

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

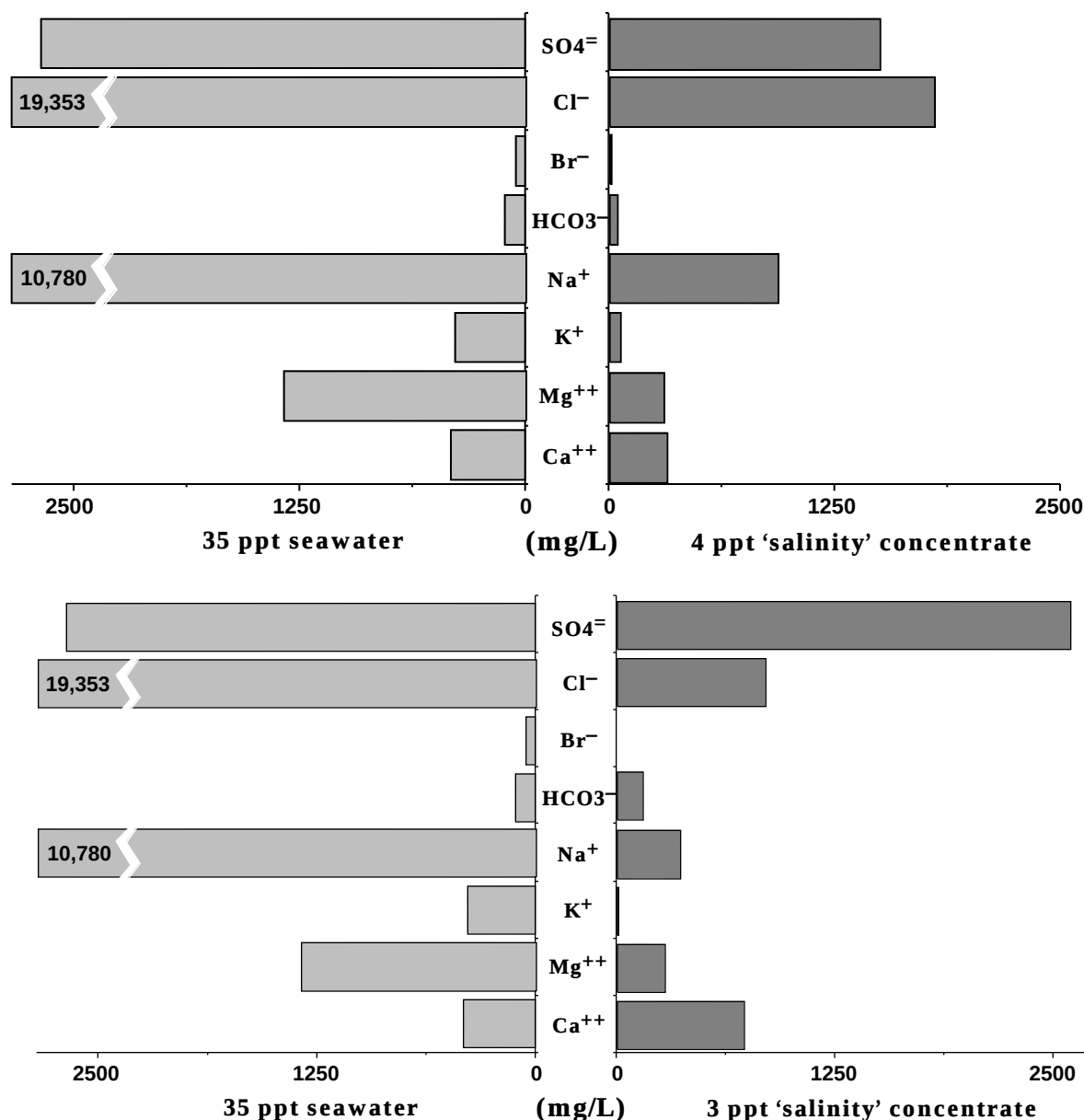


Figure 1. Comparisons of major-ion compositions of seawater and two low-salinity concentrate samples.

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.

