seawater-ion imbalances.

Figure 9 contains a summary flowchart of the test methods.

Test-Suite Findings for Studied Facilities

We tried to run each of the six membrane facilities selected through the test suite three times. Fort Myers was tested only twice because results indicated that it was not a facility best tested using marine species. In general, the first test series was less reliable as methods were being developed.

A summary of the findings is presented in Table 2. The individual test results are contained in Appendix 3.

Plant Operations Survey Results

Survey forms developed by the membrane-technology representatives on the TAC were given to all facilities when samples were taken. This was done on the expectation that information concerning the operation of the facilities being tested might help to indicate factors contributing to the presence or absence of toxicity in the concentrate. Steve Duranceau of the TAC agreed to attempt the interpretation of the survey results (summarized in Table 3). Problems with the forms not being returned or not being completely and accurately completed hinder interpretation. Fluoride data was included in the table because of the potential interference it presented and because of its electronegativity as compared to the other monovalent anions. Because of the limited data, a quantitative analysis was not possible. No qualitative correlation between post-treatment operation and toxicity was evident.

Additional Testing Possibilities

Several ideas were discussed during TAC meetings, but have not been tested. Nevertheless, these might prove useful in investigations of some concentrates.

One promising addition, dubbed Test 5c, is for those situations where substantial dilution was required in Tests 4b and 5b and where these two tests were not toxic while 4a and 5a were toxic. The proposal was to take the concentrate created in Test 5b and add back in the ions that remained out of proportion in 5a. If the resulting concentrate regained its toxicity, then the original cause of the toxicity was major seawater ions, if the sample remained nontoxic, then the original cause was unidentified toxicants.

Another option discussed is for those situations where ion adjusting the concentrate results in high salinity, and where bicarbonate is the only ion greatly out of proportion. The proposal was to lower the pH of the unaltered concentrate below 5, thereby converting the bicarbonate to CO₂ and allowing it to equilibrate with the atmosphere. The pH would then be adjusted back to the original value and bicarbonate added as necessary to achieve the desired concentration. This would have removed the necessity to split tests 4 and 5 into the a & b tests for several of the concentrates we examined. A problem with this step is the danger of removing unidentified toxicants that are volatile and/or broken down by the pH-adjustment steps or creating unidentified toxicants from unidentified nontoxic compounds through those same manipulations.

Toxicity Testing of Membrane-Technology Concentrate

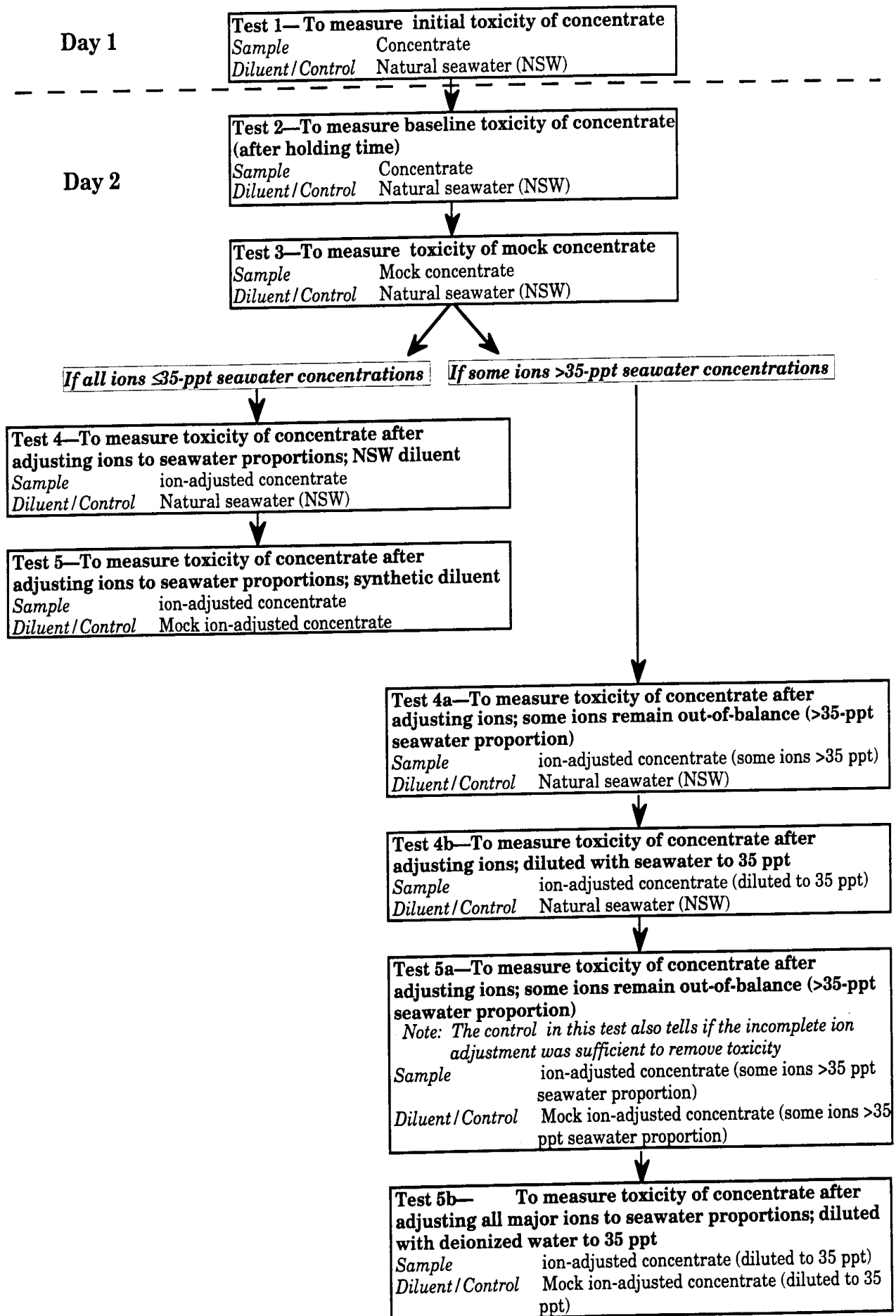


Figure 9. Test-method summary flowchart.

Table 2. Results summary for facilities tested during the study.

Facility	Test Series 1	Test Series 2	Test Series 3
Fort Myers	No reliable interpretation.	Major-seawater-ion toxicity. Not toxic to freshwater organisms.	No third test series conducted.
Vero Beach	No reliable interpretation. Na and Cl concentrations low due to calculation error. Random mortality possibly due to probe use during monitoring.	Toxicity due to unidentified toxicant(s).	Toxicity mostly due to unidentified toxicant(s). Some toxicity possibly from major-seawater-ions.
Venice	Major-seawater-ion toxicity.	Major-seawater-ion toxicity suspected. Tests 5a and 5b invalid. Cannot make definitive statement.	Major-seawater-ion toxicity.
Gasparilla	Both major-seawater-ion toxicity and unidentified toxicant(s).	Both major-seawater-ion toxicity and unidentified toxicant(s).	Both major-seawater-ion toxicity and unidentified toxicant(s).
Sailfish Point	Toxicity mostly from major-seawater-ion-toxicity. Unidentified toxicant(s) also present.	Both major-seawater-ion toxicity and unidentified toxicant(s).	Both major-seawater-ion toxicity and unidentified toxicant(s).
Jupiter	Probably no major-seawater-ion toxicity. Mostly unidentified toxicant(s).	Not certain of major-seawater-ion toxicity due to CaCO ₃ precipitate. Unidentified toxicant removed/reduced by increase in salinity or some other factor.	Toxicity due to unidentified toxicant(s). Antiscalant addition prevented CaCO ₃ precipitation without affecting toxicity.

Table 3. Plant operations and toxicity summary.

Facility Name	Fluoride (mg/L F ⁻)	Pre-treatment	Post-treatment	Toxicity interpretation
Ft. Myers	0.5	sulfuric acid, cartridge filtration	degassification, chlorination, in-line oxygen oxidation	toxic to saltwater species (major-seawater-ion toxicity), not toxic to freshwater species
Gasparilla	3.1	antiscalant, sulfuric acid, cartridge filtration	chlorination, in-line oxygen oxidation, hydrogen peroxide	major-seawater-ion toxicity and unidentified toxicity present
Jupiter	4.4	antiscalant, cartridge filtration	degassification, chlorination	unidentified toxicity present
Sailfish Point	2.3	antiscalant, sulfuric acid, cartridge filtration	degassification (gravel-bed), pH adjustment with caustic soda	major-seawater-ion toxicity and unidentified toxicity present
Venice	3.1	sand separation, antiscalant, sulfuric acid, cartridge filtration	chlorination, in-line oxygen oxidation	major-seawater-ion toxicity present
Vero Beach*	4.0	—	—	unidentified toxicity present

* No surveys returned

Miscellaneous Test Protocol Information

If the unaltered concentrate salinity is below 6 ppt (measured as conductivity), those concentrations remaining below 6 ppt after the test dilutions are prepared are adjusted to 6 ppt using a mock seawater-salt slurry. This is composed of the major seawater ions, in seawater proportions, and with sufficient deionized water added to form a thick slurry. The slurry is used to ensure that all the salts are evenly mixed and that small amounts of the slurry can be used without concern that the salts are being added out-of-proportion.

Our experience is that the toxicity tests must run for 96 hours to clearly separate test results. Also, if Test 1 shows toxicity in only the higher concentrations, then the lower dilutions (say below 25%) in the remaining definitive toxicity tests could be dropped without compromising the protocol's ability to reveal ion-balance effects.

If the pH of the manipulated or mock samples is greater than 9.0 or less than 7.5, minimal HCl or NaOH should be used to adjust the samples to fall within that range.

Following ion adjustments, it is important for follow-up chemistry analyses to be performed to confirm that the various concentrate manipulations were correctly achieved. This test suite involves numerous opportunities for error. Also, difficulties with solubility, saturation, and formation of precipitates can have substantial effects on actual final concentrations. Until the calculated ion adjustments are validated by chemistry analyses, no conclusions should be drawn as to the cause of observed toxicity.

High-quality chemistry analyses are a must for this test suite to be successfully conducted. The chemistry lab performing the analyses should preferably be able to demonstrate analytical accuracy of at least $\pm 15\%$.

Conclusions

- 1) A substantial imbalance of the major seawater ions can be toxic to mysid.
- 2) This toxicity is not directly correlated to the absolute concentrations of any of those ions, but to the relative proportions of the ions.
- 3) The suite of tests developed during this study is capable of distinguishing between toxicity caused by an imbalance in the proportions of the major seawater ions in a sample, toxicity caused by other unidentified toxics, and toxicity caused by a combination of these two factors.
- 4) It was found that certain concentrates with very low levels of dissolved solids were toxic to the mysid shrimp as a result of major-seawater-ion imbalance, but were not toxic to freshwater test organisms. The program *Salinity Toxicity Relationship* seems to accurately predict those facilities where freshwater test organisms would more appropriately be used even though the nominal salinity of the concentrate is greater than the normal tolerance range for the freshwater species. This program was developed by the ENSR consulting group of Ft. Collins, CO for the Gas Research Institute to predict the toxicity of marine salts to freshwater test organisms.

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Appendix 2. Analytical chemistry results from screening of selected Florida membrane-technology water-treatment facilities sampled weekly during December 1994.														
Facility Name	Sampling Location	Ca++ (mg/L)	Mg++ (mg/L)	K+ (mg/L)	Na+ (mg/L)	Sr+ (mg/L)	Alk (mg/L)	Cl- (mg/L)	F- (mg/L)	SO4= (mg/L)	Total Cations	Total Anions		
Anclole River	Source 1	336.0	986.0	292.0	8500.0	5.54	136	16000	1.1	2100	475.00	497.87		
Anclole River	Source 2	335.0	1030.0	299.0	9410.0	5.61	136	15000	0.9	2200	518.32	471.73		
Anclole River	Source 3	307.0	1070.0	280.0	8870.0	6.1	137	15000	0.4	2100	496.25	469.64		
Anclole River pilot	Concentrate 1	576.0	1730.0	507.0	14500.0	9.44	211	21000	1.6	3700	814.62	673.77		
Anclole River pilot, USF LAB	Concentrate 2	573.0	1660.0	504.0	14800.0	9.61	225	26000	1.6	3900	821.68	819.26		
Anclole River pilot, SWFWMD	Concentrate 3	466.0	1620.0	434.0	13400.0	9.3	205	24000	0.6	3200	750.37	747.81		
Cape Coral R/O	Source 1	68.4	88.6	17.8	362.0	11.2	184	630	2.0	200	26.91	25.72		
Cape Coral R/O	Source 2	69.5	88.1	17.9	353.0	11.8	186	640	1.7	220	26.53	26.45		
Cape Coral R/O	Source 3	61.3	81.8	18.7	316.0	9.23	204	560	1.1	160	24.02	23.27		
Cape Coral R/O	Concentrate 1	315.0	420.0	84.7	1600.0	50.7	327	2700	7.4	1400	122.06	112.26		
Cape Coral R/O	Concentrate 2	369.0	470.0	93.3	1860.0	63	347	3300	9.5	1800	140.40	138.03		
Cape Coral R/O	Concentrate 3	237.0	323.0	65.9	1290.0	36.1	166	2200	8.0	1200	96.21	90.80		
Charlotte Harbor Water Assoc	Source 1	107.0	71.6	11.9	270.0	22	110	550	1.5	230	23.29	22.59		
Charlotte Harbor Water Assoc	Source 2	112.0	78.4	12.4	296.0	24.3	82	660	1.2	250	25.24	25.53		
Charlotte Harbor Water Assoc	Source 3	103.0	67.7	12.2	257.0	20.2	113	560	0.5	240	22.21	23.08		
Charlotte Harbor Water Assoc	Concentrate 1	409.0	279.0	46.2	1020.0	84.4	246	2100	0.8	1100	88.95	87.12		
Charlotte Harbor Water Assoc	Concentrate 2	407.0	294.0	46.1	1170.0	91	267	2600	4.8	1100	96.60	101.85		
Charlotte Harbor Water Assoc	Concentrate 3	361.0	239.0	39.1	997.0	83.8	251	2100	4.6	1000	82.07	85.33		
Englewood R/O	Source 1	277.0	218.0	32.5	1400.0	35.8	119	2700	1.6	490	93.50	88.84		
Englewood R/O	Source 2	287.0	243.0	33.5	1590.0	36.7	139	3100	1.4	520	104.34	101.13		
Englewood R/O	Source 3	258.0	204.0	31.8	1340.0	34.1	136	2800	1.5	480	88.77	91.78		
Englewood R/O	Concentrate 1	807.0	648.0	95.2	4680.0	128	276	9700	4.0	1700	299.61	314.77		
Englewood R/O	Concentrate 2	957.0	765.0	99.4	5110.0	120	268	10000	4.9	1900	335.54	327.29		
Englewood R/O	Concentrate 3	935.0	749.0	98.6	4700.0	125	274	9200	2.2	1800	315.27	302.62		
Ft Myers Membrane Softening	Source 1	77.0	9.9	4.3	35.6	0.54	186	54	0.3	15	6.32	5.57		
Ft Myers Membrane Softening	Source 2	74.9	9.4	4.0	35.3	0.517	183	58	0.2	17	6.15	5.66		
Ft Myers Membrane Softening	Source 3	74.4	9.5	4.3	36.6	0.523	191	60	0.6	17	6.21	5.90		
Ft Myers Membrane Softening	Concentrate 1	308.0	49.1	13.0	90.0	2.1	543	160	0.2	220	23.69	19.97		
Ft Myers Membrane Softening	Concentrate 2	323.0	42.2	11.7	94.3	2.34	563	180	0.6	290	24.02	22.41		
Ft Myers Membrane Softening	Concentrate 3	296.0	38.5	11.7	89.8	2.09	490	170	1.0	320	22.17	21.31		

Facility Name	Sampling Location	Ca++ (mg/L)	Mg++ (mg/L)	K+ (mg/L)	Na+ (mg/L)	Sr+ (mg/L)	Alk (mg/L)	Cl- (mg/L)	F- (mg/L)	SO4= (mg/L)	Total Cations	Total Anions
Gasparilla Island Water Assoc	Source 1	278.0	231.0	33.4	1560.0	43.8	103	2800	1.0	480	101.60	91.10
Gasparilla Island Water Assoc	Source 2	264.0	232.0	33.6	1570.0	44.9	126	3100	1.4	470	101.42	99.83
Gasparilla Island Water Assoc	Source 3	264.0	219.0	32.9	1670.0	40.5	127	3000	0.4	470	104.68	96.98
Gasparilla Island Water Assoc	Concentrate 1	1080.0	899.0	131.0	5830.0	170	429	12000	3.6	1700	384.83	382.69
Gasparilla Island Water Assoc	Concentrate 2	1020.0	864.0	120.0	5670.0	164	439	11000	3.4	1800	371.71	356.76
Gasparilla Island Water Assoc	Concentrate 3	1040.0	878.0	113.0	5710.0	171	460	12000	3.5	1800	375.43	385.39
Indian River Co., North	Source 1	89.1	76.0	14.0	449.0	10.8	141	840	1.0	110	30.59	28.86
Indian River Co., North	Source 2	84.0	71.6	13.1	443.0	9.82	141	890	0.8	110	29.69	30.26
Indian River Co., North	Source 3	83.2	73.7	12.6	416.0	10.1	137	880	0.0	110	28.64	29.86
Indian River Co., North	Concentrate 1	249.0	210.0	36.5	1200.0	29.3	222	2500	2.2	460	82.84	84.66
Indian River Co., North	Concentrate 2	235.0	202.0	37.2	1210.0	24.6	209	2500	1.9	620	81.94	87.72
Indian River Co., North	Concentrate 3	266.0	234.0	35.2	1290.0	29.8	218	2600	0.0	500	89.55	88.12
Indian River Co., South *	Source 1	48.9	45.7	13.0	149.0	8.45	*****	*****	*****	*****	13.02	*****
Indian River Co., South	Source 2	49.2	44.8	12.3	148.0	8.33	103	270	1.1	170	12.90	13.28
Indian River Co., South	Source 3	51.3	46.7	11.4	148.0	9.15	158	280	0.8	120	13.14	13.60
Indian River Co., South *	Concentrate 1	170.0	163.0	36.0	419.0	30.3	*****	*****	*****	*****	41.06	*****
Indian River Co., South *	Concentrate 2	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****
Indian River Co., South	Concentrate 3	182.0	167.0	32.1	414.0	32.6	323	840	0.8	700	41.67	44.78
Island Water Assoc	Source 1	101.0	110.0	35.2	715.0	10.5	166	1100	2.0	390	46.09	42.58
Island Water Assoc	Source 2	110.0	127.0	37.7	788.0	11.9	170	1400	1.7	390	51.18	51.11
Island Water Assoc	Source 3	112.0	124.0	37.7	772.0	11.4	164	1400	0.8	390	50.34	50.94
Island Water Assoc	Concentrate 1	486.0	539.0	172.0	3440.0	50.7	608	5800	5.6	1800	222.64	213.57
Island Water Assoc	Concentrate 2	514.0	563.0	172.0	3670.0	54.6	746	6200	9.0	1900	236.01	229.87
Island Water Assoc	Concentrate 3	587.0	640.0	173.0	3910.0	56.5	771	7100	3.2	1900	256.46	255.45
Jupiter RO	Source 1	132.0	202.0	70.0	1640.0	18.4	143	2900	1.4	540	96.33	95.99
Jupiter RO	Source 2	119.0	180.0	52.4	1690.0	16.5	167	3100	1.2	530	95.59	101.89
Jupiter RO	Source 3	131.0	182.0	51.9	1500.0	16.6	143	2700	1.5	510	88.08	89.73
Jupiter RO	Concentrate 1	485.0	745.0	218.0	5900.0	68.5	536	9600	4.4	1900	347.68	321.34
Jupiter RO	Concentrate 2	406.0	650.0	176.0	5310.0	61.4	517	10000	4.2	1800	309.18	330.15
Jupiter RO	Concentrate 3	545.0	763.0	220.0	6080.0	70	596	10000	6.1	2000	360.04	336.00

Facility Name	Sampling Location	Ca++ (mg/L)	Mg++ (mg/L)	K+ (mg/L)	Na+ (mg/L)	Sr+ (mg/L)	Alk (mg/L)	Cl- (mg/L)	F- (mg/L)	SO4= (mg/L)	Total Cations	Total Anions
Marineland	Source 1	342.0	227.0	27.9	1370.0	11.7	125	2500	1.0	620	96.06	85.99
Marineland	Source 2	338.0	217.0	26.4	1350.0	11.2	124	2800	1.0	690	94.13	95.89
Marineland	Source 3	310.0	191.0	25.0	1360.0	11.5	119	2600	1.0	650	90.99	89.32
Marineland	Concentrate 1	494.0	335.0	43.9	2070.0	16.9	8	4000	1.3	1200	143.40	138.06
Marineland	Concentrate 2	523.0	340.0	44.6	2050.0	17.2	33	4000	1.3	1300	144.41	140.65
Marineland	Concentrate 3	487.0	325.0	39.4	2100.0	18.1	34	4000	1.4	1200	143.41	138.59
Sailfish Point Utilities	Source 1	89.0	89.1	21.1	591.0	6.4	160	1100	0.9	230	38.02	39.07
Sailfish Point Utilities	Source 2	92.0	91.7	22.2	597.0	6.83	166	1100	1.0	230	38.67	39.19
Sailfish Point Utilities	Source 3	96.1	95.4	21.2	605.0	6.47	164	1100	0.9	240	39.50	39.36
Sailfish Point Utilities	Concentrate 1	385.0	386.0	96.0	2450.0	27.9	319	3800	2.7	1400	160.00	142.88
Sailfish Point Utilities	Concentrate 2	353.0	353.0	80.6	2560.0	25.8	291	4300	2.8	1400	160.08	156.43
Sailfish Point Utilities	Concentrate 3	376.0	381.0	76.8	2330.0	26.1	288	4100	3.6	1400	153.43	150.77
Sarasota RO	Source 1	319.0	132.0	6.1	148.0	19.9	131	290	1.6	960	33.41	30.88
Sarasota RO	Source 2	295.0	131.0	5.4	142.0	19.2	126	280	1.4	940	31.84	30.08
Sarasota RO	Source 3	309.0	126.0	6.5	148.0	19.8	133	280	0.0	910	32.42	29.52
Sarasota RO	Concentrate 1	706.0	314.0	16.4	501.0	59.7	44	1000	2.4	2200	83.35	75.05
Sarasota RO	Concentrate 2	687.0	313.0	15.5	500.0	59.4	65	1000	2.7	2200	82.25	75.48
Sarasota RO	Concentrate 3	649.0	288.0	16.2	487.0	60.4	42	1000	0	2300	77.74	76.97
Venice RO	Source 1	514.0	200.0	9.4	311.0	16.3	131	620	1.9	1400	55.92	49.38
Venice RO	Source 2	425.0	181.0	8.0	278.0	15.5	132	610	1.7	1300	48.44	47.02
Venice RO	Source 3	412.0	137.0	4.9	57.7	15.3	115	120	0.9	1500	34.51	36.98
Venice RO	Concentrate 1	971.0	369.0	16.0	476.0	32	236	950	3.9	2800	100.03	90.06
Venice RO	Concentrate 2	891.0	347.0	13.4	412.0	30.4	246	910	3.8	2900	91.37	91.21
Venice RO	Concentrate 3	776.0	295.0	11.2	251.0	29.8	207	570	3.2	2700	74.28	76.64
Vero Beach RO	Source 1	51.2	49.2	12.1	195.0	8.88	167	340	1.2	99	15.40	15.06
Vero Beach RO	Source 2	46.9	45.5	11.2	189.0	8.24	167	400	1.0	99	14.59	16.74
Vero Beach RO	Source 3	46.3	49.4	12.0	189.0	9.65	166	320	0.8	110	14.91	14.68
Vero Beach RO	Concentrate 1	319.0	310.0	75.0	1060.0	57	112	1900	4.6	1400	89.48	85.25
Vero Beach RO	Concentrate 2	288.0	281.0	60.1	1040.0	56.8	102	2200	5.2	1500	84.29	95.62
Vero Beach RO	Concentrate 3	317.0	301.0	64.6	1060.0	54.8	100	1800	4.4	1400	88.37	82.17

* Note: Data irregularities in some samples taken at Indian River Co., South, suggested probable mislabeling and led to their being discarded.

Appendix 3—Facility Test Results From FDEP Study

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Toxicity Test Results

Date sampled
2/13/95

Facility Name
Ft Myers

NH3+NH4 =<0.017 mg/L
Fluoride = No data

Test 1— To measure: toxicity as discharged

Sample	Concentrate	1.2 ppt "salinity" adjusted to 5 ppt w/sea salts
Diluent/Control	Natural seawater (NSW)	5 ppt salinity
Test duration	96-hr Start date: 2/14/95	
	Results:	Comments:
Mysids	55% dead in 50% sample; 100% dead in 100% sample	Acclimation problems in all concs and controls, 10% dead
Menidia	40% dead in 100% sample	Controls OK

Test 2— To measure: baseline toxicity of concentrate

Sample	Concentrate	1.2 ppt "salinity" adjusted to 5 ppt w/mock salts
Diluent/Control	Natural seawater (NSW)	5 ppt salinity
Test duration	48-hr Start date : 2/15/95	
	Results:	Comments:
Mysids	90% dead in 100% sample	Controls OK

Test 3— To measure: toxicity of mock concentrate

Sample	Mock concentrate	1.2 ppt "salinity" adjusted to 5 ppt w/mock salts
Diluent/Control	Natural seawater	5 ppt salinity
Test duration	48-hr Start date : 2/15/95	
	Results:	Comments:
Mysids	20% dead in 100% sample	Controls OK

Test 4— To measure: toxicity of concentrate after adjusting ions to seawater proportions; natural seawater diluent

Sample	ion-adj concentrate	20 ppt "salinity"
Diluent/Control	Natural seawater	20 ppt salinity
Test duration	48-hr Start date : 2/15/95	
	Results:	Comments:
Mysids	None	Study-plan error resulted in use of wrong diluent (see below)

Test 5— To measure: toxicity of concentrate after adjusting ions to seawater proportions; synthetic diluent

Sample	ion-adj concentrate	20 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate	20 ppt salinity
Test duration	48-hr Start date : 2/15/95	
	Results:	Comments:
Mysids	0% dead in 100% sample; 10% dead in control	

Other Test— To measure: comparing toxicity of ion-adjusted concentrate with that of mock concentrate

Sample	ion-adj concentrate	20 ppt "salinity"
Diluent/Control	Mock concentrate	5 ppt salinity
Test duration	48-hr Start date : 2/15/95	
	Results:	Comments:
Mysids	85% dead in 100% sample; 5% dead in control (100% mock concentrate).	Study-plan-alteration error resulting in wrong diluent; good validation of Tests 2&3.

General Comments:

Concentrate salinity unexpectedly low requiring changes to plan of study and very rapid acclimation of test organisms for Test 1. Salt hydration weights not included in calcs.

Interpretation:

Test 4 and "Other Test" result of error in changing study terminology to follow that derived by TAC. Inserted "mock conc" instead of "ion-adj mock conc".

No reliable interpretation

Ft Myers

2/13/95

**errors in mock conc apparently due to errors in original calcs; e.g., hydration of salts not being included

Concentrates Component name	mg/L	% of		sea salt		% diff from	
		Concentrate	35 SW	sal adj	mock conc	sal adj	sal adj conc
Calcium	244		58.3%	405	231		-43.0%
Magnesium	53.1		4.1%	127	143		12.6%
Potassium	14.2		3.6%	63.8	66.1		3.6%
Sodium	129		1.2%	1560	1700		9.0%
Alkalinity	181		—	326	96.1		-70.5%
Carbonate	110.41		87.6%	198.86	58.621		-70.5%
Bromide	—		—	—	—		—
Chloride	150		0.8%	2500	2600		4.0%
Sulfate	1000		36.9%	1200	910		-24.2%

* not analyzed, method interferences; est @ 0.5

Ion-adjusted concentrates

	ion-adj		% of		Mock ion-adj		% of		% diff
	conc/B	20.4 SW	20.4 SW	conc/B	20.4 SW	from i-a conc			
Calcium	405	166.0%		183	75.0%			-54.8%	
Magnesium	628	83.9%		625	83.5%			-0.5%	
Potassium	408	175.4%		400	171.9%			-2.0%	
Sodium	9250	147.2%		9150	145.6%			-1.1%	
Alkalinity	202	—		98.8	—			-51.1%	
Carbonate	123.22	167.7%		60.268	82.0%			-51.1%	
Bromide	—	—		—	—			—	
Chloride	1500	13.3%		1500	13.3%			0.0%	
Sulfate	2800	177.1%		2300	145.5%			-17.9%	

Toxicity Test Results

Date sampled
3/27/95

Facility Name
Ft. Myers RO

NH3+NH4 =0.22 mg/L
Fluoride = 0.46 mg/L

Test 1— To measure toxicity as discharged

Sample	Concentrate	0.8 ppt "salinity" adjusted to 5 ppt with sea salts
Diluent/Control	Natural seawater (NSW)	5 ppt salinity for mysids
Test duration	96-hr	Start date : 3/28/95
	Results:	Comments:
Mysids	45% dead in 100% sample; 0% dead in control	Additional 100% conc with 5 ppt sea salts plus concentrate (= 5.8 ppt) had 30% dead
Menidia	10% dead in 100% sample	concentrate and diluent at 0.8 ppt "salinity"

Test 2— To measure baseline toxicity of concentrate

Sample	Concentrate	0.8 ppt "salinity" adjusted to 5 ppt with mock sea salts
Diluent/Control	Natural seawater (NSW)	5 ppt salinity
Test duration	96-hr	Start date : 3/29/95
	Results:	Comments:
Mysids	10% dead in 50% sample; 100% dead in 100% sample; 45% dead in control	no salinity gradient
C. dubia	0% dead in 100% sample	0.8 ppt "salinity" concentrate, FW diluent

Test 3— To measure toxicity of mock concentrate

Sample	Mock concentrate	0.8 ppt "salinity" adjusted to 5 ppt with mock sea salts
Diluent/Control	Natural seawater	5 ppt salinity
Test duration	96-hr	Start date : 3/29/95
	Results:	Comments:
Mysids	60% dead in 50% sample; 80% dead in 100% sample; 25% dead in control	
C. dubia	0% dead in 100% sample	0.8 ppt "salinity" concentrate, FW diluent

Test 4— To measure toxicity of concentrate after adjusting ions to seawater proportions; natural seawater diluent

Sample	ion-adjusted concentrate	19.75 ppt "salinity"
Diluent/Control	Natural seawater	19.75 ppt salinity
Test duration	96-hr	Start date : 3/29/95
	Results:	Comments:
Mysids	0% dead in 100% sample	

Test 5— To measure toxicity of concentrate after adjusting ions to seawater proportions; synthetic diluent

Sample	ion-adjusted concentrate	19.75 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate	19.75 ppt salinity
Test duration	96-hr	Start date : 3/29/95
	Results:	Comments:
Mysids	0% dead in 100% sample; 0% dead in control	

General Comments:

Test "salinity" in tests 1—3 appears to be sufficiently low as to cause some (~5%-10%) background mortality.

Interpretation:

Major-seawater-ion toxicity. Not toxic to freshwater test organism.

Ft. Myers RO 3/27/95

Concentrates

Component name	mg/L	Concentrate		% of 35 SW	Mock conc		% diff from conc	sea salt		% of 5 SW	mock slurry		% of 5 SW	sal adj		sal adj mock conc	% diff from sal ad conc
								sal adj	conc		sal adj	conc					
Calcium		236		56.4%	244		3.4%	425		710.9%	276		461.6%	276		276	-35.1%
Magnesium		34.2		2.7%	35.1		2.6%	196		106.9%	187		101.9%	187		187	-4.6%
Potassium		10.2		2.6%	10.9		6.9%	28.8		50.5%	26.3		46.1%	26.3		26.3	-8.7%
Sodium		84.4		0.8%	127		50.5%	1230		79.9%	1360		88.3%	1360		1360	10.6%
Alkalinity		345		—	162		-53.0%	191		—	158		—	158		158	-17.3%
Bicarbonate		210.45		167.0%	98.82		-53.0%	116.51		647.3%	96.38		535.4%	96.38		96.38	-17.3%
Bromide		0.4		0.6%	0.81		102.5%	8.1		84.2%	8.8		91.5%	8.8		8.8	8.6%
Chloride		140		0.7%	370		164.3%	2300		83.2%	2700		97.7%	2700		2700	17.4%
Sulfate		300		11.1%	300		0.0%	1200		309.7%	580		149.7%	580		580	-51.7%

* initial Br analysis was "I"; also need to make stock sol'ns for these low values

Ion-adjusted
concentrates

	ion-adj		% of 19.7 SW	Mock ion-adj		% of 19.7 SW	% diff from i-a c
	conc			conc			
Calcium	226		95.8%	266		112.7%	17.7%
Magnesium	682		94.2%	849		117.3%	24.5%
Potassium	222		98.7%	230		102.2%	3.6%
Sodium	5930		97.5%	7340		120.7%	23.8%
Alkalinity	377		—	171		—	-54.6%
Bicarbonate	229.97		323.7%	104.31		146.8%	-54.6%
Bromide	38		100.1%	46		121.2%	21.1%
Chloride	11000		100.8%	11000		100.8%	0.0%
Sulfate	1500		98.1%	1800		117.7%	20.0%

Seawaters

	Seawater		% of 5 SW
	diluent		
Calcium	61.5		102.9%
Magnesium	187		101.9%
Potassium	57.2		100.4%
Sodium	1510		98.1%
Alkalinity	16.2		—
Bicarbonate	9.882		54.9%
Bromide	12		124.8%
Chloride	2800		101.3%
Sulfate	400		103.2%

Toxicity Test Results

Date sampled
3/6/95

Facility Name
Gasparilla RO

NH₃+NH₄ = 0.26 mg/L
Fluoride = 3.7 J mg/L

Test 1— To measure: toxicity as discharged

Sample	Concentrate	26 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	20 ppt salinity
Test duration	96-hr	
	Results:	Comments:
Mysids	50% dead in 50% sample; 100% dead in 100% sample	26 ppt to 20 ppt gradient
Menidia	0% dead in 100% sample	26 ppt to 20 ppt gradient

Test 2— To measure: baseline toxicity of concentrate

Sample	Concentrate	26 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	26 ppt salinity
Test duration	96-hr	
	Results:	Comments:
Mysids	10% dead in 50% sample; 100% dead in 100% sample	no salinity gradient

Test 3— To measure: toxicity of mock concentrate

Sample	Mock concentrate	26 ppt "salinity"
Diluent/Control	Natural seawater	26 ppt salinity
Test duration	96-hr	
	Results:	Comments:
Mysids	100% dead in 100% sample	

Test 4a— To measure: Toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion)

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater	35 ppt salinity
Test duration	96-hr	
	Results:	Comments:
Mysids	100% dead in 100% sample	35 ppt plus some ions higher. <i>Lab error, no KCl added. No retest possible.</i>

Test 4b— To measure: Toxicity of concentrate after adjusting ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	35 ppt salinity
Test duration	96-hr	
	Results:	Comments:
Mysids	90% dead in 100% sample	Diluted from 92 ppt to 35 ppt. <i>Lab error, no KCl added. No retest possible.</i>

Test 5a— To measure: Toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion)

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (some ions >35 ppt)	35 ppt salinity
Test duration	96-hr	
	Results:	Comments:
Mysids	All dead, all concentrations	35 ppt plus some ions higher; <i>Lab error, no KCl added.</i>
Mysids retest	60% dead in 50%; 100% dead in 100% sample; 30% dead in control	35 ppt plus some ions higher. Retested left-over sample. Remaining out-of-balance ions are a partial cause of toxicity in this test.

Test 5b— To measure: Toxicity of concentrate after adjusting all major ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (diluted to 35 ppt)	35 ppt salinity
Test duration	96-hr	
	Results:	Comments:
Mysids	All dead, all concentrations	Diluted from 92 ppt to 35 ppt; <i>Lab error, no KCl added.</i>
Mysids retest	0% dead in 100% sample; 0% dead in control	Diluted from 92 ppt to 35 ppt. Retested left-over sample.

General Comments:

Lab error, no KCl added to ion-adjusted samples. Retested 5a & b using leftover sample. Insufficient volume to retest 4a & b.

Interpretation:

Both major-seawater-ion toxicity and unidentified toxicant(s).

Gasparilla RO 3/6/95

Concentrates

Component name	mg/L	% of		% diff	
Concentrate	35 SW	Mock conc	from conc		
Calcium	1100	262.8%	1140	3.6%	
Magnesium	1040	81.0%	1080	3.8%	
Potassium	178	44.6%	186	4.5%	
Sodium	7580	70.3%	8020	5.8%	
Alkalinity	443	#DIV/0!	161	-63.7%	
Carbonate	270.23	214.5%	98.21	-63.7%	
Bromide	69	102.5%	65	-5.8%	
Chloride	16000	82.7%	14000	-12.5%	
Sulfate	2400	88.5%	2300	-4.2%	

Ion-adjusted concentrates

	ion-adj conc/B	% of 35 SW	Mock ion-adj conc/B	% of 35 SW	% diff from i-a conc	ion-adj conc A	% of 35 SW	Mock ion-adj conc A	% of 35 SW	% of 92 SW	conc's %diff
Calcium	436	104.2%	424	101.3%	-2.8%	1160	277.2%	1120	267.6%	101.8%	3.5%
Magnesium	1300	101.2%	1290	100.5%	-0.8%	1330	103.6%	1310	102.0%	38.8%	1.5%
Potassium	104	26.1%	98.8	24.8%	-5.0%	178	44.6%	182	45.6%	17.4%	2.2%
Sodium	11400	105.8%	11300	104.8%	-0.9%	9890	91.7%	10000	92.8%	35.3%	1.1%
Alkalinity	186	#DIV/0!	87.9	#DIV/0!	-52.7%	439	#DIV/0!	175	#DIV/0!	#VALUE!	86.0%
Carbonate	113.46	90.0%	53.619	42.6%	-52.7%	267.79	212.5%	106.75	84.7%	32.2%	86.0%
Bromide	57	84.7%	54	80.2%	-5.3%	49	72.8%	66	98.1%	37.3%	29.6%
Chloride	18000	93.0%	17000	87.8%	-5.6%	17000	87.8%	17000	87.8%	33.4%	0.0%
Sulfate	2900	106.9%	2900	106.9%	0.0%	2600	95.9%	2900	106.9%	40.7%	10.9%

K left out of original Test 4 & 5 solutions; Readjusted and Tests 5a&b rerun (insufficient sample to retest 4).

Ion-adjusted concentrates

	ion-adj conc/B	% of 35 SW	Mock ion-adj conc/B	% of 35 SW	% diff from i-a conc	ion-adj conc A	% of 35 SW	Mock ion-adj conc A	% of 35 SW	% of 92 SW	conc's %diff
Calcium	417	99.6%	410	98.0%	-1.7%	1140	272.4%	1090	260.5%	741.6%	4.5%
Magnesium	1250	97.4%	1240	96.6%	-0.8%	1300	101.2%	1270	98.9%	#DIV/0!	2.3%
Potassium	400	100.3%	392	98.2%	-2.0%	401	100.5%	396	99.2%	9.8%	1.3%
Sodium	10800	100.2%	10800	100.2%	0.0%	10500	97.4%	10400	96.5%	#DIV/0!	1.0%
Alkalinity		#DIV/0!		#DIV/0!	#DIV/0!		#DIV/0!		#DIV/0!	#VALUE!	#DIV/0!
Carbonate	0	0.0%	0	0.0%	#DIV/0!	0	0.0%	0	0.0%	#DIV/0!	#DIV/0!
Bromide	59	87.7%	56	83.2%	-5.1%	51	75.8%	70	104.0%	#DIV/0!	31.4%
Chloride	19000	98.2%	19000	98.2%	0.0%	20000	103.3%	19000	98.2%	8175.9%	5.1%
Sulfate	2900	106.9%	3100	114.3%	6.9%	2700	99.6%	3000	110.6%	39.5%	10.5%

Toxicity Test Results

Date sampled
4/3/95

Facility Name
Gasparilla RO

NH₃+NH₄ = 1.10 mg/L
Fluoride = 3.1 J mg/L

Test 1— To measure toxicity as discharged

Sample	Concentrate	25 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	20 ppt salinity
Test duration	96-hr	Start date 4/4/95
	Results:	Comments:
Mysids	10% dead in 50% sample; 100% dead in 100% sample	25 ppt to 20 ppt gradient
Menidia	10% dead in 100% sample	25 ppt to 20 ppt gradient

Test 2— To measure baseline toxicity of concentrate

Sample	Concentrate	25 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	25 ppt salinity
Test duration	96-hr	Start date 4/5/95
	Results:	Comments:
Mysids	95% dead in 50% sample; 100% dead in 100% sample	no salinity gradient

Test 3— To measure toxicity of mock concentrate

Sample	Mock concentrate	25 ppt "salinity"
Diluent/Control	Natural seawater	25 ppt salinity
Test duration	96-hr	Start date 4/5/95
	Results:	Comments:
Mysids	100% dead in 100% sample	Precipitate noted at 24 hr

Test 4a— To measure toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion)

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater	35 ppt salinity
Test duration	96-hr	Start date 4/5/95
	Results:	Comments:
Mysids	25% dead in 50% sample; 100% dead in 100% sample	35 ppt plus some ions higher

Test 4b— To measure toxicity of concentrate after adjusting ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	35 ppt salinity
Test duration	96-hr	Start date 4/5/95
	Results:	Comments:
Mysids	50% dead in 50% sample; 100% dead in 100% sample	Diluted from 98 ppt to 35 ppt, sporadic mortality throughout test

Test 5a— To measure toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion)

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (some ions >35 ppt)	35 ppt salinity
Test duration	96-hr	Start date 4/5/95
	Results:	Comments:
Mysids	70% dead in 50% sample; 100% dead in 100% sample; 90% dead in control	35 ppt plus some ions higher; remaining out-of-balance ions are a cause of toxicity

Test 5b— To measure toxicity of concentrate after adjusting all major ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (diluted to 35 ppt)	35 ppt salinity
Test duration	96-hr	Start date 4/5/95
	Results:	Comments:
Mysids	5% dead in 100% sample; 10% dead in control	Diluted from 98 ppt to 35 ppt

General Comments:

Mock concentrate points to seawater-ion toxicity. Manipulations show toxicity from "other" sources. Precipitate in mock concentrate suggests use of antiscalant. Error in carbonate calculation formula.

Interpretation:

Both major seawater-ion toxicity and unidentified sources of toxicity appear to be present.

Gasparilla RO 4/3/95

Concentrates

Component name	mg/L		% of		% diff	
	Concentrate	35 SW	Mock conc	from conc		
00915Calcium	1170	279.6%	1120	-4.3%		
00925Magnesium	1040	81.0%	993	-4.5%		
00935Potassium	161	40.4%	158	-1.9%		
00930Sodium	7270	67.4%	7250	-0.3%		
00410Alkalinity	424	—	163	-61.6%		
Bicarbonate	258.64	205.3%	99.43	-61.6%	**carbonate for adjustments and mocks calculated incorrectly	
Bromide	50.32	74.8%	51	1.4%		
Chloride	14367	74.2%	14000	-2.6%		
Sulfate	2192	80.8%	2200	0.4%		

Ion-adjusted concentrates

	ion-adj		% of		Mock ion-adj		% of	
	conc/B	35 SW	conc/B	35 SW	conc A	35 SW	conc A	35 SW
00915Calcium	373	89.1%	394	94.1%	1070	255.7%	1090	260.5%
00925Magnesium	1180	91.9%	1180	91.9%	1210	94.2%	1180	91.9%
00935Potassium	380	95.2%	377	94.5%	392	98.2%	377	94.5%
00930Sodium	11000	102.0%	11100	103.0%	10200	94.6%	10200	94.6%
00410Alkalinity	166	—	101	—	416	—	171	—
Bicarbonate	101.26	80.4%	61.61	48.9%	253.76	201.4%	104.31	82.8%
Bromide	66	98.1%	65	96.6%	65	96.6%	66	98.1%
Chloride	19000	98.2%	20000	103.3%	18000	93.0%	20000	103.3%
Sulfate	2700	99.6%	2700	99.6%	2500	92.2%	2700	99.6%

Seawaters

Seawater		% of	
diluent	35 SW		
00915Calcium	409	97.7%	
00925Magnesium	1240	96.6%	
00935Potassium	381	95.5%	
00930Sodium	11000	102.0%	
00410Alkalinity	121	—	
Bicarbonate	73.81	58.6%	
Chloride	65	96.6%	
Sulfate	20000	103.3%	
Bromide	2400	88.5%	

Toxicity Test Results

Date sampled
5/22/95

Facility Name
Gasparilla RO

NH3+NH4 = 0.60 mg/L
Fluoride = 2.5 J mg/L

Test 1— To measure toxicity as discharged

Sample	Concentrate	25 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	20 ppt salinity
Test duration	96-hr	Start date 5/23/95
	Results:	Comments:
Mysids	25% dead in 50% sample; 100% dead in 100% sample	20 ppt to 25 ppt gradient
Menidia	5% dead in 100% sample	20 ppt to 25 ppt gradient

Test 2— To measure baseline toxicity of concentrate

Sample	Concentrate	25 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	25 ppt salinity
Test duration	96-hr	Start date 5/24/95
	Results:	Comments:
Mysids	5% dead in 50% sample; 100% dead in 100% sample	no salinity gradient

Test 3— To measure toxicity of mock concentrate

Sample	Mock concentrate	25 ppt "salinity"
Diluent/Control	Natural seawater	25 ppt salinity
Test duration	96-hr	Start date 5/24/95
	Results:	Comments:
Mysids	100% dead in 100% sample	Some CaCO3 precipitation

Test 4a— To measure toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion)

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater	35 ppt salinity
Test duration	96-hr	Start date 5/24/95
	Results:	Comments:
Mysids	90% dead in 100% sample	35 ppt plus some ions higher

Test 4b— To measure toxicity of concentrate after adjusting ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	35 ppt salinity
Test duration	96-hr	Start date 5/24/95
	Results:	Comments:
Mysids	0% dead in 100% sample	Diluted from 144 ppt to 35 ppt

Test 5a— To measure toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion)
Note: The control in this test also tells if the incomplete ion adjustment left remaining toxicity

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (some ions >35 ppt)	35 ppt "salinity"
Test duration	96-hr	Start date 5/24/95
	Results:	Comments:
Mysids	35% dead in 50% sample; 90% dead in 100% sample; 15% dead in control	35 ppt plus some ions higher; remaining out-of-balance ions are a cause of some of toxicity. Some CaCO3 precipitation in control.