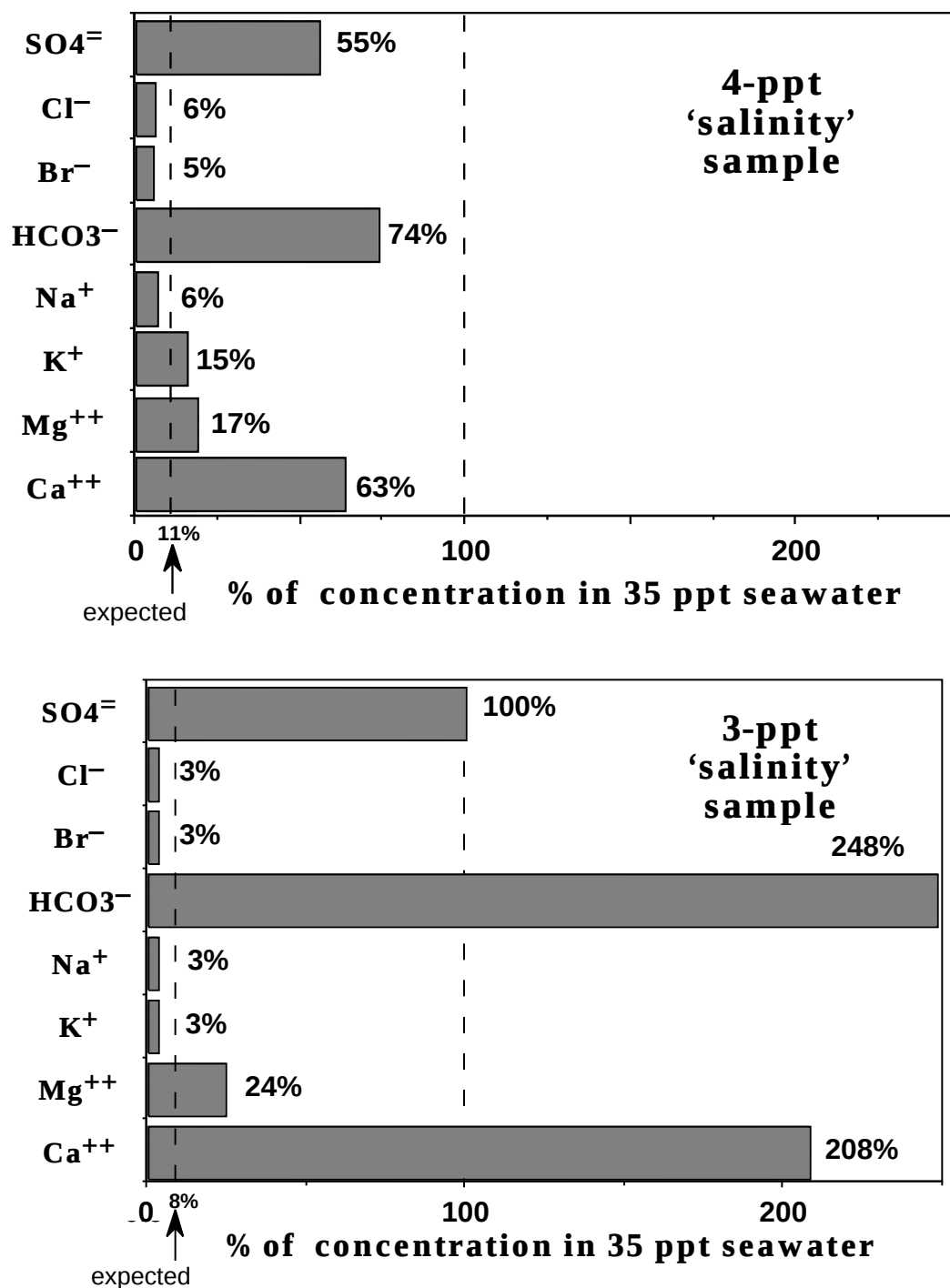


Figure 2. Comparison of proportions of the major-ion compositions of seawater and two low-salinity concentrate samples.

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

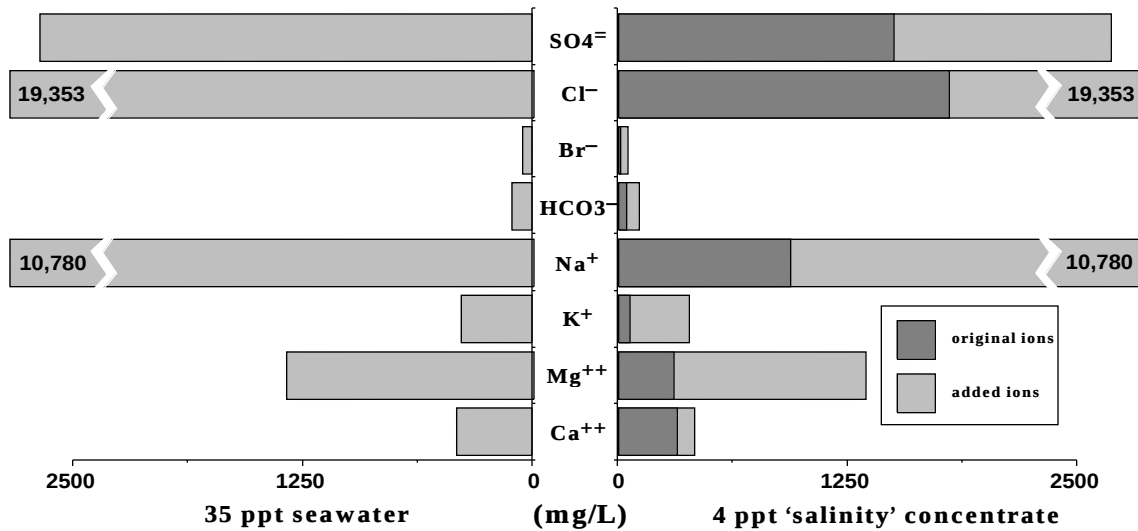


Figure 3. Major-seawater-ion correction of a 'low-salinity' concentrate sample.

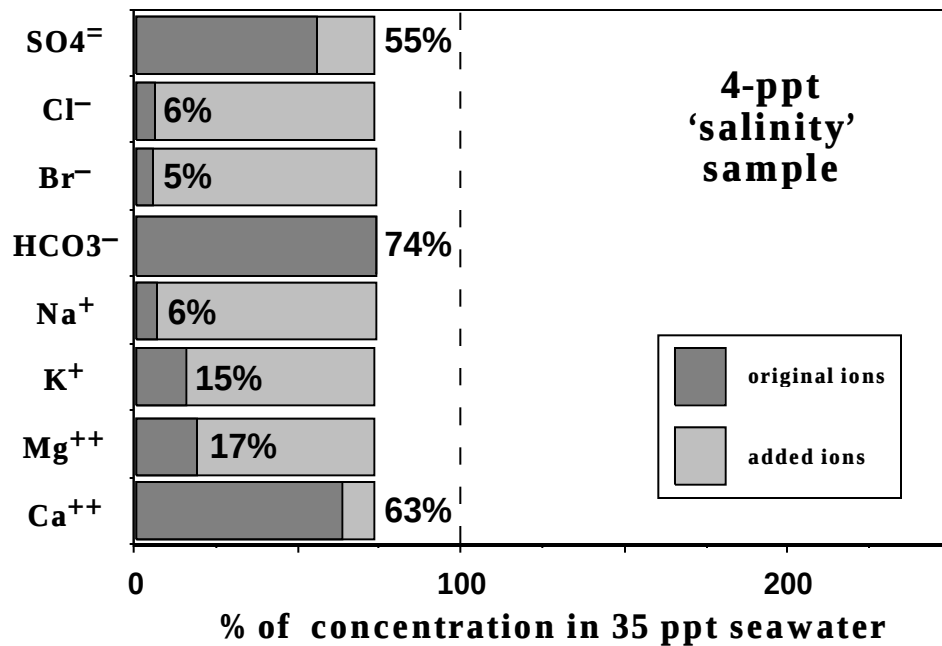


Figure 4. Relative proportions of major-seawater-ion correction of a 'low-salinity' concentrate sample.