Test 5b— To measure toxicity of concentrate after adjusting all major ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (diluted to 35 ppt)	35 ppt "salinity"
Test duration	96-hr	Start date 5/24/95
	Results:	Comments:
Mysids	0% dead in 100% sample; 0% dead in control	Diluted from 144 ppt to 35 ppt

General Comments:

Not possible to make an absolute determination of cause with this test suite because it does not control for all parameters. Mock concentrate points to seawater-ion toxicity. Manipulations show toxicity from unidentified sources. Antiscalant in

Interpretation:

Both major seawater-ion toxicity and unidentified sources of toxicity appear to be present.

Gasparilla RO 5/22/95

Concentrates

Component name	mg/L	% of 35 SW	Mock conc	% diff from conc
Calcium	1140	272.4%	995	-12.7% **
Magnesium	1080	84.1%	1030	-4.6%
Potassium	163	40.9%	156	-4.3%
Sodium	7510	69.7%	6860	-8.7%
Alkalinity	426	412.5%	333	-21.8%
Bicarbonate	519.72	412.5%	406.26	-21.8% **
Bromide	47.4	70.4%	47	-0.8%
Chloride	13000	67.2%	14000	7.7%
Sulfate	2100	77.4%	2100	0.0%

**minor CaCO3 precipitation

Ion-adjusted concentrates

	ion-adj conc/B	% of 35 SW	Mock ion-adj conc/B	% of 35 SW	% diff from i-a conc	ion-adj conc A	% of 35 SW	Mock ion-adj conc A	% of 35 SW
Calcium	377	90.1%	369	88.2%	-2.1%	1050	250.9%	971	232.0%
Magnesium	1190	92.7%	1200	93.5%	0.8%	1190	92.7%	1180	91.9%
Potassium	387	97.0%	378	94.7%	-2.3%	395	99.0%	381	95.5%
Sodium	10700	99.3%	10800	100.2%	0.9%	10800	100.2%	10300	95.5%
Alkalinity	99.9	96.7%	94	91.0%	-5.9%	429	415.4%	337	326.3%
Bicarbonate	121.878	96.7%	114.68	91.0%	-5.9%	523.38	415.4%	411.14	326.3%
Bromide	66	98.1%	64	95.1%	-3.0%	69	102.5%	66	98.1%
Chloride	19000	98.2%	19000	98.2%	0.0%	21000	108.5%	20000	103.3%
Sulfate	2700	99.6%	2700	99.6%	0.0%	2900	106.9%	2600	95.9%

Seawaters

	Seawater diluent	% of 35 SW
Calcium	398	95.1%
Magnesium	1260	98.1%
Potassium	386	96.7%
Sodium	11000	102.0%
Alkalinity	121	117.2%
Bicarbonate	147.62	117.2%
Bromide	64	95.1%
Chloride	19000	98.2%
Sulfate	2500	92.2%

Toxicity Test Results

Date sampled
3/13/95

Facility Name
Jupiter RO

NH3+NH4 = 2.16 mg/L
Fluoride = 5.1 mg/L (interference, spike recovery
only 75%. Actual amount probably higher)

Test 1— To measure: toxicity as discharged

Sample	Concentrate	21 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	25 ppt salinity
Test duration	96-hr Start date: 3/14/95	
	Results:	Comments:
Mysids	80% dead in 100% sample	21 ppt to 25 ppt gradient; pH 7.9-8.6
Menidia	65% dead in 100% sample	21 ppt to 25 ppt gradient; pH 7.9-8.6

Test 2— To measure: baseline toxicity of concentrate

Sample	Concentrate	21 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	21 ppt salinity
Test duration	96-hr Start date: 3/15/95	
	Results:	Comments:
Mysids	90% dead in 100% sample	no salinity gradient; pH 8.1-8.6

Test 3— To measure: toxicity of mock concentrate

Sample	Mock concentrate	21 ppt "salinity"
Diluent/Control	Natural seawater	21 ppt salinity
Test duration	96-hr Start date: 3/15/95	
	Results:	Comments:
Mysids	5% dead in 100% sample	pH 7.9-8.1

Test 4a— To measure: toxicity of concentrate after adjusting ions; some ions remain out-of-balance (<35-ppt proportion)

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater	35 ppt salinity
Test duration	96-hr Start date: 3/15/95	
	Results:	Comments:
Mysids	0% dead in 100% sample	35 ppt plus some ions higher; pH 8-8.5

Test 4b— To measure: toxicity of concentrate after adjusting ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	35 ppt salinity
Test duration	96-hr Start date: 3/15/95	
	Results:	Comments:
Mysids	0% dead in 100% sample	Diluted from 105 ppt to 35 ppt; pH 7.9-8.2

Test 5a— To measure: toxicity of concentrate after adjusting ions; some ions remain out-of-balance (<35-ppt proportion)

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (some ions >35 ppt)	35 ppt salinity
Test duration	96-hr Start date: 3/15/95	
	Results:	Comments:
Mysids	20% dead in 100% sample; 0% dead in control	35 ppt plus some ions higher; pH 8-8.5

Test 5b— To measure: toxicity of concentrate after adjusting ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (diluted to 35 ppt)	35 ppt salinity
Test duration	96-hr Start date: 3/15/95	
	Results:	Comments:
Mysids	0% dead in 100% sample; 0% dead in control	Diluted from 105 ppt to 35 ppt; pH 7.9-8.2

General Comments:

Carbonate calculation error, carbonate low

Interpretation:

Little or no major-seawater-ion toxicity. Most of toxicity from unidentified toxicant(s).

Jupiter RO 3/13/95

Concentrates

Component name	mg/L	Concentrate	% of 35 SW	Mock conc	% diff from conc
Calcium	522	522	124.7%	549	5.0%
Magnesium	798	798	62.1%	831	4.1%
Potassium	226	226	56.6%	235	3.9%
Sodium	6380	6380	59.2%	6260	-1.9%
Alkalinity	620	620	—	297	-70.4%
Carbonate	378.2	378.2	300.2%	181.17	-70.4%
Bromide	36	36	53.5%	36	0.0%
Chloride	11000	11000	56.8%	11000	0.0%
Sulfate	2100	2100	77.4%	2200	4.7%

Ion-adjusted concentrates

	ion-adj conc/B	% of 35 SW	Mock ion-adj conc/B	% of 35 SW	conc's %diff	ion-adj conc A	% of 35 SW	Mock ion-adj conc A	% of 35 SW
Calcium	426	101.8%	423	101.1%	0.7%	528	126.2%	528	126.2%
Magnesium	1330	103.6%	1310	102.0%	1.5%	1320	102.8%	1310	102.0%
Potassium	411	103.0%	405	101.5%	1.5%	414	103.8%	411	103.0%
Sodium	11500	106.7%	11100	103.0%	3.5%	11400	105.8%	11000	102.0%
Alkalinity	190	—	92.9	—	68.6%	621	—	300	—
Carbonate	115.9	92.0%	56.7	45.0%	68.6%	378.8	300.6%	183	145.2%
Bromide	58	86.2%	55	81.7%	5.3%	69	102.5%	68	101.0%
Chloride	19000	98.2%	19000	98.2%	0.0%	19000	98.2%	18000	93.0%
Sulfate	2900	106.9%	2800	103.2%	3.5%	2800	103.2%	2700	99.6%

Seawaters

Seawater diluent	% of 35 SW
Calcium	415
Magnesium	1310
Potassium	394
Sodium	11000
Alkalinity	119
Carbonate	72.59
Bromide	65
Chloride	19000
Sulfate	2300

Toxicity Test Results

Date sampled
5/1/95

Facility Name
Jupiter RO

NH3+NH4 = 6.0 mg/L
Fluoride = 4.4 mg/L

Test 1— To measure toxicity as discharged

Sample	Concentrate	20 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	24 ppt salinity
Test duration	96-hr	Start date 5/2/95
	Results:	Comments:
Mysids	10% dead in 50% sample; 100% dead in 100% sample	24 ppt to 20 ppt gradient
Menidia	10% dead in 100% sample	24 ppt to 20 ppt gradient

Test 2— To measure baseline toxicity of concentrate

Sample	Concentrate	20 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	20 ppt salinity
Test duration	96-hr	Start date 5/3/95
	Results:	Comments:
Mysids	10% dead in 50% sample; 90% dead in 100% sample	no salinity gradient

Test 3— To measure toxicity of mock concentrate

Sample	Mock concentrate	20 ppt "salinity"
Diluent/Control	Natural seawater	20 ppt salinity
Test duration	96-hr	Start date 5/3/95
	Results:	Comments:
Mysids	10% dead in 100% sample	

Test 4a— To measure toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion)

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater	35 ppt salinity
Test duration	96-hr	Start date 5/3/95
	Results:	Comments:
Mysids	60% dead in 100% sample	35 ppt plus some ions higher

Test 4b— To measure toxicity of concentrate after adjusting ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	35 ppt salinity
Test duration	96-hr	Start date 5/3/95
	Results:	Comments:
Mysids	0% dead in 100% sample	Diluted from 219 ppt to 35 ppt

Test 5a— To measure toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion)
Note: The control in this test also tells if the incomplete ion adjustment left remaining toxicity

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (some ions >35 ppt)	35 ppt salinity
Test duration	96-hr	Start date 5/3/95
	Results:	Comments:
Mysids	15% dead in 100% sample; 0% dead in control	35 ppt plus some ions higher; remaining out-of-balance ions are not a cause of toxicity

Test 5b— To measure toxicity of concentrate after adjusting all major ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (diluted to 35 ppt)	35 ppt salinity
Test duration	96-hr	Start date 5/3/95
	Results:	Comments:
Mysids	0% dead in 100% sample	Diluted from 219 ppt to 35 ppt

General Comments:

Interpretation:

Not sure of major-seawater-ion toxicity since CaCO3 precipitated. Unidentified toxicant seems to have toxicity removed/reduced by increasing salinity or some other factor.

Jupiter RO 5/1/95

Concentrates

Component name	mg/L		% of		% diff	
	Concentrate	35 SW	Mock conc	from conc		
Calcium	498	119.0%	326	-34.5%		
Magnesium	771	60.0%	759	-1.6%		
Potassium	215	53.9%	214	-0.5%		
Sodium	6310	58.5%	6390	1.3%		
Alkalinity	647	—	369	-43.0%		
Bicarbonate	789.34	626.5%	450.18	-43.0%		
Bromide	35.6	52.9%	34	-4.5%		
Chloride	10590	54.7%	10000	-5.6%		
Sulfate	2120	78.2%	2100	-0.9%		

Ion-adjusted concentrates	ion-adj conc/B		% of		Mock ion-adj conc/B		% of		% diff	
		35 SW		35 SW		from i-a conc		35 SW		
Calcium	391	93.4%	393	93.9%	0.5%		504	120.4%	394	94.1%
Magnesium	1200	93.5%	1220	95.0%	1.7%		1260	98.1%	1210	94.2%
Potassium	380	95.2%	387	97.0%	1.8%		401	100.5%	383	96.0%
Sodium	11000	102.0%	11200	103.9%	1.8%		11100	103.0%	11200	103.9%
Alkalinity	94.9	—	83.2	—	-12.3%		626	—	507	—
Bicarbonate	115.778	91.9%	101.504	80.6%	-12.3%		763.72	606.1%	618.54	490.9%
Bromide	65	96.6%	64	95.1%	-1.5%		66	98.1%	65	96.6%
Chloride	19000	98.2%	19000	98.2%	0.0%		19000	98.2%	19000	98.2%
Sulfate	2600	95.9%	2600	95.9%	0.0%		2600	95.9%	2700	99.6%

Seawaters	Seawater diluent		% of	
		35 SW		
Calcium	412	98.4%		
Magnesium	1270	98.9%		
Potassium	387	97.0%		
Sodium	11100	103.0%		
Alkalinity	128	—		
Bicarbonate	156.16	123.9%		
Bromide	65	96.6%		
Chloride	19000	98.2%		
Sulfate	2200	81.1%		

Toxicity Test Results

Date sampled 6/6/95	Facility Name Jupiter RO	NH3+NH4 = 2.7 mg/L 6/7;4.4 mg/L 6/8;2.1 mg/L 6/12 Fluoride = 3.6J mg/L
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Test 1— To measure toxicity as discharged

Sample	Concentrate	18.3 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	20 ppt salinity
Test duration	96-hr	Start date 6/7/95
	Results:	Comments:
Mysids	60% dead in 100% sample	20 ppt to 18.3 ppt gradient
Menidia	0% dead in 100% sample	20 ppt to 18.3 ppt gradient

Test 2— To measure baseline toxicity of concentrate

Sample	Concentrate	18.3 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	18 ppt salinity
Test duration	96-hr	Start date 6/8/95
	Results:	Comments:
Mysids	60% dead in 100% sample	no salinity gradient

Test 3— To measure toxicity of mock concentrate

Sample	Mock concentrate	18.3 ppt "salinity"
Diluent/Control	Natural seawater	18 ppt salinity
Test duration	96-hr	Start date 6/8/95
	Results:	Comments:
Mysids	0% dead in 100% sample	CaCO3 precipitated

Test 4a— To measure toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion)

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater	35 ppt salinity
Test duration	96-hr	Start date 6/8/95
	Results:	Comments:
Mysids	15% dead in 100% sample	35 ppt plus some ions higher

Test 4b— To measure toxicity of concentrate after adjusting ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	35 ppt salinity
Test duration	96-hr	Start date 6/8/95
	Results:	Comments:
Mysids	0% dead in 100% sample	Diluted from 188 ppt to 35 ppt

Test 5a— To measure toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion) Note: The control in this test also tells if the incomplete ion adjustment left remaining toxicity

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (some ions >35 ppt)	35 ppt "salinity"
Test duration	96-hr	Start date 6/8/95
	Results:	Comments:
Mysids	25% dead in 100% sample; 0% dead in control	35 ppt plus some ions higher; remaining out-of-balance ions are not a cause of toxicity

Test 5b— To measure toxicity of concentrate after adjusting all major ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (diluted to 35 ppt)	35 ppt "salinity"
Test duration	96-hr	Start date 6/8/95
	Results:	Comments:
Mysids	0% dead in 100% sample; 0% dead in control	Diluted from 188 ppt to 35 ppt

General Comments:

CaCO3 precipitated in prep of mock concentrate. Reduced Ca relative to concentrate could result in lack of toxicity in mock concentrate, incorrectly indicating no major-seawater-ion toxicity.

Found that antiscalant was resulting in calcium supersaturation. Antiscalant tested and found nontoxic. It's addition to mock conc resulted in CaCO3 staying in solution. Resulting mock conc w/antiscalant (see chem data) showed no toxicity.

Interpretation:

No toxicity from major seawater ions. Unidentified toxicant source of toxicity.

Jupiter RO 6/6/95

Concentrates

Component name	mg/L		% of		Mock conc		% diff	
	Concentrate	35 SW	Mock conc	from conc	w/antiscalant	from conc		
Calcium	443	105.9%	398	-10.2%	465	5.0%		
Magnesium	689	53.7%	697	1.2%	701	1.7%		
Potassium	196	49.1%	199	1.5%	204	4.1%		
Sodium	5667	52.6%	5720	0.9%	5990	5.7%		
Alkalinity	554	536.4%	430	-22.4%	543	-2.0%		
Bicarbonate	675.88	536.4%	524.6	-22.4%	662.46	-2.0%		
Bromide	32	47.5%	32	0.0%	31	-3.1%		
Chloride	9700	50.1%	10000	3.1%	9900	2.1%		
Sulfate	1800	66.4%	1800	0.0%	1800	0.0%		

Ion-adjusted concentrates

	ion-adj		% of		Mock ion-adj		% diff		ion-adj		% of		Mock ion-adj		% of	
	conc/B	35 SW	conc/B	35 SW	conc/B	from i-a conc			conc A	35 SW	conc A	35 SW	conc A	35 SW		
Calcium	417	99.6%	414	98.9%	414	-0.7%			459	109.7%	388	92.7%	388	92.7%		
Magnesium	1270	98.9%	1270	98.9%	1270	0.0%			1300	101.2%	1260	98.1%	1260	98.1%		
Potassium	397	99.5%	398	99.7%	398	0.3%			405	101.5%	398	99.7%	398	99.7%		
Sodium	11000	102.0%	11000	102.0%	11000	0.0%			11100	103.0%	11000	102.0%	11000	102.0%		
Alkalinity	94	91.0%	92	89.1%	92	-2.1%			540	522.9%	434	420.2%	434	420.2%		
Bicarbonate	114.68	91.0%	112.24	89.1%	112.24	-2.1%			658.8	522.9%	529.48	420.2%	529.48	420.2%		
Bromide	64	95.1%	66	98.1%	66	3.1%			66	98.1%	65	96.6%	65	96.6%		
Chloride	20000	103.3%	20000	103.3%	20000	0.0%			19000	98.2%	20000	103.3%	20000	103.3%		
Sulfate	2800	103.2%	2700	99.6%	2700	-3.6%			2800	103.2%	2700	99.6%	2700	99.6%		

Seawaters

	Seawater		% of	
	diluent	35 SW		
Calcium	429	102.5%		
Magnesium	1320	102.8%		
Potassium	403	101.0%		
Sodium	11300	104.8%		
Alkalinity	127	123.0%		
Bicarbonate	154.94	123.0%		
Bromide	68	101.0%		
Chloride	20000	103.3%		
Sulfate	2800	103.2%		

Toxicity Test Results

Date sampled
3/20/95

Facility Name
Sailfish Point

NH3+NH4 = 1.83 mg/L
Fluoride = 2.5 mg/L (slight interference, actual amount may be somewhat greater)

Test 1— To measure: toxicity as discharged

Sample	Concentrate	8 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	20 ppt salinity
Test duration	96-hr Start date: 3/21/95	
	Results:	Comments:
Mysids	85% dead in 100% sample	8 ppt to 34 ppt salinity gradient
Menidia	0% dead in 100% sample	8 ppt to 34 ppt salinity gradient

Test 2— To measure: baseline toxicity of concentrate

Sample	Concentrate	8 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	8 ppt salinity
Test duration	96-hr Start date: 3/22/95	
	Results:	Comments:
Mysids	85% dead in 100% sample	no salinity gradient

Test 3— To measure: toxicity of mock concentrate

Sample	Mock concentrate	8 ppt "salinity"
Diluent/Control	Natural seawater	8 ppt salinity
Test duration	96-hr Start date: 3/22/95	
	Results:	Comments:
Mysids	20% dead in 100% sample	

Test 4a— To measure: toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion)

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater	34 ppt salinity
Test duration	96-hr Start date: 3/22/95	
	Results:	Comments:
Mysids	5% dead in 100% sample	35 ppt plus some ions higher

Test 4b— To measure: toxicity of concentrate after adjusting ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	34 ppt salinity
Test duration	96-hr Start date: 3/22/95	
	Results:	Comments:
Mysids	0% dead in 100% sample	Diluted from 51.5 ppt to 35 ppt

Test 5a— To measure: toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion)

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate	35 ppt "salinity"
Test duration	96-hr Start date: 3/22/95	
	Results:	Comments:
Mysids	0% dead in 100% sample; 0% dead in control*	35 ppt plus some ions higher

Test 5b— To measure: toxicity of concentrate after adjusting ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate	35 ppt "salinity"
Test duration	96-hr Start date: 3/22/95	
	Results:	Comments:
Mysids	0% dead in 100% sample; 0% dead in control	Diluted from 51.5 ppt to 35 ppt

General Comments:

* Test 5a—in 1 rep of control all dead.
Probable contamination. pH consistent across all tests.

Interpretation:

Main cause of toxicity is major-seawater-ion toxicity. Some unidentified toxicant is also present.