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

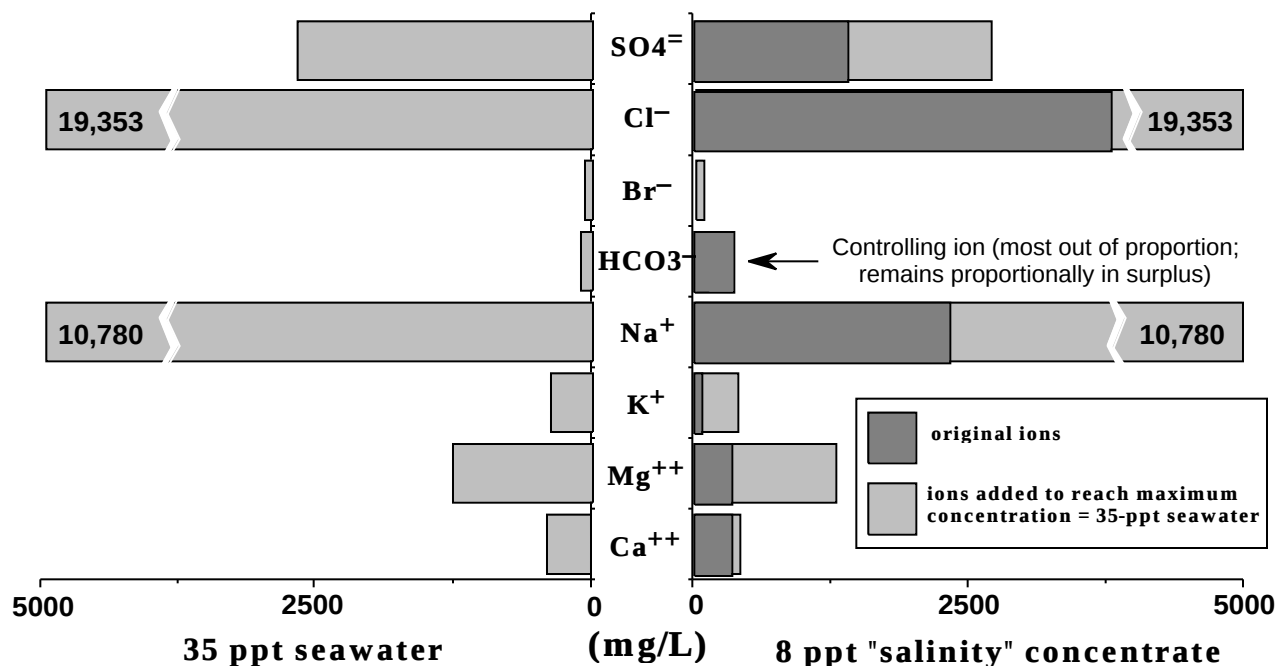


Figure 5. Example of ion correcting a moderate-‘salinity’ concentrate sample having one ion present at a concentration greater than that in 35-ppt seawater. This ion remains proportionally in surplus, with all other ions made proportional at 35-ppt seawater concentrations (per Tests 4A and 5A).

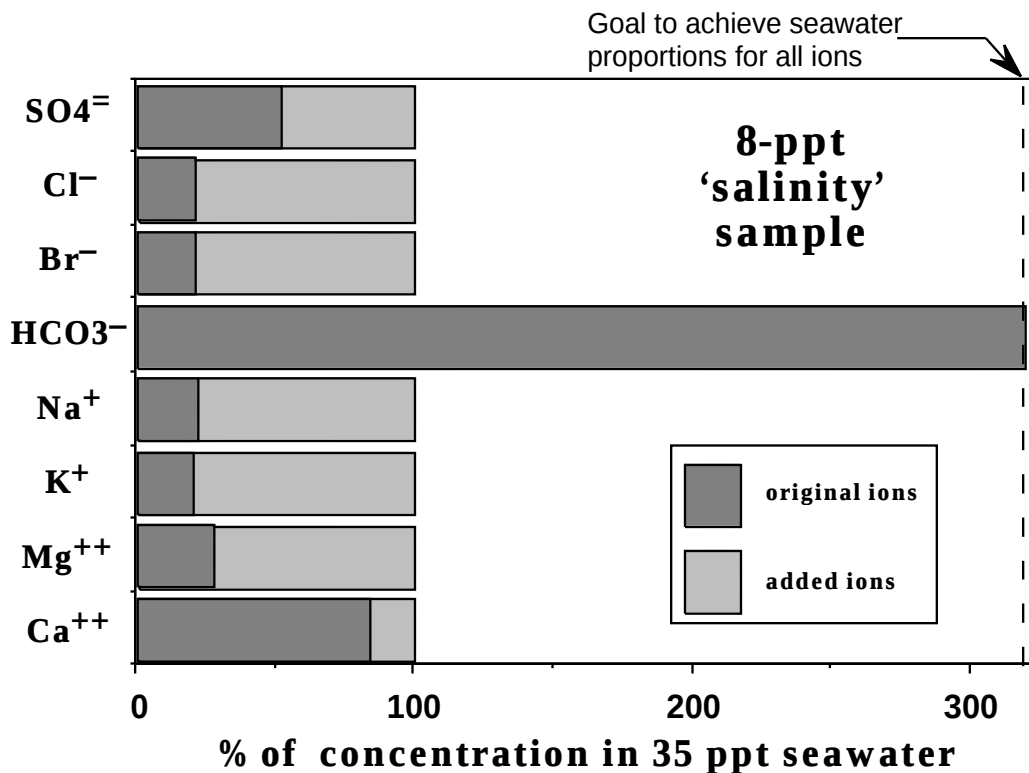


Figure 6. Proportion example of ion correction of a moderate-salinity concentrate sample, leaving one ion proportionally in surplus, with all

**other ions made proportional at 35-ppt
seawater concentrations (per Tests 4A and 5A).**

Toxicity Testing of Membrane-Technology Concentrate

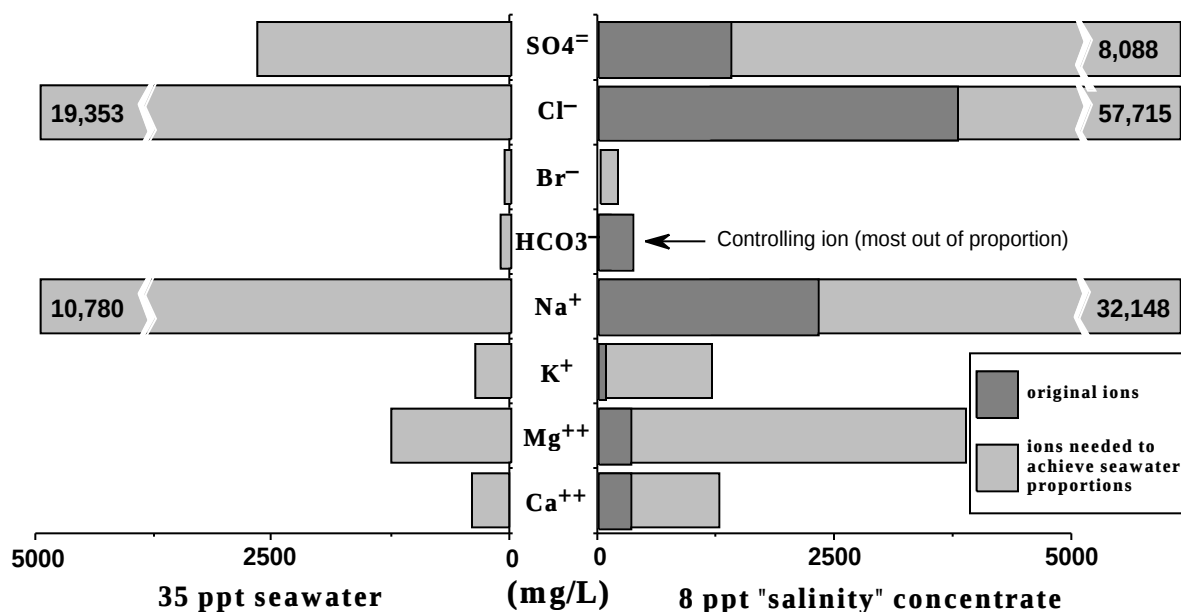


Figure 7. Ion correction of a moderate-salinity concentrate sample, requiring dilution (per Test 4B and 5B).