Sailfish Point 3/20/95

Concentrates

Component name	mg/L	% of		% diff
		Concentrate	35 SW	
Calcium	374	89.4%	374	0.0%
Magnesium	365	28.4%	361	-1.1%
Potassium	84.2	21.1%	83.2	-1.2%
Sodium	2310	21.4%	2080	-10.5%
Alkalinity	305	—	136	-76.6%
Carbonate	186.05	147.7%	82.96	-76.6%
Bromide	13	19.3%	13	0.0%
Chloride	3800	19.6%	3900	2.6%
Sulfate	1400	51.6%	1500	6.9%

Ion-adjusted
concentrates

	ion-adj conc/B	% of 35 SW	Mock ion-adj conc/B	% of 35 SW	conc's %diff	ion-adj		% of	
						conc A	conc A	35 SW	35 SW
Calcium	400	95.6%	408	97.5%	2.0%	391	410	93.4%	98.0%
Magnesium	1240	96.6%	1230	95.8%	0.8%	1230	1240	95.8%	96.6%
Potassium	388	97.2%	388	97.2%	0.0%	383	392	96.0%	98.2%
Sodium	10500	97.4%	10700	99.3%	1.9%	10600	10800	98.3%	100.2%
Alkalinity	197	—	96.8	—	68.2%	299	145	—	—
Carbonate	120.17	95.4%	59.048	46.9%	68.2%	182.39	88.45	144.8%	70.2%
Bromide	70	104.0%	68	101.0%	2.9%	70	69	104.0%	102.5%
Chloride	19000	98.2%	19000	98.2%	0.0%	19000	19000	98.2%	98.2%
Sulfate	2600	95.9%	2700	99.6%	3.8%	2700	2800	99.6%	103.2%

Seawaters

	% of	
	Seawater diluent	35 SW
Calcium	429	102.5%
Magnesium	1310	102.0%
Potassium	398	99.7%
Sodium	10800	100.2%
Alkalinity	126	—
Carbonate	76.86	61.0%
Bromide	69	102.5%
Chloride	20000	103.3%
Sulfate	2300	84.8%

Toxicity Test Results

Date sampled
4/24/95

Facility Name
Sailfish Point

NH3+NH4 = 1.97 mg/L
Fluoride = 2.3 mg/L

Test 1— To measure toxicity as discharged

Sample	Concentrate	8 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	20 ppt salinity
Test duration	96-hr	Start date 4/25/95
	Results:	Comments:
Mysids	90% dead in 100% sample	20 ppt to 8 ppt gradient
Menidia	5% dead in 100% sample	20 ppt to 8 ppt gradient

Test 2— To measure baseline toxicity of concentrate

Sample	Concentrate	8 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	8 ppt salinity
Test duration	96-hr	Start date 4/26/95
	Results:	Comments:
Mysids	80% dead in 100% sample	no salinity gradient

Test 3— To measure toxicity of mock concentrate

Sample	Mock concentrate	8 ppt "salinity"
Diluent/Control	Natural seawater	8 ppt salinity
Test duration	96-hr	Start date 4/26/95
	Results:	Comments:
Mysids	45% dead in 100% sample	

Test 4a— To measure toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion)

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater	35 ppt salinity
Test duration	96-hr	Start date 4/26/95
	Results:	Comments:
Mysids	0% dead in 100% sample	35 ppt plus some ions higher

Test 4b— To measure toxicity of concentrate after adjusting ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	35 ppt salinity
Test duration	96-hr	Start date 4/26/95
	Results:	Comments:
Mysids	0% dead in 100% sample	Diluted from 104 ppt to 35 ppt

Test 5a— To measure toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion) Note: The control in this test also tells if the incomplete ion adjustment left remaining toxicity

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (some ions >35 ppt)	35 ppt salinity
Test duration	96-hr	Start date 4/26/95
	Results:	Comments:
Mysids	0% dead in 100% sample; 0% dead in control	35 ppt plus some ions higher; remaining out-of-balance ions are not a cause of toxicity

Test 5b— To measure toxicity of concentrate after adjusting all major ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (diluted to 35 ppt)	35 ppt salinity
Test duration	96-hr	Start date 4/26/95
	Results:	Comments:
Mysids	0% dead in 100% sample	Diluted from 104 ppt to 35 ppt

General Comments:

Interpretation:

Major-seawater-ion toxicity and possible unidentified toxicity source

RQ#: RQ-95-Apr-24-11

4/24/95

Sailfish Point

Concentrates

Component name

mg/L	% of		% diff	
Concentrate	35 SW	Mock conc	from conc	
Calcium	349	338	-3.2%	
Magnesium	351	359	2.3%	
Potassium	77.9	83.8	7.6%	
Sodium	2330	2260	-3.0%	
Alkalinity	308	289	-6.2%	
Bicarbonate	375.76	352.58	-6.2%	
Bromide	14	14	0.0%	
Chloride	3800	3800	0.0%	
Sulfate	1400	1400	0.0%	

Ion-adjusted
concentrates

ion-adj conc/B	% of 35 SW	Mock ion-adj conc/B	% of 35 SW	% diff from i-a conc		ion-adj conc A	% of 35 SW	Mock ion-adj conc A	% of 35 SW	conc's %diff
411	98.2%	402	96.1%	-2.2%		424	101.3%	396	94.6%	6.8%
1280	99.7%	1260	98.1%	-1.6%		1290	100.5%	1270	98.9%	1.6%
412	103.3%	408	102.3%	-1.0%		415	104.0%	409	102.5%	1.5%
11100	103.0%	11000	102.0%	-0.9%		11000	102.0%	11000	102.0%	0.0%
92.9	90.0%	101	97.8%	8.7%		283	274.0%	305	295.3%	7.5%
113.338	90.0%	123.22	97.8%	8.7%		345.26	274.0%	372.1	295.3%	7.5%
66	98.1%	66	98.1%	0.0%		65	96.6%	66	98.1%	1.5%
20000	103.3%	19000	98.2%	-5.0%		20000	103.3%	19000	98.2%	5.1%
2700	99.6%	2600	95.9%	-3.7%		2700	99.6%	2700	99.6%	0.0%

Seawater diluent	% of 35 SW
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Seawaters

Calcium	410	98.0%
Magnesium	1290	100.5%
Potassium	400	100.3%
Sodium	11000	102.0%
Alkalinity	128	123.9%
Bicarbonate	156.16	123.9%
Bromide	67	99.6%
Chloride	20000	103.3%
Sulfate	2400	88.5%

Toxicity Test Results

Date sampled
5/30/95

Facility Name
Sailfish Point RO

NH3+NH4 = 1.90 mg/L
Fluoride = 2.0 J mg/L

Test 1— To measure toxicity as discharged

Sample	Concentrate	8.2 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	20 ppt salinity
Test duration	96-hr	Start date 5/31/95
	Results:	Comments:
Mysids	5% dead in 50% sample; 100% dead in 100% sample	8.2 ppt to 20 ppt gradient
Menidia	0% dead in 100% sample	8.2 ppt to 20 ppt gradient

Test 2— To measure baseline toxicity of concentrate

Sample	Concentrate	8.2 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	8 ppt salinity
Test duration	96-hr	Start date 6/2/95
	Results:	Comments:
Mysids	90% dead in 100% sample	no salinity gradient

Test 3— To measure toxicity of mock concentrate

Sample	Mock concentrate	8.2 ppt "salinity"
Diluent/Control	Natural seawater	8 ppt salinity
Test duration	96-hr	Start date 6/2/95
	Results:	Comments:
Mysids	40% dead in 100% sample	

Test 4a— To measure toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion)

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater	35 ppt salinity
Test duration	96-hr	Start date 6/2/95
	Results:	Comments:
Mysids	0% dead in 100% sample	35 ppt plus some ions higher

Test 4b— To measure toxicity of concentrate after adjusting ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	35 ppt salinity
Test duration	96-hr	Start date 6/2/95
	Results:	Comments:
Mysids	0% dead in 100% sample	Diluted from 112 ppt to 35 ppt

Test 5a— To measure toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion) Note: The control in this test also tells if the incomplete ion adjustment left remaining toxicity

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (some ions >35 ppt)	35 ppt "salinity"
Test duration	96-hr	Start date 6/2/95
	Results:	Comments:
Mysids	0% dead in 100% sample; 0% dead in control	35 ppt plus some ions higher; remaining out-of-balance ions are not a cause of toxicity

Test 5b— To measure toxicity of concentrate after adjusting all major ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (diluted to 35 ppt)	35 ppt "salinity"
Test duration	96-hr	Start date 6/2/95
	Results:	Comments:
Mysids	0% dead in 100% sample; 0% dead in control	Diluted from 112 ppt to 35 ppt

General Comments:

Interpretation:

Both major-seawater-ion and unidentified toxicity source present

Sailfish Point RO

5/30/95

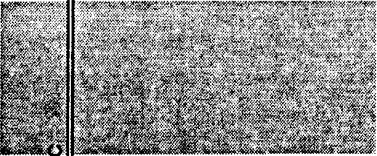
RQ#: RQ-95-May-29-05

Concentrates

Component name	mg/L		% of		% diff	
	Concentrate	35 SW	Mock conc	from conc		
Calcium	350	83.6%	308	-12.0%		
Magnesium	360	28.0%	381	5.8%		
Potassium	81.2	20.4%	80.4	-1.0%		
Sodium	2360	21.9%	2410	2.1%		
Alkalinity	330	319.5%	273	-17.3%		
Bicarbonate	402.6	319.5%	333.06	-17.3%		
Bromide	14.2	21.1%	14	-1.4%		
Chloride	4100	21.2%	4200	2.4%		
Sulfate	1400	51.6%	1400	0.0%		

Ion-adjusted
concentrates

	ion-adj		% of		% diff		ion-adj conc A	% of		Mock ion-adj conc A	% of	
	conc/B	35 SW	Mock ion-adj conc/B	35 SW	from i-a conc			35 SW			35 SW	
Calcium	435	103.9%	430	102.7%	-1.1%		443	105.9%	405	96.8%		
Magnesium	1280	99.7%	1270	98.9%	-0.8%		1290	100.5%	1280	99.7%		
Potassium	396	99.2%	396	99.2%	0.0%		398	99.7%	394	98.7%		
Sodium	10800	100.2%	10900	101.1%	0.9%		11000	102.0%	10800	100.2%		
Alkalinity	102	98.8%	90.9	88.0%	-10.9%		323	312.7%	307	297.3%		
Bicarbonate	124.44	98.8%	110.898	88.0%	-10.9%		394.06	312.7%	374.54	297.3%		
Bromide	68	101.0%	72	107.0%	5.9%		70	104.0%	67	99.6%		
Chloride	18000	93.0%	19000	98.2%	5.6%		19000	98.2%	19000	98.2%		
Sulfate	2600	95.9%	2700	99.6%	3.8%		2600	95.9%	2600	95.9%		



Seawaters

	Seawater diluent		% of	
		35 SW		
Calcium	430	102.7%		
Magnesium	1270	98.9%		
Potassium	396	99.2%		
Sodium	10900	101.1%		
Alkalinity	129	124.9%		
Bicarbonate	157.38	124.9%		
Bromide	76	112.9%		
Chloride	20000	103.3%		
Sulfate	2300	84.8%		

Toxicity Test Results

Date sampled
2/27/95

Facility Name
Venice RO

NH3+NH4 =<0.017 mg/L
Fluoride = 3.2 mg/L

Test 1— To measure: toxicity as discharged

Sample	Concentrate	3.2 ppt "salinity" adjusted to 5 ppt with sea salts
Diluent/Control	Natural seawater (NSW)	20 ppt salinity
Test duration	96-hr <i>Start date: 2/28/95</i>	
	Results:	Comments:
Mysids	50% dead in 50% sample; 100% dead in 100% sample	5 ppt to 20 ppt gradient; 5 ppt & 20 ppt controls
Menidia	0% dead in 100% sample	5 ppt to 20 ppt gradient; 5 ppt & 20 ppt controls

Test 2— To measure: baseline toxicity of concentrate

Sample	Concentrate	3.2 ppt "salinity" adjusted to 5 ppt with mock sea salts
Diluent/Control	Natural seawater (NSW)	5 ppt salinity
Test duration	48-hr <i>Start date: 3/1/95</i>	
	Results:	Comments:
Mysids	95% dead in 50% sample; 100% dead in 100% sample	no salinity gradient

Test 3— To measure: toxicity of mock concentrate

Sample	Mock concentrate	3.2 ppt "salinity" adjusted to 5 ppt with mock sea salts
Diluent/Control	Natural seawater	5 ppt salinity
Test duration	48-hr <i>Start date: 3/1/95</i>	
	Results:	Comments:
Mysids	95% dead in 50% sample; 100% dead in 100% sample	

Test 4a— To measure: toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion)

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater	35 ppt salinity
Test duration	48-hr <i>Start date: 3/1/95</i>	
	Results:	Comments:
Mysids	0% dead in 100% sample	35 ppt plus some ions higher

Test 4b— To measure: toxicity of concentrate after adjusting ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	35 ppt salinity
Test duration	48-hr <i>Start date: 3/1/95</i>	
	Results:	Comments:
Mysids	0% dead in 100% sample	Diluted from 62 ppt to 35 ppt

Test 5a— To measure: toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion)

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (some ions >35ppt)	35 ppt salinity
Test duration	48-hr <i>Start date: 3/1/95</i>	
	Results:	Comments:
Mysids	0% dead in 100% sample; 0% dead in control	35 ppt plus some ions higher. Remaining out-of-balance ions are not a cause of toxicity

Test 5b— To measure: Toxicity of concentrate after adjusting all major ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (diluted to 35 ppt)	35 ppt salinity
Test duration	48-hr <i>Start date: 3/1/95</i>	
	Results:	Comments:
Mysids	0% dead in 100% sample; 0% dead in control	Diluted from 62 ppt to 35 ppt

General Comments:

Precipitate formed when salinity adjusting concentrate. Required pH manipulation to create mock concentrate. Mock concentrate poor duplicate of concentrate.

Interpretation:

Probable major-seawater-ion toxicity. No sign of unidentified toxicants.

Venice RO

2/27/95

Concentrates

Component name	mg/L Concentrate	% of		sea salt		mock slurry	
		35 SW	% diff	sal adj	% of	sal adj	% of
			from conc	conc	5 SW	conc	5 SW
Calcium	724	173.0%	-0.3%	853	1426.8%	846	1415.1%
Magnesium	281	21.9%	-0.7%	368	200.6%	386	210.4%
Potassium	14.3	3.6%	37.5%	32.2	56.5%	20.8	36.5%
Sodium	354	3.3%	52.0%	983	63.8%	931	60.5%
Alkalinity	232	—	-65.1%	242	—	254	—
Carbonate	141.52	112.3%	-65.1%	147.62	820.1%	154.94	860.8%
Bromide	3.2	4.8%	125.6%	5.6	58.2%	5.9	61.4%
Chloride	860	4.4%	15.1%	1800	65.1%	1800	65.1%
Sulfate	2600	95.9%	0.0%	2700	696.9%	2700	696.9%

Ion-adjusted
concentrates

concentrates	ion-adj	% of	Mock ion-adj	% of	conc's	ion-adj	% of	Mock ion-adj
	conc/B	35 SW	conc/B	35 SW	%diff	conc A	35 SW	conc A
Calcium	437	104.4%	397	94.9%	9.6%	780	186.4%	
Magnesium	1270	98.9%	1230	95.8%	3.2%	1290	100.5%	
Potassium	378	94.7%	387	97.0%	2.4%	387	97.0%	*insufficient
Sodium	10600	98.3%	10700	99.3%	0.9%	11000	102.0%	sample,
Alkalinity	172	—	101	—	52.0%	240	—	broken
Carbonate	104.92	83.3%	61.61	48.9%	52.0%	146.4	116.2%	shipping
Bromide	69	102.5%	61	90.6%	12.3%	69	102.5%	container
Chloride	20000	103.3%	19000	98.2%	5.1%	19000	98.2%	
Sulfate	2700	99.6%	2700	99.6%	0.0%	2700	99.6%	

Double NaHCO3
pH adjustment
added to mock conc
because HCO3
with HCl
gassed off at pH 2.
and NaOH
Accounts for part of
contributed
excess Na.
to excess
Na and Cl

Toxicity Test Results

Date sampled
5/15/95

Facility Name
Venice RO

NH3+NH4 = .41 mg/L
Fluoride = 2.7J mg/L

Test 1— To measure toxicity as discharged

Sample	Concentrate	3.7 ppt "salinity" adjusted to 6 ppt for mysid tests
Diluent/Control	Natural seawater (NSW)	20 ppt salinity
Test duration	96-hr	Start date 5/16/95
	Results:	Comments:
Mysids	100% dead in 50% sample; 100% dead in 100% sample	20 ppt to 6 ppt gradient
Menidia	10% dead in 50% sample; 10% dead in 100% sample	20 ppt to 3.7 ppt gradient; mortality same as low-salinity control

Test 2— To measure baseline toxicity of concentrate

Sample	Concentrate	6 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	6 ppt salinity
Test duration	96-hr	Start date 5/17/95
	Results:	Comments:
Mysids	50% dead in 25% sample; 100% dead in 50% sample; 100% dead in 100% sample	no salinity gradient

Test 3— To measure toxicity of mock concentrate

Sample	Mock concentrate	6 ppt "salinity"
Diluent/Control	Natural seawater	6 ppt salinity
Test duration	96-hr	Start date 5/17/95
	Results:	Comments:
Mysids	50% dead in 25% sample; 100% dead in 50% sample; 100% dead in 100% sample	

Test 4a— To measure toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion)

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater	35 ppt salinity
Test duration	96-hr	Start date 5/17/95
	Results:	Comments:
Mysids	0% dead in 100% sample	35 ppt plus some ions higher

Test 4b— To measure toxicity of concentrate after adjusting ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	35 ppt salinity
Test duration	96-hr	Start date 5/17/95
	Results:	Comments:
Mysids	0% dead in 100% sample	Diluted from 76 ppt to 35 ppt

Test 5a— To measure toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion) Note: The control in this test also tells if the incomplete ion adjustment left remaining toxicity

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (some ions >35 ppt)	35 ppt "salinity"
Test duration	96-hr	Start date 5/17/95
	Results:	Comments:
Mysids	5% dead in 100% sample; 0% dead in control	35 ppt plus some ions higher; remaining out-of-balance ions are not a cause of toxicity

Test 5b— To measure toxicity of concentrate after adjusting all major ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (diluted to 35 ppt)	35 ppt "salinity"
Test duration	96-hr	Start date 5/17/95
	Results:	Comments:
Mysids	0% dead in 100% sample	Diluted from 76 ppt to 35 ppt

General Comments:

Interpretation:

Toxicity caused by major-seawater-ion imbalance

Chemistry Analyses

Component name	mg/L	% of				% diff		sal adj		% of		sal adj		% diff	
		Concentrate	35 SW	Mock conc	from conc.	% diff	from conc.	conc	5 SW	mock conc	from sal adj	conc	5 SW	mock conc	from sal adj
Calcium		913	218.2%	966	5.8%			856	1431.8%	911	6.4%				
Magnesium		326	25.4%	326	0.0%			408	222.4%	391	-4.2%				
Potassium		13.2	3.3%	12.4	-6.1%			45.3	79.5%	32.8	-27.6%				
Sodium		433	4.0%	857	97.9%			1130	73.4%	1300	15.0%				
Alkalinity		191	184.9%	184	-3.7%			202	1369.1%	195	-3.5%				
Bicarbonate		233.02	184.9%	224.48	-3.7%			246.44	1369.1%	237.9	-3.5%				
Bromide		2.6	3.9%	2.5	-3.8%			8.1	84.2%	5.5	-32.1%				
Chloride		820	4.2%	1600	95.1%			2300	83.2%	2300	0.0%				
Sulfate		2800	103.2%	2700	-3.6%			3100	800.1%	2700	-12.9%				

Ion-adjusted concentrates

	ion-adj conc/B	% of		Mock ion-adj conc/B	% of		% diff from i-a conc	ion-adj conc A	% of		Mock ion-adj conc A	% of	
		35 SW	35 SW		35 SW	35 SW			35 SW	35 SW		35 SW	35 SW
Calcium	374	89.4%		412	98.4%	10.2%		855	204.3%		919	219.6%	
Magnesium	1250	97.4%		1250	97.4%	0.0%		1280	99.7%		1260	98.1%	
Potassium	396	99.2%		395	99.0%	-0.3%		401	100.5%		398	99.7%	
Sodium	11000	102.0%		11300	104.8%	2.7%		10900	101.1%		11300	104.8%	
Alkalinity	97.7	94.6%		102	98.8%	4.4%		203	196.6%		186	180.1%	
Bicarbonate	119.194	94.6%		124.44	98.8%	4.4%		247.66	196.6%		226.92	180.1%	
Bromide	65	96.6%		64	95.1%	-1.5%		62	92.1%		62	92.1%	
Chloride	18000	93.0%		18000	93.0%	0.0%		19000	98.2%		19000	98.2%	
Sulfate	2500	92.2%		2500	92.2%	0.0%		2600	95.9%		2600	95.9%	

Seawaters

Seawater		% of	
diluent	35 SW		
Calcium	432	103.2%	
Magnesium	1310	102.0%	
Potassium	396	99.2%	
Sodium	11100	103.0%	
Alkalinity	126	122.0%	
Bicarbonate	153.72	122.0%	
Bromide	64	95.1%	
Chloride	19000	98.2%	
Sulfate	2400	88.5%	

Toxicity Test Results

Date sampled
6/12/95

Facility Name
Venice RO

NH3+NH4 = 0.46 mg/L
Fluoride = 2.9 J mg/L

Test 1— To measure toxicity as discharged

Sample	Concentrate	3.6 ppt "salinity", raised to 6 ppt for mysid test
Diluent/Control	Natural seawater (NSW)	20 ppt salinity
Test duration	96-hr	Start date 6/13/95
	Results:	Comments:
Mysids	85% dead in 50% sample; 100% dead in 100% sample	6 ppt to 20 ppt gradient
Menidia	0% dead in 100% sample	3.6 ppt to 20 ppt gradient

Test 2— To measure baseline toxicity of concentrate

Sample	Concentrate	3.6 ppt "salinity" raised to 6 ppt
Diluent/Control	Natural seawater (NSW)	6 ppt salinity
Test duration	96-hr	Start date 6/14/95
	Results:	Comments:
Mysids	95% dead in 50% sample; 100% dead in 100% sample	no salinity gradient

Test 3— To measure toxicity of mock concentrate

Sample	Mock concentrate	3.6 ppt "salinity" raised to 6 ppt
Diluent/Control	Natural seawater	6 ppt salinity
Test duration	96-hr	Start date 6/14/95
	Results:	Comments:
Mysids	100% dead in 50% sample; 100% dead in 100% sample	

Test 4a— To measure toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion)

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater	35 ppt salinity
Test duration	96-hr	Start date 6/14/95
	Results:	Comments:
Mysids	20% dead in 100% sample	35 ppt plus some ions higher; Test 5a control indicates remaining out-of-balance ions may be a cause of part of toxicity.

Test 4b— To measure toxicity of concentrate after adjusting ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	35 ppt salinity
Test duration	96-hr	Start date 6/14/95
	Results:	Comments:
Mysids	0% dead in 100% sample	Diluted from 75 ppt to 35 ppt

Test 5a— To measure toxicity of concentrate after adjusting ions; some ions remain out-of-balance (>35-ppt proportion) Note: The control in this test also tells if the incomplete ion adjustment left remaining toxicity

Sample	ion-adj conc (some ions >35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (some ions >35 ppt)	35 ppt "salinity"
Test duration	96-hr	Start date 6/14/95
	Results:	Comments:
Mysids	15% dead in 100% sample; 5% dead in control	35 ppt plus some ions higher; remaining out-of-balance ions may be a cause of part of toxicity.

Test 5b— To measure toxicity of concentrate after adjusting all major ions; diluted with DI water to 35 ppt

Sample	ion-adj conc (diluted to 35 ppt)	35 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate (diluted to 35 ppt)	35 ppt "salinity"
Test duration	96-hr	Start date 6/14/95
	Results:	Comments:
Mysids	0% dead in 100% sample; 0% dead in control	Diluted from 75 ppt to 35 ppt

General Comments:

Na & Cl concentrations somewhat high in mock concentrate prior to salinity adjustment. Shouldn't affect results.

Interpretation:

Primarily major seawater ion toxicity. Possible minor toxicity from other unidentified source(s) indicated.

Venice RO 6/12/95

Concentrates
Component name

mg/L Concentrate	% of 35 SW	% diff		sea salt		% of 5 SW		mock slurry		% diff from mock slurry	
		Mock conc	from conc	sal adj conc		5 SW	sal adj conc	mock conc	mock slurry		
895	213.9%	924	3.2%			0.0%	892	853	-4.4%		
329	25.6%	335	1.8%			0.0%	495	437	-11.7%		
14	3.5%	11.4	-18.6%			0.0%	57.8	40	-30.8%		
365	3.4%	635	74.0%			0.0%	1190	1260	5.9%		
188	182.0%	187	-0.5%			0.0%	195	190	-2.6%		
229.36	182.0%	228.14	-0.5%	0		0.0%	237.9	231.8	-2.6%		
2.6	3.9%	2.4	-7.7%			0.0%	9.3	65	598.9%		
830	4.3%	1200	44.6%			0.0%	2200	2300	4.5%		
2900	106.9%	2900	0.0%			0.0%	3100	2900	-6.5%		

Ion-adjusted
concentrates

ion-adj conc/B	% of 35 SW	Mock ion-adj conc/B	% of 35 SW	% diff from i-a conc	ion-adj conc A	% of 35 SW	Mock ion-adj conc A	% of 35 SW
378	90.3%	396	94.6%	4.8%	809	193.3%	859	205.3%
1210	94.2%	1270	98.9%	5.0%	1290	100.5%	1310	102.0%
362	90.7%	379	95.0%	4.7%	388	97.2%	390	97.7%
10000	92.8%	10700	99.3%	7.0%	10700	99.3%	11000	102.0%
92.9	90.0%	93.3	90.3%	0.4%	191	184.9%	191	184.9%
113.338	90.0%	113.826	90.3%	0.4%	233.02	184.9%	233.02	184.9%
60	89.2%	62	92.1%	3.3%	66	98.1%	64	95.1%
18000	93.0%	19000	98.2%	5.6%	20000	103.3%	20000	103.3%
2500	92.2%	2600	95.9%	4.0%	2800	103.2%	2800	103.2%

Seawaters

Seawater diluent	% of 35 SW
Calcium	405 96.8%
Magnesium	1320 102.8%
Potassium	385 96.5%
Sodium	10900 101.1%
Alkalinity	127 123.0%
Bicarbonate	154.94 123.0%
Bromide	65 96.6%
Chloride	20000 103.3%
Sulfate	2600 95.9%

Toxicity Test Results

Date sampled
 2/20/95

Facility Name
 Vero Beach

NH3+NH4 =1.07 mg/L
 Fluoride = 4.2 mg/L

Test 1— To measure: toxicity as discharged

Sample	Concentrate	4.2 ppt "salinity" adjusted to 5 ppt with sea salts
Diluent/Control	Natural seawater (NSW)	20 ppt salinity
Test duration	96-hr	Start date: 2/21/95
	Results:	Comments:
Mysids	20% dead in 50% sample; 100% dead in 100% sample	5 ppt to 20 ppt gradient; 2 cntrls; OK at 20 ppt, 80% dead at 5 ppt
Menidia	45% dead in 100% sample	5 ppt to 20 ppt gradient; 15% dead in controls

Test 2— To measure: baseline toxicity of concentrate

Sample	Concentrate	4.2 ppt "salinity" adjusted to 5 ppt with mock sea salts
Diluent/Control	Natural seawater (NSW)	5 ppt salinity
Test duration	72-hr	Start date: 2/22/95
	Results:	Comments:
Mysids	90% dead in 100% sample	no salinity gradient; 25% dead in control

Test 3— To measure: toxicity of mock concentrate

Sample	Mock concentrate	4.2 ppt "salinity" adjusted to 5 ppt with mock sea salts
Diluent/Control	Natural seawater	5 ppt salinity
Test duration	72-hr	Start date: 2/22/95
	Results:	Comments:
Mysids	70% dead in 100% sample	Control OK

Test 4— To measure: toxicity of concentrate after adjusting ions to seawater proportions; natural seawater diluent

Sample	ion-adusted concentrate	26 ppt "salinity"
Diluent/Control	Natural seawater	26 ppt salinity
Test duration	72-hr	Start date: 2/22/95
	Results:	Comments:
Mysids	50% dead in 100% sample	Control OK

Test 5— To measure: toxicity of concentrate after adjusting ions to seawater proportions; synthetic diluent

Sample	ion-adusted concentrate	26 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate	26 ppt salinity
Test duration	72-hr	Start date: 2/22/95
	Results:	Comments:
Mysids	75% dead in 100% sample; 0% dead in control	Control OK

General Comments:
 Problems with random test-organism mortality. Suspect damage from probes inserted to measure phys-chem parameters in small (200 mL) test volumes. Measurements now taken only before and after tests.

Interpretation:
 Na & Cl are low because of calc error in original spreadsheet template. Test conclusions not reliable.

Chemistry Analyses

Vero Beach 2/20/95

Concentrates

Component name	mg/L Concentrate	% of 35 SW	Mock conc	% diff from conc	sal adj conc	% of 5 SW	Seawaters			
							35 ppt salt slurry # 1	35 ppt salt slurry # 2	% diff	% of 35 SW
00915Calcium	312	74.6%	346	10.9%	328	548.6%	385	370	-3.9%	88.4%
00925Magnesium	300	23.4%	340	13.3%	328	178.8%	1160	1130	-2.6%	88.0%
00935Potassium	65.1	16.3%	70.3	8.0%	68.9	120.9%	122	120	-1.6%	30.1%
00930Sodium	1040	9.6%	1320	26.9%	1260	81.8%	10000	9890	-1.1%	91.7%
00410Alkalinity	80	—	45.4	-43.3%	82.8	—	84.4	91.9	8.9%	—
Carbonate	48.8	38.7%	27.694	-43.3%	50.508	280.6%	51.484	56.059	8.9%	44.5%
Bromide	6.2	9.2%	8.2	32.3%	7.9	82.2%	63	62	-1.6%	92.1%
Chloride	1800	9.3%	2500	38.9%	2300	83.2%	18000	18000	0.0%	93.0%
Sulfate	1500	55.3%	1600	6.7%	1500	387.2%	2200	2200	0.0%	81.1%

Ion-adjusted concentrates

	ion-adj conc/B	% of 26.1 SW	Mock ion-adj conc/B	% of 26.1 SW	conc's %diff	
00915Calcium	300	96.2%	315	101.0%	5.0%	
00925Magnesium	942	98.4%	943	98.5%	0.1%	
00935Potassium	295	99.2%	295	99.2%	0.0%	
00930Sodium	7030	87.5%	7060	87.8%	0.4%	
00410Alkalinity	113	—	82.4	—	-27.1%	
Carbonate	68.93	73.4%	50.264	53.5%	-27.1%	
Bromide	55	109.6%	54	107.6%	-1.8%	
Chloride	13000	90.1%	13000	90.1%	0.0%	
Sulfate	1900	94.0%	2000	98.9%	5.3%	

**Na & Cl are low in ion adj conc because of calc error in original spreadsheet template

Toxicity Test Results

Date sampled
4/17/95

Facility Name
Vero Beach RO

NH3+NH4 = 1.07 mg/L

Test 1— To measure toxicity as discharged

Sample	Concentrate	3 ppt "salinity" (adjusted to 6 for mysids)
Diluent/Control	Natural seawater (NSW)	20 ppt salinity
Test duration	96-hr	Start date 4/18/95
	Results:	Comments:
Mysids	75% dead in 100% sample	20 ppt to 6 ppt gradient
Menidia	0% dead in 100% sample	20 ppt to 3 ppt gradient

Test 2— To measure baseline toxicity of concentrate

Sample	Concentrate	6 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	6 ppt salinity
Test duration	96-hr	Start date 4/19/95
	Results:	Comments:
Mysids	35% dead in 50% sample; 90% dead in 100% sample	no salinity gradient

Test 3— To measure toxicity of mock concentrate

Sample	Mock concentrate	6 ppt "salinity"
Diluent/Control	Natural seawater	6 ppt salinity
Test duration	96-hr	Start date 4/19/95
	Results:	Comments:
Mysids	0% dead in 100% sample	

Test 4— To measure toxicity of concentrate after adjusting ions to seawater proportions; natural seawater diluent

Sample	ion-adjusted concentrate	26 ppt "salinity"
Diluent/Control	Natural seawater	26 ppt salinity
Test duration	96-hr	Start date 4/19/95
	Results:	Comments:
Mysids	40% dead in 100% sample	

Test 5— To measure toxicity of concentrate after adjusting ions to seawater proportions; synthetic diluent

Sample	ion-adjusted concentrate	26 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate	26 ppt salinity
Test duration	96-hr	Start date 4/19/95
	Results:	Comments:
Mysids	55% dead in 100% sample	

General Comments:

Interpretation:

Toxicity resulting from unidentified toxicants

Vero Beach RO 4/17/95

Concentrates
Component name

mg/L	Concentrate	% of 35 SW	Mock conc	% diff from conc	sea salt sal adj conc	% of 5 SW	mock slurry sal adj conc	% of 5 SW	sal adj mock conc	% diff from sal ad conc
	265	63.3%	280	5.7%	284	475.0%	294	491.8%	308	8.1%
Calcium	240	18.7%	245	2.1%	316	172.3%	347	189.2%	357	12.2%
Magnesium	60.7	15.2%	62.5	3.0%	101	177.2%	92.5	162.3%	98	-3.0%
Potassium	673	6.2%	705	4.8%	1670	108.4%	1610	104.5%	1610	-3.7%
Sodium	76.1	73.7%	79.8	4.9%	82.8	561.2%	82.8	561.2%	86.9	4.8%
Alkalinity	92.842	73.7%	97.356	4.9%	101.016	561.2%	101.016	561.2%	106.018	4.8%
Bicarbonate	3.6	5.3%	4.1	13.9%	9.2	95.7%	9	93.6%	10	8.3%
Bromide	1100	5.7%	1100	0.0%	2700	97.7%	2700	97.7%	2800	3.6%
Chloride	1500	55.3%	1500	0.0%	1700	438.8%	1700	438.8%	1800	5.7%
Sulfate										

Ion-adjusted
concentrates

ion-adj conc	% of 26 SW	Mock ion-adj conc	% of 26 SW	% diff from i-a conc
291	94.4%	301	97.6%	3.4%
Calcium				
921	97.3%	913	96.5%	-0.9%
Magnesium				
287	97.6%	286	97.3%	-0.3%
Potassium				
8030	101.1%	8070	101.6%	0.5%
Sodium				
63.6	83.5%	75.1	98.7%	18.1%
Alkalinity				
77.592	83.5%	91.622	98.7%	18.1%
Bicarbonate				
52	104.9%	50	100.8%	-3.8%
Bromide				
15000	105.2%	14000	98.2%	-6.7%
Chloride				
2000	100.1%	1900	95.1%	-5.0%
Sulfate				

Seawaters

Seawater diluent	% of 26 SW
303	98.3%
Calcium	
944	99.8%
Magnesium	
283	96.3%
Potassium	
8230	103.6%
Sodium	
94.9	124.7%
Alkalinity	
115.778	124.7%
Bicarbonate	
51	102.8%
Bromide	
14000	98.2%
Chloride	
2100	105.1%
Sulfate	

Toxicity Test Results

Date sampled
5/8/95

Facility Name
Vero Beach RO

NH3+NH4 = 1.1 mg/L
Fluoride = 4.0 mg/L

Test 1— To measure toxicity as discharged

Sample	Concentrate	3 ppt "salinity" adjusted to 6 ppt for mysid tests
Diluent/Control	Natural seawater (NSW)	20 ppt salinity
Test duration	96-hr	Start date 5/9/95
	Results:	Comments:
Mysids	5% dead in 50% sample; 95% dead in 100% sample	20 ppt to 6 ppt gradient
Menidia	5% dead in 50% sample; 25% dead in 100% sample	20 ppt to 3 ppt gradient; mortality same as in low-salinity-control

Test 2— To measure baseline toxicity of concentrate

Sample	Concentrate	6 ppt "salinity"
Diluent/Control	Natural seawater (NSW)	6 ppt salinity
Test duration	96-hr	Start date 5/10/95
	Results:	Comments:
Mysids	30% dead in 50% sample; 100% dead in 100% sample	no salinity gradient

Test 3— To measure toxicity of mock concentrate

Sample	Mock concentrate	6 ppt "salinity"
Diluent/Control	Natural seawater	6 ppt salinity
Test duration	96-hr	Start date 5/10/95
	Results:	Comments:
Mysids	15% dead in 100% sample	

Test 4— To measure toxicity of concentrate after adjusting ions to seawater proportions; natural seawater diluent

Sample	ion-adjusted concentrate	25.3 ppt "salinity"
Diluent/Control	Natural seawater	25 ppt salinity
Test duration	96-hr	Start date 5/10/95
	Results:	Comments:
Mysids	10% dead in 50% sample; 60% dead in 100% sample	

Test 5— To measure toxicity of concentrate after adjusting ions to seawater proportions; synthetic diluent

Sample	ion-adjusted concentrate	25.3 ppt "salinity"
Diluent/Control	Mock ion-adj concentrate	25.3 ppt "salinity"
Test duration	96-hr	Start date 5/10/95
	Results:	Comments:
Mysids	65% dead in 100% sample	

General Comments:

Tox of mock conc possibly resulting from insufficient Br- in makeup (see Followup Chem).

Interpretation:

Some toxicity possibly from major seawater ions, most from other unidentified toxicants.

Chemistry Analyses

Vero Beach RO

5/8/95

Concentrates
Component name

mg/L Concentrate	% of 35 SW	Mock conc	% diff from conc	sea salt		% of 5 SW	sal adj mock conc	% diff from sal adj conc
				sal adj conc	conc			
Calcium	270	64.5%	265	-1.9%	286	478.4%	293	2.4%
Magnesium	244	19.0%	238	-2.5%	332	181.0%	351	5.7%
Potassium	61.4	15.4%	61.5	0.2%	90.1	158.1%	98.6	9.4%
Sodium	681	6.3%	691	1.5%	1580	102.6%	1550	-1.9%
Alkalinity	74.8	72.4%	75.8	1.3%	82.4	558.5%	86.9	5.5%
Bicarbonate	91.256	72.4%	92.476	1.3%	100.528	558.5%	106.018	5.5%
Bromide	3.5	5.2%	2.3	-34.3%	7.7	80.1%	8.1	5.2%
Chloride	1100	5.7%	1200	9.1%	2700	97.7%	2600	-3.7%
Sulfate	1520	56.0%	1600	5.3%	1700	438.8%	1700	0.0%

Ion-adjusted
concentrates

ion-adj conc	% of 25.3 SW	Mock ion-adj conc	% of 25.3 SW	% diff from i-a conc
Calcium	273	90.1%	275	0.7%
Magnesium	889	95.6%	876	-1.5%
Potassium	280	96.9%	280	0.0%
Sodium	7820	100.2%	7740	-1.0%
Alkalinity	72.7	97.2%	74.3	2.2%
Bicarbonate	88.694	97.2%	90.646	2.2%
Bromide	47	96.4%	50	6.4%
Chloride	14000	99.9%	13000	-7.1%
Sulfate	1900	96.7%	1900	0.0%

Seawaters

Seawater diluent	% of 25.3 SW
289	95.3%
912	98.1%
278	96.2%
7650	98.0%
89.9	120.1%
109.678	120.1%
47	96.4%
14000	99.9%
1800	91.6%

SOP #1
Acute Toxicity Testing of
Membrane-Technology (Reverse Osmosis) Water-Treatment Concentrates:
Preparation of Mock Sea-Salt Slurry

STEPS		COMMENTS
Materials		
1. Mock slurry salts:		Store salts in a dessicator.
a. Sodium chloride, ACS grade		
b. Sodium sulfate, ACS grade		
c. Potassium chloride, ACS grade		
d. Potassium bicarbonate, ACS grade		
e. Potassium bromide, ACS grade		
g. Magnesium chloride, ACS grade		
h. Calcium chloride, ACS grade		
2. Deionized (DI) water		
3. Balance capable of weighing to 0.001g		
4. Balance capable of weighing to 0.00001g		
5. Metal spatula		
6. Magnetic stirrer		
7. Magnetic stir bar, 2.5 cm		
8. Magnetic stir-bar retriever		
9. Small weigh boats		
10. Medium weigh boats		
11. 500-mL Erlenmeyer flask		
12. 100-mL graduated cylinder		
13. Calibrated Epindorf™ or equivalent pipette		
14. Parafilm™		
Methods		
1. Add 100 mL of DI water to the 500-mL Erlenmeyer flask. Place the flask on the magnetic stirrer, put the stir bar into the flask, and turn the stirrer on.		A 500-mL Erlenmeyer flask is used to allow room for mixing before each use.
2. Measure an additional 100 mL of DI water to use as rinse water when adding salts to the flask.		
3. Use a balance capable of weighing to 0.001g to weigh each of the following salts. Use separate medium sized weigh boats for each and use a small amount of DI water to rinse the clinging salts into the flask. Clean the spatula before and after each salt.		Use DI water previously measured as the rinse water.
NaCl	24.083 ±0.001g	
Na ₂ SO ₄	4.010 ±0.001g	
MgCl ₂ •6H ₂ O	10.774 ±0.001g	
CaCl ₂ •2H ₂ O	1.534 ±0.001g	

STEPS

COMMENTS

4. Use a balance capable of weighing to 0.00001g to weigh the following salt. Use a small-sized weigh boat and use a small amount of DI water to rinse the salt into the flask. Clean the spatula before and after use.

KCl 0.54347 ±0.00001g

5. Use stock salt solutions to add the following salts.

Add 9.996 mL of 0.01 g/mL KBr stock.

Add 2.073 mL of 0.1 g/mL KHCO₃ stock.

6. Add any remaining DI water from the graduated cylinder into the Erlenmeyer flask and mix.
7. Label the flask with “Mock Slurry”, the date, and your initials and store at 4°C.
8. Mix thoroughly before and during each use.

Use previously measured DI water as rinse water.

For stock salt solutions see SOP # 9.

Use a calibrated Epindorf pipette.

These volumes of stock salts equals:

KBr 0.09996 ±0.00001g

KHCO₃ 0.20725 ±0.00001g

The mock slurry is very concentrated and will not dissolve completely.

SOP #2
Acute Toxicity Testing of
Membrane-Technology (Reverse-Osmosis) Water-Treatment Concentrates
Advance Test Preparation

(General test methods based on Weber, 1993, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 4th Edition, EPA/600/4-90/027F).

STEPS	COMMENTS
Materials	
1. A minimum of 140 inland silverside minnows, <i>Menidia beryllina</i> , (9–14 days old, 24-hr age range) acclimated to appropriate test salinities.	Acclimate <i>Menidia</i> to a range of salinities between that of the concentrate and 35 ppt (or highest salinity indicated by the spreadsheet <i>Ion_Calc</i>— see SOP #12). If salinity adjustments are large, start the adjustments 2 to 3 days in advance. 3 ppt adjustments may be made in the morning, then again in the evening. This allows for adjustments of 6 ppt per day. The test organisms are cultured at 20 ppt. EPA methods recommend that mysids and <i>Menidia</i> should not be subjected to a change of more than 3 ppt in 12 hours (EPA/600/4-90/027). This recommendation is extremely conservative. If time constraints require faster acclimation, do the largest salinity changes first, and smaller ones as the test initiation approaches. Generally, more frequent but smaller salinity changes are preferred to less frequent but larger salinity changes. Leave test organisms at the final salinity for at least 3 hours before test loading begins.
2. A minimum of 900 young mysids, <i>Mysidopsis bahia</i> , (1–5 days old, 24-hr age range) acclimated to appropriate test salinities.	Acclimate 140 mysids for Test 1 like the <i>Menidia</i> above. Acclimate 240 mysids for Tests 2 and 3 to the salinity of the concentrate. Acclimate 260 mysids for Tests 4 and 5 (520 mysids if performing tests 4A & 4B, 5A & 5B) to the salinity of the ion-adjusted concentrates as determined by <i>Ion_Calc</i>. The salinity of the ion-adjustments will be higher than the concentrates but will not exceed 35 ppt. Records of monthly SRT (Standard Reference Toxicant) tests should be maintained for both test species.
3. Approximately 200 1-L glass bioassay jars. Each test should have jars labeled using a different colored tape to avoid lab errors.	Four replicates per concentration plus 4 replicates for the controls are used for each of the tests, for a total of 24 jars per test. As many as 8 tests may be required. In test 1, where the possibility of a wide range of salinities exists among the dilutions, an extra control should be run at the concentrate salinity if it differs from the control salinity. Wash 1-L glass bioassay jars with soap and water, then acid, acetone, and DI-water rinse, then oven dry, and label.
4. Natural Seawater (NSW)	The NSW is used for diluent and will need to be available at different salinities depending on the test and concentrate. Adjust it by adding deionized water (DI) to lower the salinity or by adding hypersaline brine to raise the salinity.
5. Hypersaline brine.	