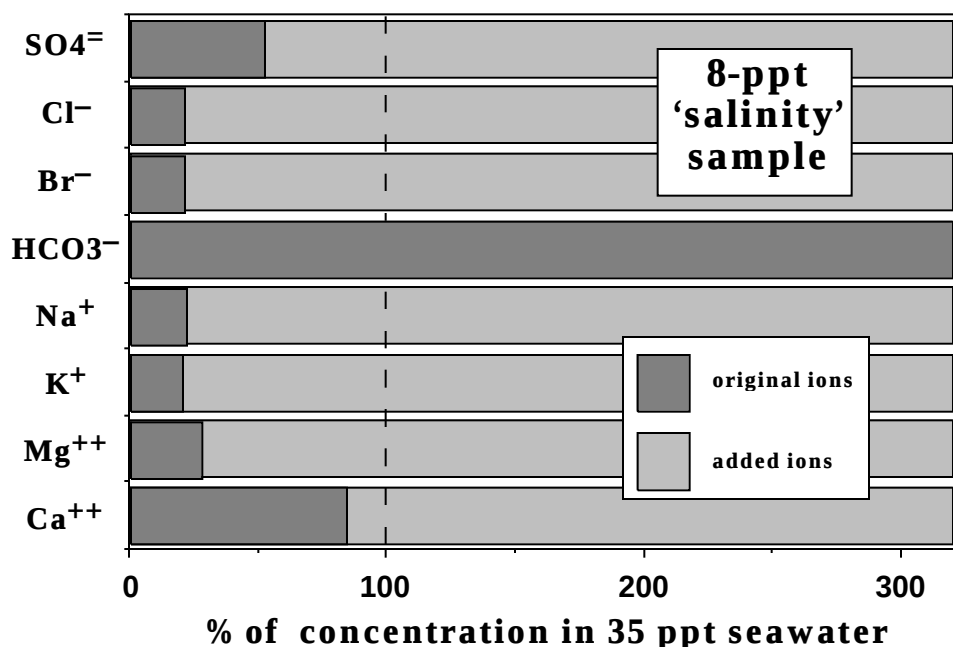


Figure 8. Proportion example of ion correction of a moderate-salinity concentrate sample, requiring dilution (per Test 4B and 5B).