STEPS	COMMENTS
6. Mock sea-salt slurry	Prepare per SOP # 1.
Materials (continued)	
7. Stock HCl and NaOH solutions for pH adjustments	Prepare per SOP #10 and #11.
8. ACS-grade salts: NaCl, Na ₂ SO ₄ , NaHCO ₃ , NaBr, KCl, K ₂ SO ₄ , KHCO ₃ , KBr, MgCL ₂ •6H ₂ O, MgSO ₄ , CaCl•2H ₂ O, CaSO ₄ •2H ₂ O, CaCO ₃ .	Store salts in dessicator.
9. Stock salt solutions.	If less than 0.5g of a salt is needed, it should be added as a concentrated stock solution. Prepare per SOP #9.
10. 8" Carolina dishes	Transfer mysids from generator larvae cups to Carolina dishes daily. Also, hold the test animals in Carolina dishes during the different salinity adjustments, and when loading bioassay jars.
11. 18" length of Tygon® tubing (minimum I.D. 1/4") with a 4" fire-polished glass tube in one end.	Used to transfer <i>Menidia</i> from a Carolina dish to the test jars. Nets cannot be used as they damage the larvae. A short piece of Tygon tubing can be slipped over the end of the glass tube to prevent chipping.
12. Small hand held screens, 500-µm Nitex™.	Used to transfer mysids from Carolina dish to test jars.
13. Meters to measure pH, temperature, D.O., and conductivity	
14. Chlorine test kit.	Calibrate per manufacturer's directions.
15. Alkalinity test kit.	Calibrate per manufacturer's directions. Hardness is typically not measured if conductivity >5,000 µmhos/cm.
16. Specific-ion meter with ammonia probe.	Calibrate per manufacturer's directions.
17. Temperature controlled testing area.	Room should maintain 25±1°C and 16h:8h light:dark.
18. Live <i>Artemia</i> nauplii for feeding.	
19. Two 20-L carboys	
20. Six 6-L Erlenmeyer flasks	For mixing the ion-adjusted concentrates and mock ion-adjusted concentrates.
21. Graduated Cylinders: Two 1,000-mL; One 500-mL; Two 250-mL; One 100-mL	For measuring the dilution volumes.
22. Pipettes: Mechanical pipettor or pipette bulb; Calibrated 1-mL Epindorf™ or equivalent pipettor with disposable plastic pipette tips; Calibrated Epindorf or equivalent adjustable micropipettor with disposable plastic pipette tips	For small volume measurements. Up to 1mL, capable of reading to three decimal places.

STEPS	COMMENTS
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Materials (continued)

- | | |
|---|---|
| 24. Parafilm™ | Used to cover open containers. |
| 25. Balance capable of measuring to 0.001g | Calibrate per manufacturer's instructions. |
| 26. Balance capable of measuring to 0.00001g. | Calibrate per manufacturer's instructions. |
| 27. Weigh boats, assorted sizes | |
| 28. Dessicator | |
| 29. Spatulas | For transferring chemicals during weighing. |
| 30. Assorted stir bars | |
| 31. Magnetic stirrer | |
| 32. Magnetic stir-bar retriever | |

Documentation

- | | |
|--|--|
| 1. Log concentrate receipt, temperature, salinity, conductivity, dissolved oxygen, and condition into appropriate log book. | Use only black, waterproof pen for all log-book and bench-sheet entries. All entries must be initialed and dated. |
| 2. Fill in sample information on bench sheets as completely as possible—including daily calibrations—prior to test initiation. | Include full name of facility, date and time collected, field TRC, and chamber numbers. This step usually can be partially done ahead of time. |
| 4. As the test progresses, record any unusual occurrences or procedures in the bench sheets and/or log book. The procedures used for doing the salt additions for the ion-adjustments are also recorded. | Typical entries might be a salt or pH adjustment or a precipitate noted. |

Methods—Advance preparation

Before the concentrate arrives

- | | |
|---|--|
| 1. Clean and label with color-coded tape all the necessary glassware and organize them on carts | 8 possible tests, 5 concentrations +1 control per test, and 4 replicates per test + the possibility of extra controls. Approximately 200 jars need to be prepared. |
| 2. Prepare bench sheets | |
| 3. Prepare a 20-L carboy of NSW adjusted to the concentrate salinity (if known). | |
| 4. Acclimate the test organisms to the expected salinities. | |

On arrival of sample (Day 0)

- | | |
|---|--|
| 1. Warm 5 L of concentrate up to test temperature (25°C) | On day 0, approximately 4.5 L of concentrate will be needed for test 1. |
| 2. Perform and record preliminary physical-chemical analyses. | These include chlorine, alkalinity, pH , temperature, D.O., conductivity, and salinity |
| 3. Perform any final salinity acclimation of test animals in the Carolina dishes. | See Step 1 of <i>Materials</i> for this procedure. |

STEPS

- 3. Perform any final salinity acclimation of test animals in the Carolina dishes.
- 4. Adjust a 20-L carboy of NSW to the salinity of the concentrate.

COMMENTS

See Step 1 of *Materials* for this procedure.

This is the diluent for Tests 1, 2, and 3.

On receipt of chemical analyses (Day 1)

- 1. If needed, perform final salinity acclimation of mysids.
- 2. Warm the remaining concentrate to test temperature (25°C).
- 3. Calculate the required salt additions for the mock concentrate and for the ion-adjustments.
- 4. Weigh out all salts needed for each test in separate weigh boats. If the required weight is less than 0.5g, then use a stock solution and measure a corresponding volume.
- 5. Group the salts by test.

See SOP # 12 for calculation methods.

Salts are kept in the dessicator.

Salts ≥1g are weighed on the balance which can weigh to 0.001g.

Weights between 0.5g and 1g are weighed on the balance capable of weighing to 0.00001 g.

For weights <0.5g, prepare stock solutions per SOP # 9)

For example, if 0.47790g of KBr is needed and the concentration of the stock is 0. 1 g/mL then the amount of stock necessary would be 4.779 mL. Use calibrated Epindorf pipettes. Accuracy is very important in salt measurements.

The chemicals going into the mock concentrate should be in one area. The chemicals going into the ion-adjusted concentrate should be in a separate area and so on. This is to help prevent errors.

Methods

Day 0

Test 1- NSW at the concentrate salinity is the diluent. Concentrate is the effluent.

Refer to SOP # 3.

Day 1

Test 2 - NSW at the concentrate salinity is the diluent. Concentrate is the effluent.

Refer to SOP # 4.

Test 3 - NSW at the concentrate salinity is the diluent. Mock concentrate is the effluent.

Refer to SOP # 5.

Test 4 - NSW at the salinity of the ion-adjusted concentrate is the diluent. Ion-adjusted concentrate is the effluent

Refer to SOP # 6.

There is the possibility of 2 tests (4A and 4B).

Test 5 - Mock ion-adjusted concentrate is the diluent. Ion-adjusted concentrate is the effluent.

Refer to SOP # 7.

There is the possibility of 2 tests (5A and 5B).

SOP # 3
Acute Toxicity Testing of
Membrane-Technology (Reverse Osmosis) Water-Treatment Concentrates:
Test 1—Initial Test

(based on Weber, 1993, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 4th Edition, EPA/600/4-90/027F).

STEPS	COMMENTS																												
Materials	Refer to SOP #2.																												
Documentation	Refer to SOP #2.																												
Advance Preparation	Refer to SOP #2.																												
Methods																													
<i>Test Initiation</i>																													
1. Pour 50 mL of concentrate into a clean plastic cup and check the salinity. If necessary, adjust the salinity of the test concentrate.	This is done to prevent contaminating the concentrate in the sample bottle. For <i>Menidia</i> , no salinity adjustments are necessary. For mysids, if the salinity is <6 ppt, then add 40 Fathoms® artificial sea salts as needed to bring 2.5L of concentrate up to 6 ppt. Allow at least one-half hour to dissolve salts completely.																												
2. Measure the pH of the cup of concentrate used in Step 1. If salinity adjustment was necessary, replace it with salinity-adjusted concentrate before measuring pH. If necessary, adjust the test-concentrate pH to 7.5–9.0.	Measuring the pH is done after any salinity adjustments because they may raise the pH. The acceptable range for pH is 7.5–9.0. Adjust as needed by adding a minimal amount of acid or base. Use only ACS-grade acid or base stock solutions. Refer to SOP #10 and #11 for preparation of HCl and NaOH stock solutions.																												
3. If adjustments to the concentrate were required, save samples for chemical analysis. Label the bottles with the sample name, test number, date, and your initials.	Have these bottles analysed for the dissolved major seawater ions (Ca ⁺⁺ , Mg ⁺⁺ , Na ⁺ , K ⁺ , Br ⁻ , Cl ⁻ , SO4 ⁼ , and alkalinity).																												
4. Put 250 mL of NSW adjusted to the concentrate salinity into each of the four replicate control jars.																													
5. The test concentrations are 6.25%, 12.5%, 25%, 50%, and 100%. Starting with the lowest concentration, measure the desired volume of concentrate using a graduated cylinder of the appropriate size and add it to a 1,000-mL graduated cylinder. Add diluent to bring the volume of the cylinder up near the 1,000 mL mark. Add the final amount of diluent with a 10-mL pipette so as not to overshoot.	Use four 250-mL replicates for each test concentration. <table><tr><th>Concentration</th><th>Diluent</th><th>Sample</th><th>Total Volume</th></tr><tr><td>Control</td><td>1,000 mL</td><td>0 mL</td><td>1,000 mL</td></tr><tr><td>6.25%</td><td>937.5 mL</td><td>62.5 mL</td><td>1,000 mL</td></tr><tr><td>12.5%</td><td>875 mL</td><td>125 mL</td><td>1,000 mL</td></tr><tr><td>25%</td><td>750 mL</td><td>250 mL</td><td>1,000 mL</td></tr><tr><td>50%</td><td>500 mL</td><td>500 mL</td><td>1,000 mL</td></tr><tr><td>100%</td><td>0 mL</td><td>1,000 mL</td><td>1,000 mL</td></tr></table>	Concentration	Diluent	Sample	Total Volume	Control	1,000 mL	0 mL	1,000 mL	6.25%	937.5 mL	62.5 mL	1,000 mL	12.5%	875 mL	125 mL	1,000 mL	25%	750 mL	250 mL	1,000 mL	50%	500 mL	500 mL	1,000 mL	100%	0 mL	1,000 mL	1,000 mL
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100%	0 mL	1,000 mL	1,000 mL																										

STEPS	COMMENTS
6. For each concentration, seal the cylinder opening with Parafilm and invert 5 or 6 times. Pour 250 mL of the solution into each of the four replicate test jars for that test concentration. Repeat for each test concentration.	
7. Measure pH, temperature, DO, and conductivity of the solutions in all jars and record on bench sheets.	Read pH, conductivity, DO, and temperature to one decimal place; however, read low-conductivity measurements (<10 mmhos/cm) to two decimal places. Take measurements proceeding from the lowest to the highest concentration.
8. Record any comments pertaining to the condition or appearance of the sample.	Record all readings in appropriate columns on the bench sheet.
9. Load test organisms.	Per SOP #8.

SOP # 4
Acute Toxicity Testing of
Membrane-Technology (Reverse Osmosis) Water-Treatment Concentrates:
Test 2—Baseline Test

(Toxicity testing based on Weber, 1993, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 4th Edition, EPA/600/4-90/027F).

STEPS	COMMENTS																												
Materials	Refer to SOP #2.																												
Documentation	Refer to SOP #2.																												
Advance Preparation	Refer to SOP #2.																												
Methods																													
<i>Test Initiation</i>																													
1. Pour 50 mL of concentrate into a clean plastic cup and check the salinity. If the salinity is <6 ppt, then add mock slurry salts as needed to bring 2.5L of concentrate up to 6 ppt.	This is done to prevent contaminating concentrate in sample bottle. Allow at least one-half hour to dissolve salts completely. To make mock-sea-salt slurry refer to SOP #1.																												
2. Measure the pH of the cup of concentrate used in Step 1. If salinity adjustment was necessary, replace it with salinity adjusted concentrate before measuring pH. If necessary, adjust the test-concentrate pH to 7.5–9.0.	Measuring the pH is done after any salinity adjustments because they may raise the pH. The acceptable range for pH is 7.5–9.0. Adjust as needed by adding a minimal amount of acid or base. Use only those acid or base stock solutions designated for the RO Project. Refer to SOP #10 and #11 for preparation of HCl and NaOH stock solutions.																												
3. Save samples for chemical analysis. Label the bottles with the sample name, test number, date, and your initials.	Have these samples analysed for the dissolved major seawater ions (Ca ⁺⁺ , Mg ⁺⁺ , Na ⁺ , K ⁺ , Br ⁻ , Cl ⁻ , SO4 ⁻ , and alkalinity).																												
4. Put 250 mL of NSW adjusted to the concentrate salinity into each of the four replicate control jars.																													
5. The test concentrations are 6.25%, 12.5%, 25%, 50%, and 100%. Starting with the lowest concentration, measure the desired volume of concentrate using a graduated cylinder of the appropriate size and add it to a 1,000-mL graduated cylinder. Add diluent to bring the volume of the cylinder up near the 1,000 mL mark. Add the final amount of diluent with a 10-mL pipette so as not to overshoot.	Use four 250-mL replicates for each test concentration. <table><tr><th>Concentration</th><th>Diluent</th><th>Sample</th><th>Total Volume</th></tr><tr><td>Control</td><td>1,000 mL</td><td>0 mL</td><td>1,000 mL</td></tr><tr><td>6.25%</td><td>937.5 mL</td><td>62.5 mL</td><td>1,000 mL</td></tr><tr><td>12.5%</td><td>875 mL</td><td>125 mL</td><td>1,000 mL</td></tr><tr><td>25%</td><td>750 mL</td><td>250 mL</td><td>1,000 mL</td></tr><tr><td>50%</td><td>500 mL</td><td>500 mL</td><td>1,000 mL</td></tr><tr><td>100%</td><td>0 mL</td><td>1,000 mL</td><td>1,000 mL</td></tr></table>	Concentration	Diluent	Sample	Total Volume	Control	1,000 mL	0 mL	1,000 mL	6.25%	937.5 mL	62.5 mL	1,000 mL	12.5%	875 mL	125 mL	1,000 mL	25%	750 mL	250 mL	1,000 mL	50%	500 mL	500 mL	1,000 mL	100%	0 mL	1,000 mL	1,000 mL
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100%	0 mL	1,000 mL	1,000 mL																										

STEPS	COMMENTS
6. For each concentration, seal the cylinder opening with Parafilm and invert 5 or 6 times. Pour 250 mL of the solution into each of the four replicate test jars for that test concentration. Repeat for each test concentration.	
7. Measure pH, temperature, DO, and conductivity of the solutions in all jars and record on bench sheets.	Read pH, conductivity, DO, and temperature to one decimal place; however, read low-conductivity measurements (<10 mmhos/cm) to two decimal places. Take measurements proceeding from the lowest to the highest concentration.
8. Record any comments pertaining to the condition or appearance of the sample.	Record all readings in appropriate columns on the bench sheets.
9. Load test organisms.	Per SOP #8.

SOP # 5
Acute Toxicity Testing of
Membrane-Technology (Reverse Osmosis) Water-Treatment Concentrates:
Test 3—Mock Concentrate

(based on Weber, 1993, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 4th Edition, EPA/600/4-90/027F).

STEPS	COMMENTS
Materials	Refer to SOP #2.
Documentation	Refer to SOP #2.
Advance Preparation	Refer to SOP #2.
Methods	
<i>Note: if anti-scalants are used by the facility from which the concentrate sample was taken, it may not be possible to match the concentrations of major seawater ions in the concentrate because of precipitation. The anti-scalants allow salts to remain in solution that exceed the normal saturation concentration. It may be necessary to test the anti-scalant for toxicity and, if not toxic, include the anti-scalant in the makeup of the mock concentrate.</i>	
Test Initiation	
1. Gather the salts prepared for the mock concentrate.	Refer to SOP #2.
2. Fill a 20L carboy with 16 liters of DI water.	This carboy will be used to make 18 liters of mock concentrate. Part of this will be used in Tests 4 and 5.
3. If CaSO ₄ is needed, it must be dissolved at low pH. Bring 16L of DI water down to pH 2 using HCl. After dissolving the CaSO ₄ , bring the pH up to 5 using NaOH. The CaSO ₄ should be the first salt dissolved.	Refer to SOP #10 and #11 for preparation of HCl and NaOH stock solutions. It is very important to adjust the pH above 5 before adding additional salts. Carbonates will be lost to the atmosphere as CO ₂ below pH 5. Any acid or base used in this step must be recorded. The amount of NaCl needed to create the mock concentrate is likely to change and must be recalculated.
4. In separate 1,000-mL Erlenmeyer flasks, pre-dissolve each remaining salt that is hard to dissolve (such as MgSO ₄ , CaCO ₃ , Na ₂ SO ₄ , and K ₂ SO ₄) and NaCl on a magnetic stirrer with a stir bar. Portions of the 16L from Step 3, now at pH 5, can be used to pre-dissolve the other salts except NaCl.	MgSO ₄ tends to solidify into large flakes without adequate agitation. Adding individual salts using a stir plate gets difficult-to-dissolve compounds more readily into solution.
5. Add the pre-dissolved salts and the salts not needing pre-dissolving to the mock concentrate. Bring the total volume to 18L with DI water.	NaCl should always be dissolved separately then added slowly and last to minimize precipitation of any salts that may be near saturation.

STEPS

COMMENTS

6. Pour 50 mL of mock concentrate into a clean plastic cup and check the salinity.
- If the salinity is <6 ppt, then first save 500 mL of the mock concentrate in two separate 250-mL bottles for chemical analysis. In a 4-L Erlenmeyer flask, bring 2.5L of mock concentrate up to 6 ppt by adding mock slurry salts as needed.
7. Measure the pH of the cup of mock concentrate used in Step 6. If salinity adjustment was necessary, replace it with salinity-adjusted mock concentrate before measuring pH.
- If necessary, adjust the mock concentrate pH to 7.5–9.0.
8. Save samples for chemical analysis. If salinity adjustment was necessary, then save samples from before and after adjustment. Label the bottles with the sample name, test number, date, and your initials.
9. Put 250 mL of NSW adjusted to the concentrate salinity into each of the four replicate control jars.
10. The test concentrations are 6.25%, 12.5%, 25%, 50%, and 100%. Starting with the lowest concentration, measure the desired volume of mock concentrate (sample) using a graduated cylinder of the appropriate size and add it to a 1,000-mL graduated cylinder. Add diluent to bring the volume of the cylinder up near the 1,000 mL mark. Add the final amount of diluent with a 10-mL pipette so as not to overshoot.
11. For each concentration, seal the cylinder opening with Parafilm and invert 5 or 6 times. Pour 250 mL of the solution into each of the four replicate test jars for that test concentration. Repeat for each test concentration.
12. Measure pH, temperature, DO, and conductivity of the solutions in all jars and record on bench sheets.

This is done to prevent contaminating the concentrate in the sample bottle.

Allow at least one-half hour to dissolve salts completely.

To make mock-sea-salt slurry refer to SOP #1.

Measuring the pH is done after any salinity adjustments because they may raise the pH.

The acceptable range for pH is 7.5–9.0. Adjust as needed by adding a minimal amount of acid or base. Use only those acid or base stock solutions designated for the RO Project.

Refer to SOP #10 and #11 for preparation of HCl and NaOH stock solutions.

Have these samples analysed for the dissolved major seawater ions (Ca⁺⁺, Mg⁺⁺, Na⁺, K⁺, Br⁻, Cl⁻, SO₄⁻, and alkalinity).

If precipitation occurred during the synthesis of the mock concentrate, make sure that the analytical lab does not acidify the sample without first filtering it. Otherwise the analyses may be misleading.

Use four 250-mL replicates for each test concentration.

Concentration	Diluent	Sample	Total Volume
Control	1,000 mL	0 mL	1,000 mL
6.25%	937.5 mL	62.5 mL	1,000 mL
12.5%	875 mL	125 mL	1,000 mL
25%	750 mL	250 mL	1,000 mL
50%	500 mL	500 mL	1,000 mL
100%	0 mL	1,000 mL	1,000 mL

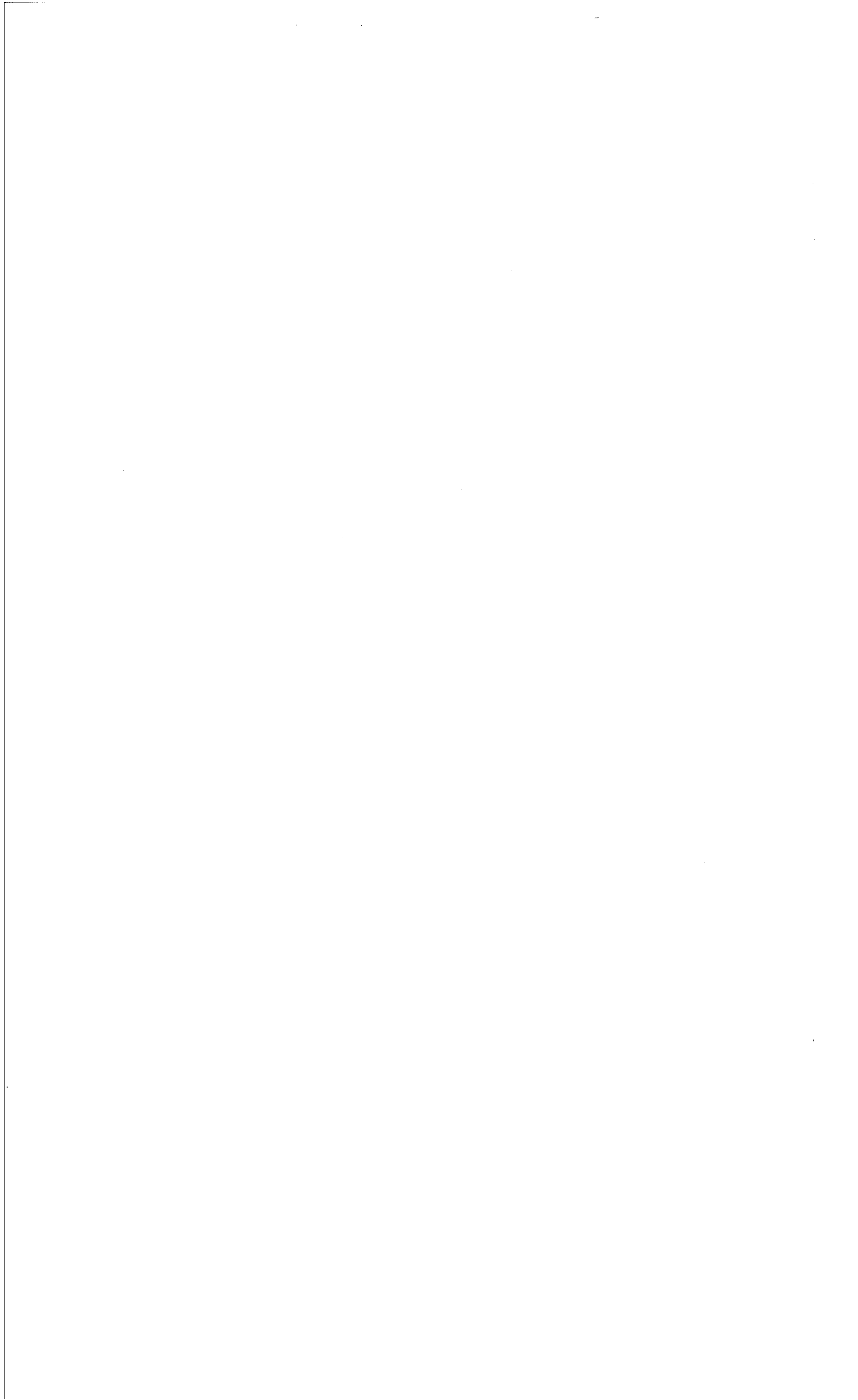
Read pH, conductivity, DO, and temperature to one decimal place; however, read low-conductivity measurements (<10 mmhos/cm) to two decimal places. Take measurements proceeding from the lowest to the highest concentration.

- STEPS**
13. Record any comments pertaining to the condition or appearance of the sample.
14. Load test organisms.

COMMENTS

Record all readings in appropriate columns on the bench sheet.

Per SOP #8.



SOP # 6
Acute Toxicity Testing of
Membrane-Technology (Reverse Osmosis) Water-Treatment Concentrates:
Test 4—Ion-adjusted Concentrate with
Natural Seawater as Diluent

(Toxicity testing based on Weber, 1993, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 4th Edition, EPA/600/4-90/027F).

STEPS	COMMENTS
Materials	Refer to SOP #2.
Documentation	Refer to SOP #2.
Advance Preparation	Refer to SOP #2.
Methods	
<i>If the ion-adjusted concentrate calculations (see SOP #12) show that all the major seawater ions can be adjusted to seawater proportions without exceeding their 35-ppt seawater concentrations, then skip the instructions for Test 4A and 4B. Otherwise, skip the instructions for Test 4.</i>	
Test 4 Create fully ion-adjusted concentrate	Use Test 4 only if the resulting ion-adjusted concentrate will have no major seawater ion concentrations greater than their 35-ppt seawater concentrations. Otherwise go to Test 4A.
1. Gather the salts prepared for the ion-adjusted concentrate.	Refer to SOP #2. The salts in ion-adjusted concentrate should result in major seawater ions that are proportional to seawater.
2. Pour 250 mL of the unaltered concentrate into a 6-L Erlenmeyer flask and add any easy-to-dissolve salts.	Salts added in small amounts, such as KBr and KCl, can be added directly to this portion.
3. In separate 1,000-mL Erlenmeyer flasks, pre-dissolve each remaining hard-to-dissolve salt (such as MgSO ₄ , CaCO ₃ , Na ₂ SO ₄ , and K ₂ SO ₄) and NaCl on a magnetic stirrer with a stir bar. It may be possible to dissolve more than one salt in a single flask.	The volume of concentrate needed will vary based upon the concentrate characteristics. About 250 mL will probably be enough to dissolve most salts. MgSO ₄ tends to solidify into large flakes without adequate agitation. Pre-dissolving the salts on a stir plate allows difficult to dissolve compounds to go into solution more readily. NaCl should always be dissolved separately in a full liter of concentrate.
4. After salts are dissolved, accurately measure the volume of each pre-dissolved-salt solution and add to the 6-L Erlenmeyer flask.	It is necessary to measure the concentrate to ensure accurate volume. Erlenmeyer flasks are not accurately calibrated and the salt additions will slightly increase the volumes. The NaCl solution should always added slowly and last to minimize precipitation of any salts that may be near saturation.
5. Bring the total amount of ion-adjusted concentrate up to the goal volume by adding unaltered concentrate.	The goal volume is determined in the spreadsheet <i>Ion_Calc</i> per SOP #12. Part of the ion-adjusted concentrate will be saved for use in Test 5.

STEPS

COMMENTS

Test 4A
Create ion-adjusted concentrate A: some ions remaining out-of-proportion.

1. Gather the salts prepared for the ion-adjusted concentrate A.
2. Pour 2L of the unaltered concentrate into a 6-L Erlenmeyer flask and add any easy-to-dissolve salts.
3. In separate 1,000-mL Erlenmeyer flask(s), pre-dissolve each remaining hard-to-dissolve salts (such as $MgSO_4$, $CaCO_3$, Na_2SO_4 , and K_2SO_4) and NaCl on a magnetic stirrer with a stir bar.

It may be possible to dissolve more than one salt in a single flask.
4. After salts are dissolved, accurately measure the volume of each pre-dissolved-salt solution and add to the 6-L Erlenmeyer flask.
5. Bring the total amount of ion-adjusted concentrate A up to the goal volume (generally 5L) with additional unaltered concentrate.

Test 4B
Create ion-adjusted concentrate B: ions fully adjusted, but diluted to 35 ppt.

1. Gather the salts prepared for the ion-adjusted concentrate B.
2. Pour about 500 mL less than the needed volume of the unadjusted concentrate into a 6-L Erlenmeyer flask and add any easy-to-dissolve salts.

Tests 4A and 4B are for use with concentrate samples in which the ion adjustment will create a salinity >35 ppt.

Refer to SOP #2.

The salts in this ion-adjusted concentrate A are not fully balanced. They are balanced as far as possible without the individual ions going over their 35-ppt seawater concentration.

Salts added in small amounts, such as KBr and KCl, can be added directly to this portion.

The volume of concentrate needed will vary based upon the concentrate characteristics. About 250 mL will probably be enough to dissolve most salts.

$MgSO_4$ tends to solidify into large flakes without adequate agitation.

Pre-dissolving the salts on a stir plate allows difficult to dissolve compounds to go into solution more readily.

NaCl should always be dissolved separately in a full liter of concentrate.

It is necessary to measure the concentrate to ensure accurate volume. Erlenmeyer flasks are not accurately calibrated and the salt additions will slightly increase the volumes.

The NaCl solution should always added slowly and last to minimize precipitation of any salts that may be near saturation.

The goal volume is determined in the spreadsheet *Ion_Calc* per SOP #12.

Part of the ion-adjusted concentrate A will be saved for use in Test 5.

Refer to SOP # 2.

The salts in ion-adjusted concentrate B should result in major seawater ions that are proportional to seawater.

The volumes of concentrate and DI water needed are calculated by the spreadsheet *Ion_Calc* per SOP #12. Adjust volumes for dissolving the salts such that these total volumes are not exceeded.

Salts added in small amounts, such as KBr and KCl, can be added directly to this portion.

STEPS**COMMENTS**

3. In separate 1,000-mL Erlenmeyer flasks, pre-dissolve in DI water each remaining hard-to-dissolve salt (such as MgSO_4 , CaCO_3 , Na_2SO_4 , and K_2SO_4) and NaCl on a magnetic stirrer with a stir bar.

It may be possible to dissolve more than one salt in a single flask.

4. After salts are dissolved, accurately measure the volume of each pre-dissolved-salt solution and add to the 6-L Erlenmeyer flask. Any *increase* in the volume of the DI water as a result of adding the salts should be counted as part of the needed concentrate volume. The initial DI water volume should be counted as part of the needed DI water.
5. Bring the total amount of ion-adjusted concentrate B up to the goal volume by first adding any remaining concentrate and then adding the necessary DI water.

A total of about 250 mL of concentrate and/or DI water will probably be enough to dissolve most salts.

MgSO_4 tends to solidify into large flakes without adequate agitation.

As it is often difficult to get all salts dissolved in this concentrate before diluting, add most of the dilution water before adding the salts.

Pre-dissolving the salts on a stir plate allows difficult to dissolve compounds to go into solution more readily.

NaCl should always be dissolved separately in a full liter of concentrate or D.I. because past experience indicates that tests sing high-salinity concentrates required slightly over a liter of concentrate in this test.

It is necessary to measure the concentrate to ensure accurate volume. Erlenmeyer flasks are not accurately calibrated and the salt additions will slightly increase the volumes.

The NaCl solution should always be added slowly and last to minimize precipitation of any salts that may be near saturation.

The goal volume is determined in the spreadsheet *Ion_Calc* per SOP #12.

The DI water used to dissolve the salts is part of the DI volume determined by the spreadsheet.

Part of the ion-adjusted concentrate B will be saved for use in Test 5.

Test Initiation

If running Tests 4A and 4B, repeat the following instructions using the ion-adjusted concentrates created for each test.

6. Pour 50 mL of ion-adjusted concentrate into a plastic cup and check the salinity to confirm that it is ≤ 35 ppt.
7. Measure the pH of the cup of ion-adjusted concentrate used in Step 6.

If necessary, adjust the ion-adjusted concentrate pH to 7.5–9.0.

This is done to prevent contaminating concentrate in sample bottle.

Measure the pH after salt additions because the additions may affect the pH.

The acceptable range for pH is 7.5–9.0. Adjust as needed by adding a minimal amount of acid or base. Use only those acid or base stock solutions designated for the RO Project.

Refer to SOP #10 and #11 for preparation of HCl and NaOH stock solutions.

8. Save samples for chemical analysis. Label the bottles with the sample name, test number, date, and your initials.

Have these samples analysed for the dissolved major seawater ions (Ca^{++} , Mg^{++} , Na^+ , K^+ , Br^- , Cl^- , SO_4^{--} , and alkalinity).

If precipitation occurred during the synthesis of the mock concentrate, make sure that the analytical lab does not acidify the samples without first filtering them. Otherwise the analyses may be misleading.

STEPS

COMMENTS

9. Put 250 mL of natural seawater (NSW) adjusted to the ion-adjusted concentrate salinity into each of the four replicate control jars for each test.
10. The test concentrations are 6.25%, 12.5%, 25%, 50%, and 100%. Starting with the lowest concentration, measure the desired volume of sample (ion-adjusted concentrate) using a graduated cylinder of the appropriate size and add it to a 1,000-mL graduated cylinder. Add diluent (mock ion-adjusted concentrate) to bring the volume of the cylinder up near the 1,000 mL mark. Add the final amount of diluent with a 10-mL pipette so as not to overshoot.
11. For each concentration, seal the cylinder opening with Parafilm and invert 5 or 6 times. Pour 250 mL of the solution into each of the four replicate test jars for that test concentration. Repeat for each test concentration.
12. Measure pH, temperature, DO, and conductivity of the solutions in all jars and record on bench sheets.
13. Record any comments pertaining to the condition or appearance of the sample.
14. Load test organisms.

Use four 250-mL replicates for each test concentration.

Concentration	Diluent	Sample	Total Volume
Control	1,000 mL	0 mL	1,000 mL
6.25%	937.5 mL	62.5 mL	1,000 mL
12.5%	875 mL	125 mL	1,000 mL
25%	750 mL	250 mL	1,000 mL
50%	500 mL	500 mL	1,000 mL
100%	0 mL	1,000 mL	1,000 mL

Save any remaining ion-adjusted concentrate for use in Test 5.

Read pH, conductivity, DO, and temperature to one decimal place; however, read low-conductivity measurements (<10 mmhos/cm) to two decimal places. Take measurements proceeding from the lowest to the highest concentration.

Record all readings in appropriate columns on the bench sheet.

Per SOP #8.

SOP # 7
Acute Toxicity Testing of
Membrane-Technology (Reverse Osmosis) Water-Treatment Concentrates:
Test 5—Ion-adjusted Concentrate with
Mock Ion-adjusted Concentrate as Diluent

(Toxicity testing based on Weber, 1993, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 4th Edition, EPA/600/4-90/027F).

STEPS	COMMENTS
Materials	Refer to SOP #2.
Documentation	Refer to SOP #2.
Advance Preparation	Refer to SOP #2.
Methods	
<i>If the ion-adjusted concentrate calculations (see SOP #12) show that all the major seawater ions can be adjusted to seawater proportions without exceeding their 35-ppt seawater concentrations, then skip the instructions for Test 5A and 5B. Otherwise, skip the instructions for Test 5.</i>	
Test 5—fully ion-adjusted concentrate	Use Test 5 only if the resulting ion-adjusted concentrate will have no major seawater ion concentrations greater than their 35-ppt seawater concentrations. Otherwise go to Test 5A.
1. Use the remaining ion-adjusted concentrate created in Test 4.	
Create mock ion-adjusted concentrate	
2. Gather the salts prepared for the mock ion-adjusted concentrate.	Refer to SOP #2.
3. Pour 2L of the mock concentrate into a 6-L Erlenmeyer flask and add any easy-to-dissolve salts.	Salts added in small amounts, such as KBr and KCl, can be added directly to this portion.
4. In separate 1,000-mL Erlenmeyer flasks, pre-dissolve each remaining hard-to-dissolve salt (such as MgSO ₄ , CaCO ₃ , Na ₂ SO ₄ , and K ₂ SO ₄) and NaCl on a magnetic stirrer with a stir bar. It may be possible to dissolve more than one salt in a single flask.	The volume of mock concentrate needed will vary based upon the concentrate characteristics. About 250 mL will probably be enough to dissolve most salts. MgSO ₄ tends to solidify into large flakes without adequate agitation. Pre-dissolving the salts on a stir plate allows difficult to dissolve compounds to go into solution more readily. NaCl should always be dissolved separately in a full liter of mock concentrate.
5. After salts are dissolved, accurately measure the volume of each pre-dissolved-salt solution and add to the 6-L Erlenmeyer flask.	It is necessary to measure the concentrate to ensure accurate volume. Erlenmeyer flasks are not accurately calibrated and the salt additions will slightly increase the volumes. The NaCl solution should always added slowly and last to minimize precipitation of any salts that may be near saturation.
6. Bring the total volume of mock ion-adjusted concentrate up to the goal volume (generally 6L) by adding mock concentrate.	The goal volume is determined in the spreadsheet <i>Ion_Calc</i> per SOP #12.

STEPS

COMMENTS

***Test 5A—ion-adjusted concentrate A:
some ions remaining out-of-proportion.***

1. Use remaining ion-adjusted concentrate A created in Test 4A as the sample.

The mock ion-adjusted concentrate A that is prepared next will be the diluent.

Create mock ion-adjusted concentrate A

2. Gather the salts prepared for the mock ion-adjusted concentrate A.
3. Pour 2L of the mock concentrate into a 6-L Erlenmeyer flask and add any easy-to-dissolve salts.
4. In separate 1,000-mL Erlenmeyer flasks, pre-dissolve each remaining hard-to-dissolve salt (such as MgSO_4 , CaCO_3 , Na_2SO_4 , and K_2SO_4) and NaCl on a magnetic stirrer with a stir bar.

It may be possible to dissolve more than one salt in a single flask.
5. After salts are dissolved, accurately measure the volume of each pre-dissolved-salt solution and add to the 6-L Erlenmeyer flask.
6. Bring the total volume of mock ion-adjusted concentrate A up to the goal volume (generally 6L) by adding mock concentrate.

Refer to SOP #2.

Salts added in small amounts, such as KBr and KCl, can be added directly to this portion.

The volume of mock concentrate needed will vary based upon the concentrate characteristics. About 250 mL will probably be enough to dissolve most salts.

MgSO_4 tends to solidify into large flakes without adequate agitation.

Pre-dissolving the salts on a stir plate allows difficult to dissolve compounds to go into solution more readily.

NaCl should always be dissolved separately in a full liter of mock concentrate.

It is necessary to measure the concentrate to ensure accurate volume. Erlenmeyer flasks are not accurately calibrated and the salt additions will slightly increase the volumes.

The NaCl solution should always added slowly and last to minimize precipitation of any salts that may be near saturation.

The goal volume is determined in the spreadsheet *Ion_Calc* per SOP #12.

***Test 5B— ion-adjusted concentrate B:
ions fully adjusted, but diluted to 35 ppt.***

1. Use remaining ion-adjusted concentrate B created in Test 4B as the sample.

The mock ion-adjusted concentrate B that is prepared next will be the diluent.

Create mock ion-adjusted concentrate B

2. Gather the salts prepared for the mock ion-adjusted concentrate B.
3. Pour about 500 mL less than the needed volume of the mock concentrate into a 6-L Erlenmeyer flask and add any easy-to-dissolve salts.

Refer to SOP #2.

The volumes of concentrate and DI water needed are calculated by the spreadsheet *Ion_Calc* per SOP #12.

Adjust volumes for dissolving the salts such that these total volumes are not exceeded.

Salts added in small amounts, such as KBr and KCl, can be added directly to this portion.

STEPS

4. In separate 1,000-mL Erlenmeyer flasks, pre-dissolve in DI water each remaining hard-to-dissolve salt (such as MgSO_4 , CaCO_3 , Na_2SO_4 , and K_2SO_4) and NaCl on a magnetic stirrer with a stir bar.

It may be possible to dissolve more than one salt in a single flask.

5. After salts are dissolved, accurately measure the volume of each pre-dissolved-salt solution and add to the 6-L Erlenmeyer flask. Any *increase* in the volume of the DI water as a result of adding the salts should be counted as part of the needed mock concentrate volume. The initial DI water volume should be counted as part of the needed DI water.
6. Bring the total volume of mock ion-adjusted concentrate B up to the goal volume (generally 6L) by first adding any remaining mock concentrate and then adding the necessary DI water.

Test Initiation

If running Tests 5A and 5B, repeat the following instructions using the ion-adjusted concentrates and mock ion-adjusted concentrates created for each test.

6. Pour 50 mL of mock ion-adjusted concentrate into a plastic cup and check the salinity to confirm that it is ≤ 35 ppt.
7. Measure the pH of the cup of mock ion-adjusted concentrate used in Step 6.

If necessary, adjust the mock ion-adjusted concentrate pH to 7.5–9.0.

8. Save samples for chemical analysis. Label the bottles with the sample name, test number, date, and your initials.

9. Put 250 mL of mock ion-adjusted concentrate into each of the four replicate control jars for each test.

COMMENTS

A total of about 250 mL of mock concentrate and/or DI water will probably be enough to dissolve most salts.

MgSO_4 tends to solidify into large flakes without adequate agitation.

Pre-dissolving the salts on a stir plate allows difficult to dissolve compounds to go into solution more readily.

NaCl should always be dissolved separately in a full liter of concentrate or D.I. because past experience indicates that tests sing high-salinity concentrates required slightly over a liter of concentrate in this test.

It is necessary to measure the concentrate to ensure accurate volume. Erlenmeyer flasks are not accurately calibrated and the salt additions will slightly increase the volumes.

The NaCl solution should always added slowly and last to minimize precipitation of any salts that may be near saturation.

The goal volume is determined in the *Ion-Adjustment Calculation Spreadsheet* per SOP #12.

The DI water used to dissolve the salts is part of the DI volume determined by the spreadsheet.

This is done to prevent contaminating concentrate in sample bottle.

Measure the pH after salt additions because the additions may affect the pH.

The acceptable range for pH is 7.5–9.0. Adjust as needed by adding a minimal amount of acid or base. Use only those acid or base stock solutions designated for the RO Project.

Refer to SOP #10 and #11 for preparation of HCl and NaOH stock solutions.

Have these samples analysed for the dissolved major seawater ions (Ca^{++} , Mg^{++} , Na^+ , K^+ , Br^- , Cl^- , SO_4^{--} , and alkalinity).

If precipitation occurred during the synthesis of the mock concentrate, make sure that the analytical lab does not acidify the samples without first filtering them. Otherwise the analyses may be misleading.

STEPS

10. The test concentrations are 6.25%, 12.5%, 25%, 50%, and 100%. Starting with the lowest concentration, measure the desired volume of sample (ion-adjusted concentrate) using a graduated cylinder of the appropriate size and add it to a 1,000-mL graduated cylinder. Add diluent (mock ion-adjusted concentrate) to bring the volume of the cylinder up near the 1,000 mL mark. Add the final amount of diluent with a 10-mL pipette so as not to overshoot.
11. For each concentration, seal the cylinder opening with Parafilm and invert 5 or 6 times. Pour 250 mL of the solution into each of the four replicate test jars for that test concentration. Repeat for each test concentration.
12. Measure pH, temperature, DO, and conductivity of the solutions in all jars and record on bench sheets.
13. Record any comments pertaining to the condition or appearance of the sample.
14. Load test organisms.

COMMENTS

Use four 250-mL replicates for each test concentration.

Concentration	Diluent	Sample	Total Volume
Control	1,000 mL	0 mL	1,000 mL
6.25%	937.5 mL	62.5 mL	1,000 mL
12.5%	875 mL	125 mL	1,000 mL
25%	750 mL	250 mL	1,000 mL
50%	500 mL	500 mL	1,000 mL
100%	0 mL	1,000 mL	1,000 mL

Read pH, conductivity, DO, and temperature to one decimal place; however, read low-conductivity measurements (<10 mmhos/cm) to two decimal places. Take measurements proceeding from the lowest to the highest concentration.

Record all readings in appropriate columns on the bench sheet.

Per SOP #8.

SOP # 8
Acute Toxicity Testing of
Membrane-Technology (Reverse Osmosis) Water Treatment Concentrates:
Test-Organism Loading, Interim Readings, and Test Termination

(Toxicity testing based on Weber, 1993, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 4th Edition, EPA/600/4-90/027F).

STEPS	COMMENTS
Materials	Refer to SOP #2.
Documentation	Refer to SOP #2.
Advance Preparation	Refer to SOP #2.
Test Setup	Refer to SOP #3 through #7.
Test-Organism Loading	
Menidia loading	
1. Using a <i>Menidia</i> siphon, load five <i>Menidia</i> from the Carolina dish of the appropriate salinity into each test jar of similar salinity. Start at the lowest concentration and rinse the siphon in DI water between transfers. Verify loading counts over a light table.	<i>Menidia</i> siphon described in SOP #2. “Appropriate salinity” refers to test organisms acclimated to within $\pm 2\%$ of the test salinity. See SOP #2 for test-organism acclimation. (5 <i>Menidia</i> per replicate) x (4 replicates per test concentration) = 20 <i>Menidia</i> per concentration tested. Record counts and physical-chemical readings in appropriate columns on the bench sheets. <i>Menidia</i> are normally used only for Test 1 as they do not seem to be as sensitive to major-seawater-ion toxicity. However, they may help to indicate the presence of unidentified toxicants.
2. Add one to two drops of <i>Artemia</i> concentrate daily to each test jar (approximately 50 <i>Artemia</i> nauplii per <i>Menidia</i>).	<i>Menidia</i> are fed only once at 48 hr. Prepare <i>Artemia</i> nauplii by light separation, then pour onto a 60- μ m mesh screen and rinse with DI water to remove the saltwater. Rinse nauplii off the screen with a small volume of DI water to obtain a concentrated <i>Artemia</i> solution.
3. Cover test jars with a sheet of clear acrylic, allowing a 1/4” gap between the lips of the test jars and the acrylic for air circulation.	Covers prevent accidental contamination and minimize evaporation.
Mysid shrimp loading	
1. Using a 4 cm x 6 cm piece of 100- μ m Nitex® screen, lift 5 mysids from the Carolina dish of the appropriate salinity into each test jar. Start at the lowest test concentration and rinse the screen in DI water between transfers. Verify loading counts over a light table. Record counts in appropriate columns on acute bioassay bench sheets in laboratory notebook	“Appropriate salinity” refers to test organisms which have been acclimated to a salinity within $\pm 2\%$ of the test salinity. See SOP #2 for test-organism acclimation. (5 mysids per replicate) x (4 replicates per test concentration) = 20 mysids per concentration tested. Record counts and physical-chemical readings in appropriate columns in the bench sheets.