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

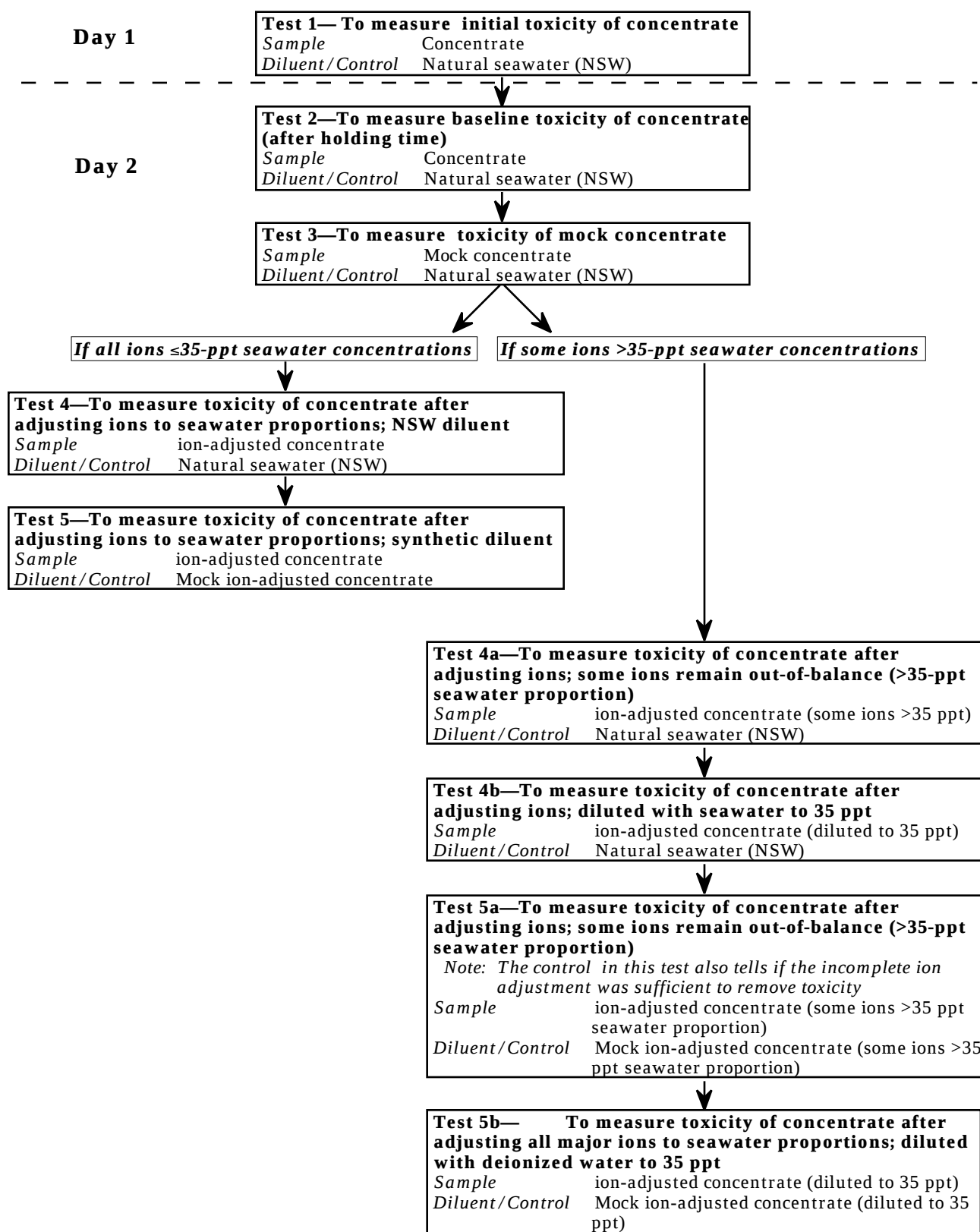


Figure 9. Test-method summary flowchart.

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 3—Facility Test Results From FDEP Study

Facility	Date tested	Page
Ft. Myers Membrane Softening	2/13/95	A3-1
Ft. Myers Membrane Softening	3/27/95	A3-3
Gasparilla RO	3/6/95	A3-5
Gasparilla RO	4/3/95	A3-7
Gasparilla RO	5/22/95	A3-9
Jupiter RO	3/13/95	A3-11
Jupiter RO	5/1/95	A3-13
Jupiter RO	6/6/95	A3-15
Sailfish Point RO	3/20/95	A3-17
Sailfish Point RO	4/24/95	A3-19
Sailfish Point RO	5/30/95	A3-21
Venice RO	2/27/95	A3-23
Venice RO	5/15/95	A3-25
Venice RO	6/12/95	A3-27
Vero Beach RO	2/21/95	A3-29
Vero Beach RO	4/17/95	A3-31
Vero Beach RO	5/8/95	A3-33

Appendix 4—Standard Operating Procedures (SOP's)

SOP #1: Preparation of Mock Sea-Salt Slurry

SOP #2: Advance Test Preparation

SOP #3: Test 1—Initial Test

SOP #4: Test 2—Baseline Test

SOP #5: Test 3—Mock Concentrate

SOP #6: Test 4—Ion-adjusted Concentrate with Natural Seawater as Diluent

SOP #7: Test 5—Ion-adjusted Concentrate with Mock Ion-adjusted Concentrate as Diluent

SOP #8: Test-Organism Loading, Interim Readings, and Test Termination

SOP #9: Preparation of Stock Salt Solutions

SOP #10: Preparation of Stock HCl (Acid) Solutions

SOP #11: Preparation of Stock NaOH (Base) Solutions

SOP #12: Calculation of Ion Additions