STEPS

2. Add one to two drops of *Artemia* concentrate daily to each test jar (approximately 50 *Artemia* nauplii per mysid).
3. Cover test jars with a sheet of clear acrylic, allowing a 3-mm gap between the lips of the test jars and the acrylic for air circulation.

Interim Readings

1. At 24, 48, and 72 hrs into the test, record the number of surviving *Menidia* or mysids in all jars and the pH, temperature, DO, and conductivity from jars that have 100% mortality. After recording parameters, feed the *Menidia* (if appropriate) and mysids and cover.

Record in small superscript next to the number alive the status of any animals that are dead or missing

2. As the test progresses, record any unusual observations or procedures in the logbook and/or benchsheets.

Test Termination

1. On termination day, $t = 96$ hr, count the number of surviving *Menidia* and mysids. Afterward, measure parameters of the solutions in all test jars. Record all counts and readings. Record any comments pertaining to the appearance or condition of the *Menidia* and mysids. Record test termination date and time.
2. Pour contents of all jars into sink and flush drain with water. Remove labels and take glassware to be washed.
3. Enter test results into statistical program to obtain the LC_{50} if 50% or greater mortality occurs in any test concentration.

COMMENTS

Mysids are fed daily throughout test.

Prepare *Artemia* nauplii by light separation, then pour onto a 60- μ m mesh screen and rinse with DI water to remove the saltwater. Rinse nauplii off the screen with a small volume of DI water to obtain a concentrated *Artemia* solution.

Covers prevent accidental contamination and minimize evaporation.

Counting is done over a light table.

Mysids are recorded as alive if they move when lightly prodded or appendage movement is seen.

Menidia are recorded as alive if they move when lightly prodded or gill movement is seen.

Record counts and physical-chemical readings in appropriate columns in the bench sheet.

D = Dead animals, body seen.

M = Missing animals, no body found.

An example of an entry might be a precipitate noted or unusual behavior or appearance of the test organisms. Any observations, whether seemingly important or not, may prove invaluable for later test interpretation.

Counting is done over a light table. Physical-chemical readings (pH, temperature, D.O., and conductivity) are taken in all test jars. If, at $t = 0$, the total ammonia was greater than 1 mg/L, then a final ammonia reading must be taken.

Record counts, readings, date, and time in appropriate columns in the bench sheets.

SOP # 9
Preparation of Stock Salt Solutions

STEPS	COMMENTS
Materials	
1. ACS-grade salts, as desired	Keep salts in a dessicator.
2. Deionized (DI) water	
3. Balance capable of measuring to 0.001g.	Calibrate per manufacturer's instructions.
4. Metal spatula	
5. Magnetic stirrer	
6. Magnetic stir bar, 1.0 cm	
7. Magnetic stir-bar retriever	
8. Medium weigh boats	
9. 100-mL volumetric flasks	
10. Calibrated Epindorf™ or equivalent pipette	
11. Parafilm™	
Method to create 0.1g/mL salt solutions	
1. Use the balance to weigh 10g of a salt into a weigh boat.	Multiples of 10 are easy numbers to work with.
2. Fill a 100-mL volumetric flask half way with DI water.	
3. Add the 10g of salt to the 100-mL volumetric flask using a little DI water to rinse the salt into the flask.	
4. Add a stir bar and place the flask on the magnetic stirrer. Allow the salt to dissolve.	It may be difficult to dissolve 10g of some salts. These salts may be made up in 0.01g/mL solutions.
5. When the salt is dissolved, remove the stir bar and add DI water to bring the flask up to the 100-mL mark.	
6. Label the flask with "0.1g/mL [salt] stock" and appropriate QC information (e.g., batch number, the date, and your initials). Store at 4°C.	
8. Mix thoroughly before each use.	
Method to create 0.01g/mL salt solutions from 0.1 g/mL stock salt solution	
1. If you were able to make a 0.1g/mL stock solution of the desired salt and you wish to make a 0.01g/mL stock solution, remove 10 mL of the 0.1g/mL salt stock solution and put it into a 100-mL volumetric flask.	Use a calibrated Epindorf pipette.
2. Fill the flask with DI water to the 100-mL mark. Shake vigorously.	

STEPS**COMMENTS**

3. Label the flask with "0.01g/mL (salt) stock and appropriate QC information (e.g., batch number, the date, and your initials). Store at 4°C.

Method to create 0.01g/mL salt solutions from reagent salts

4. If a 0.1g/mL stock solution was not able to be made (i.e. 10g would not dissolve in 100 mL of DI water), a 0.01g/mL stock solution can be made. Use the balance to weigh 1.0g of a salt.
5. Fill a 100-mL volumetric flask half way with DI water.
6. Add the 1.0g of salt to the 100-mL volumetric flask using a little DI water to rinse the salt into the flask.
7. Add a stir bar and place the flask on the magnetic stirrer. Allow the salt to dissolve.
8. When salt is dissolved, remove the stir bar and add DI water to bring the flask up to the 100-mL mark.
9. Label the flask with "0.01g/mL (salt) stock and appropriate QC information (e.g., batch number, the date, and your initials). Store at 4°C.
11. Mix salt solutions thoroughly before each use.

It is preferable to make this stock solution as a serial dilution to maximize the accuracy of the resulting concentration; however, in those cases where the salt has low solubility, they can be made using the dry salt.

SOP # 10
Preparation of Stock HCl (Acid) Solution

STEPS		COMMENTS
Materials		
1. Technical Grade 12N hydrochloric acid 2. Deionized (DI) water 3. 1-L volumetric flask 4. Fume hood 5. Parafilm™		
Methods		
1. Add 800 mL of DI water to the 1-L volumetric flask.		
Caution! Exothermic Reaction! HOT! Wear Goggles! Wear Gloves Use the Fume Hood!		
2. Under the fume hood, slowly add 100 mL of 12N HCl to the flask, making sure to mix the solution well.		Handle the HCl with care! Strong acid.
3. Carefully, bring the total volume of the flask up to 1-L with DI water. Tightly seal with a teflon stopper or acid resistant plastic.		Seal the flask tightly, any evaporation will result in the HCl concentration increasing.
4. Label the flask with “1.2N HCl Acid Stock Solution and appropriate QC information (e.g., batch number, the date, and your initials).		

SOP # 11
Preparation of Stock NaOH (Base) Solutions

STEPS	COMMENTS
Materials	
1. Sodium hydroxide, ACS grade	Keep salts in a dessicator.
2. Deionized (DI) water	
3. 1-L volumetric flask	Calibrate per manufacturer's instructions.
4. 250-mL volumetric flask	
4. Balance capable of measuring to 0.001g.	
5. Metal spatula	
6. Medium weigh boats	
7. Teflon or plastic stopper	
8. Parafilm™	

Method to make 10N NaOH stock solution

1. Use the balance to weigh 100g of NaOH into a weight boat.
2. Add 100 mL of DI water to a 250-mL volumetric flask.

Caution!
Exothermic Reaction!
HOT!
Wear Goggles!
Wear Gloves
Use the Fume Hood!

- | | |
|---|---|
| 3. Under the fume hood, slowly add 100g of NaOH crystals to the flask, 2–3g at a time. Allow NaOH to dissolve completely before adding more. | <i>Do not allow flask to get too hot!</i> |
| 4. Once all the NaOH crystals are added and dissolved, carefully bring the volume up to 250-mL with DI water. | |
| 5. Label the flask with “10N NaOH Base Stock Solution and appropriate QC information (e.g., batch number, the date, and your initials). | |

Method to make 1.0N NaOH stock solution

1. Add 800 mL of DI water to a 1-L volumetric flask.

COMMENTS

STEPS
Caution!**Exothermic Reaction!****HOT!****Wear Goggles!****Wear Gloves****Use the Fume Hood!**

2. Under the fume hood, **slowly** add 100 mL of 10N NaOH stock solution to the flask, mixing the solution well. *Do not allow flask to get too hot!*
3. Carefully, bring the total volume of the flask to 1-L with DI water.
4. Do not use a glass stopper to seal the volumetric flask as it will fuse to the flask. Use a teflon or plastic stopper, or cover with Parafilm. *Seal the flask tightly, any evaporation will result in the NaOH concentration increasing.*
5. Label the flask with "1.0N NaOH Base Stock Solution and appropriate QC information (e.g., batch number, the date, and your initials).

SOP #12

Acute Toxicity Testing of

Membrane-Technology (Reverse Osmosis) Water-Treatment Concentrates:

Calculation of Ion Additions

STEPS	COMMENTS
Materials	
1. Microsoft Excel™ 5.0 spreadsheet template, <i>Ion_Calc</i> .	Available for Macintosh or Windows from Florida Dept. of Environmental Protection, Biology Section, Tallahassee, FL 32399.
2. Results of chemical analyses of the concentrate sample for major seawater ions.	To include a minimum of: Na ⁺ , Ca ⁺⁺ , Mg ⁺⁺ , K ⁺ , Cl ⁻ , Br ⁻ , SO ₄ ⁼ , and alkalinity.
Method	Note: the term ions will be used to indicate the major seawater ions being manipulated by this test protocol.
1. Open spreadsheet file <i>Ion_Calc</i> , which consists of a set of eight work sheets in a 'workbook'.	
2. Click on the tab labeled <i>Salts 1 (4&5, 4B & 5B)</i> at the bottom of the workbook if it is not already selected.	If salts with hydration numbers different than those included are needed, unlock the spreadsheet and alter the appropriate value in the lookup tables on worksheets <i>Salts 1</i> (headed by cell H41) and <i>Salts 2</i> (headed by cell G39).
Worksheet <i>Salts 1 (4&5, 4B & 5B)</i>	This worksheet is used with all samples and tests
<i>Enter basic data and determine test protocol</i>	
1. Enter the facility name, sampling date, salinity, and concentrate alkalinity in the appropriate cells at the top of the worksheet.	Determine salinity by measuring conductivity. Spreadsheet cells where data is to be entered are light blue with a bold black border. Other cells are locked. Cells containing important information needed for the calculations are generally light yellow.
2. In column B under <i>Sample Concentration</i> , enter the amounts of each ion occurring in the unaltered concentrate sample.	Note that the carbonate concentration is calculated from the alkalinity and is not entered manually.
3. Column I under <i>Key ion for adjustment</i> indicates the ion most out of proportion relative to seawater.	This ion will not be altered in subsequent manipulations and will determine the amount of adjustment necessary to bring the other ions into seawater proportions.
4. Cell E17 shows the 'salinity' of the concentrate that would result from adjusting the ions to seawater proportions.	If this salinity is greater than 35 ppt, then the A&B portions of Tests 4 & 5 will be necessary and the worksheet <i>Salts 2</i> must also be used.

STEPS

Determine salts for creating ion-adjusted concentrate (or ion-adjusted concentrate B)

5. In the black-bordered blue cells headed by cell B40, enter the number of moles of salts needed to ion adjust the concentrate.

Note: multiple combinations of salts can provide the same results. There is no one correct set of salts. The goal is to select the combination of salts that minimizes or eliminates any 'leftover' ions.

Determine salts for creating mock concentrate

6. In the black-bordered blue cells headed by cell B75, enter the number of moles of salts needed to create the mock concentrate.

Note: multiple combinations of salts can provide the same results. There is no one correct set of salts. The goal is to select the combination of salts that minimizes or eliminates any 'leftover' ions.

7. If it is necessary to add acid or base in the process of dissolving the salts, the acid and base amounts (in mL) should be entered into the appropriate black-bordered blue cell (headed by cell D89) before the NaCl is added. Any resulting corrections to the amount of NaCl needed should be made on the spreadsheet and in the lab.

COMMENTS

Ion-adjusted concentrate is used in tests 4 & 5 or in tests 4B and 5B.

Beginning with cell F27, the light-yellow cells within the double red border show the needed moles of each ion needed to create a balanced, ion-adjusted concentrate. All concentrations are in molar units so that charges can be simply balanced.

Typically, entering the moles of KBr (into cell B47) equivalent to the needed moles of Br⁻ (cell F34) is an appropriate first step. Entering the salts to provide the smallest needed-ion concentrations first generally works best.

Leave the Na⁺ and Cl⁻ ions until last. One should normally be able to reduce all the needed ions to zero with the exception of Na⁺ and/or Cl⁻. However, these ions are in much greater concentrations and the error should be minor. Cells G27 and G31 will give you information on the percent difference between these two ions.

If the 'salinity' is high or moderate, split any charge imbalance between Na⁺ and Cl⁻. If it is low, leave either Na⁺ or Cl⁻ in excess and neither in deficit. This is to provide the highest 'salinity' practical.

Mock concentrate is used in tests 3 and in preparing solutions for Tests 4B & 5B.

Proceed similarly to step 5.

Beginning with cell D62, the light-yellow cells within the double red border show the needed moles of each ion needed to create a balanced, ion-adjusted concentrate.

Typically, entering the moles of KBr (into cell B82) equivalent to the needed moles of Br⁻ (cell D69) is an appropriate first step. Entering the salts to provide the smallest needed-ion concentrations first generally works best.

Leave the Na⁺ and Cl⁻ ions until last. One should normally be able to reduce all the needed ions to zero with the exception of Na⁺ and/or Cl⁻. However, these ions are in much greater concentrations and the error should be minor. Cells E62 and E66 will give you information on the percent difference between these two ions.

If "salinity" is low, don't leave Na⁺ or Cl⁻ in deficit. If "salinity" is high, don't leave Na⁺ or Cl⁻ in excess. This is to minimize very high or low ionic strengths.

Be sure that the acid and base amounts are entered into the cell for the correct strength of acid or base.

With some concentrates, all Na⁺ and Cl⁻ may be added using salts other than NaCl. In those cases, the corrections will need to be made for the appropriate Na⁺ and Cl⁻ salts.

STEPS**COMMENTS**

8. If the salinity in cell E17 was 35 ppt or less, proceed to *Worksheet Makeup 1* and click on the tab labeled *Makeup 1* at the bottom of the workbook, otherwise click on the tab labeled *Salts 2 (4A & 5A)* at the bottom of the workbook and continue with the next step.

Worksheet Salts 2 (4A & 5A)***Determine salts for ion-adjusted concentrate A***

1. The information at the top of the worksheet duplicates that on worksheet *Salts 1*.
2. In the black-bordered blue cells headed by cell B38, enter the number of moles of salts needed to ion adjust the concentrate.

Ion-adjusted concentrate A is used in tests 4A & 5A.

Proceed similarly to step 5.

Beginning with cell F25, the light-yellow cells within the double red border show the needed moles of each ion needed to create a balanced, ion-adjusted concentrate. Some of the ions will show negative numbers. These ions are the ones being left unbalanced. Ignore them.

Typically, entering the moles of KBr (into cell B45) equivalent to the needed moles of Br⁻ (cell F32) is an appropriate first step. Entering the salts to provide the smallest needed-ion concentrations first generally works best.

Leave the Na⁺ and Cl⁻ ions until last. One should normally be able to reduce all the needed ions to zero with the exception of Na⁺ and/or Cl⁻. However, these ions are in much greater concentrations and the error should be minor. Cells G25 and G29 will give you information on the percent difference between these two ions.

If the 'salinity' is high or moderate, split any charge imbalance between Na⁺ and Cl⁻. If it is low, leave either Na⁺ or Cl⁻ in excess and neither in deficit. This is to provide the highest 'salinity' practical.

Note: multiple combinations of salts can provide the same results. There is no one correct set of salts. The goal is to select the combination of salts that minimizes or eliminates any 'leftover' ions.

8. Click on the tab labeled *Makeup 1* at the bottom of the workbook.

Worksheet Makeup 1

This worksheet calculates the amount of each salt needed for the solutions in *Salts 1* based upon the volume of each solution to be made. This volume will depend in part on the volume of the test containers. The default volumes can serve as starting points.

Ion-adjusted concentrate (or ion-adjusted concentrate B)

1. Cell C24 (dark yellow with double red border) will show the total volume of ion-adjusted concentrate (or ion-adjusted concentrate B) that will result. Enter various volumes of concentrate into cell C5 (blue with bold black border) until cell C24 shows the desired total volume.

This is a somewhat indirect method, but avoids the need for an iterative calculation and is quick to perform.

STEPS

- 2. The light-yellow cells within the double red border headed by cell C8 show the amount of each salt to weigh out.
- 3. If the ‘salinity’ of the resulting ion-adjusted concentrate would be >35 ppt, cell C23 (light yellow with double red border) shows the amount of DI water to include in producing the final solution.

Mock ion-adjusted concentrate (or mock ion-adjusted concentrate B)

- 4. As above, cell H24 (dark yellow with double red border) will show the total volume of mock ion-adjusted concentrate (or mock ion-adjusted concentrate B) that will result. Enter various volumes of mock concentrate into cell H5 (blue with bold black border) until cell H24 shows the desired total volume.
- 5. The light-yellow cells within the double red border headed by cell H8 show the amount of each salt to weigh out.
- 6. If the ‘salinity’ of the resulting mock ion-adjusted concentrate would be >35 ppt, cell H23 (light yellow with double red border) shows the amount of DI water to include in producing the final solution.

Mock concentrate

- 7. Enter the needed volume of mock concentrate into cell C29 (blue with bold black border).
- 8. The light-yellow cells within the double red border headed by cell C32 show the amount of each salt to weigh out.
- 9. This sheet is made to be printed out and includes columns to help QA/QC during the test manipulations.
- 10. Click on the tab labeled *Makeup 2* at the bottom of the workbook.

Worksheet Makeup 2

COMMENTS

Add these amounts of salts to the appropriate amount of unaltered concentrate in order to produce the appropriate ion proportions.

Salts will normally dissolve more easily if most of the DI water is added before the salts.

Add these amounts of salts to the appropriate amount of mock concentrate in order to produce the appropriate ion proportions.

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Add these amounts of salts to the appropriate amount of DI water in order to produce the appropriate ion proportions.

There is some saturation information to the right of some of the salts. It may sometimes be necessary to assemble the mock concentrate from a different array of salts to get the needed ions into solution.

Columns are included for the initials of the staff weighing out each salt, the balance used to weigh the salt, and the initials of the staff adding the salt to the solution. It is strongly recommended that these steps be taken to minimize errors. More than one copy of the sheets may be used.

This worksheet calculates the amount of each salt needed for the solutions in *Salts 2* based upon the volume of each solution to be made. This volume will depend in part on the volume of the test containers. The default volumes can serve as starting points.

STEPS

COMMENTS

Ion-adjusted concentrate A

- 1. Enter the needed volume of ion-adjusted concentrate A into cell C4 (blue with bold black border).
- 2. The light-yellow cells within the double red border headed by cell C7 show the amount of each salt to weigh out.

Add these amounts of salts to the appropriate amount of unaltered concentrate in order to produce the appropriate ion proportions.

Mock ion-adjusted concentrate A

- 3. Enter the needed volume of mock concentrate into cell H4 (blue with bold black border).
- 4. The light-yellow cells within the double red border headed by cell H7 show the amount of each salt to weigh out.
- 5. This sheet is made to be printed out and includes columns to help QA/QC during the test manipulations.
- 6. Click on the tab labeled *For Chem, no A&B* if Tests 4A &4B and 5A & 5B will not be necessary. Click on the tab labeled *For Chem, A&B* if Tests 4A &4B and 5A & 5B are necessary.

Add these amounts of salts to the appropriate amount of mock concentrate in order to produce the appropriate ion proportions.

Columns are included for the initials of the staff weighing out each salt, the balance used to weigh the salt, and the initials of the staff adding the salt to the solution. It is strongly recommended that these steps be taken to minimize errors. More than one copy of the sheets may be used.

Worksheet *For Chem, no A&B*
or
Worksheet *For Chem, A&B*

- 1. Print whichever of these sheets is appropriate and include them with samples being analyzed to confirm the final major seawater ion concentrations. All values are approximate and are generated automatically.
- 2. Click on the tab labeled *Follow-up Chem*.

These follow-up analyses are necessary to confirm the major seawater ion concentrations in the final solutions and the information on these sheets will assist the chemists in determining appropriate dilutions prior to analysis.

Note: these values are approximations.

Worksheet *Follow-up Chem*

- 1. When the results of the follow-up analytical chemistry are received, enter them into the appropriate cells of this worksheet. Blocks of cells where data can be entered are blue surrounded by a bold black border.

Carbonate concentrations are calculated by the spreadsheet from alkalinity values.

STEPS

2. Beside the columns of data for the various solutions are calculations comparing the various solutions to each other or to seawater of an appropriate salinity. Cells containing these calculations are light yellow.
3. Click on the tab labeled *Tox Results*.

Worksheet *Tox Results*

1. Enter the results of the toxicity tests into the appropriate places on this worksheet.
2. Compare test results to interpret cause(s) of toxicity. This interpretation requires a thorough understanding of the test methodology and rationale. Read *Protocols for Determining Major-Seawater-Ion Toxicity in Membrane-Technology Water-Treatment Concentrate* for more information.

COMMENTS

Good lab methods and good analytical chemistry should provide final ion concentrations within approximately 10% of the goals.

Results too far from those expected probably indicate laboratory errors in synthesis or analysis.

The cells that are editable are blue.

This sheet is intended to juxtapose the toxicity test results as an aid to interpretation. No calculations are performed by this sheet.

Protocols for Determining Major-Seawater-Ion Toxicity in Membrane-Technology Water-Treatment Concentrate is available from the Florida Department of Environmental Protection, Central Biology Laboratory, Tallahassee.