September 2003

Ammonia Water Effects Ratios and Site-Specific Objectives for Los Angeles County Waterbodies-Final Results

Submitted to:
County Sanitation Districts of Los Angeles County
City of Los Angeles
City of Burbank

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Executive Summary

In 1999, the City of Los Angeles, County Sanitation Districts of Los Angeles County (CSDLAC), and the City of Burbank began the development of a site-specific freshwater objective for ammonia. The chosen approach was to develop a Water Effects Ratio (WER) downstream of ten wastewater treatment plant (POTW) discharges to effluent dominated water bodies in the Los Angeles River, San Gabriel River, and Santa Clara River watersheds. This report provides a summary of the results of that study and the proposed WERs and site-specific objectives (SSOs) for these waterbodies. Based on the results of the study, the WERs will be used to calculate chronic site-specific objectives. Acute site-specific objectives are not being proposed as a part of this study. The complete work plan for the study is included in Appendix 1-*Ammonia Water Effects Ratio and Site Specific Objective Work Plan for the Los Angeles County Waterbodies*.

SAMPLING SCHEDULE AND LOCATIONS

Samples were collected at ten stations, each downstream of a wastewater treatment plant. At all but one station, four acute *Hyalella azteca* toxicity tests and one chronic *Pimephales promelas* (fathead minnow) test were collected. Additionally, at five stations, a chronic *Hyalella azteca* test was conducted to confirm that the use of acute tests to establish WER values was appropriately conservative for the purposes of this study. As a result of some QA/QC problems with the analysis of some samples, four acute *Hyalella* tests, two chronic *Hyalella* tests and three chronic fathead minnow tests were rejected and not used in the study analysis. Therefore, a total of 35 acute *Hyalella* tests, three *Hyalella* chronic tests, and seven chronic fathead minnow tests were successfully conducted during this study. The acute *Hyalella* tests were conducted during both dry and wet weather to assess the impacts of different seasons on the WER. Sampling began in January 2002 and was completed in February 2003. In addition, an initial study to assess the potential for developing a WER for ammonia was conducted in October 2000 at two sites on the Los Angeles River and at two sites on the San Gabriel River. The following table (ES-1) summarizes the sampling locations for the study and a map of the sampling locations is included as Figure ES-1.

Table ES- 1. POTW Characteristics and Associated Sampling Locations

Name	Agency	Main Receiving Water	Design / Permitted Flow (mgd)	Typical Dry Weather Upstream Flow (mgd)	Sampling Location ID	Description
DC Tillman	City of Los Angeles	Los Angeles River	80	NA	LA-1, LA-R8	Downstream of DC Tillman at Van Nuys Blvd. and Coldwater Canyon
LA-Glendale	City of Los Angeles	Los Angeles River	20	51	LA-2, LA-R7	Downstream of LA Glendale at Los Feliz
Burbank WWTP	City of Burbank	Burbank Western Wash/Los Angeles River	9	NA	BW-1	Downstream of Burbank at Riverside Dr.
Saugus	CSDLAC	Santa Clara River	6.5	0	SCR-1	Downstream of Saugus- 25 feet downstream of discharge
Valencia	CSDLAC	Santa Clara River	12.6	5.4	SCR-2	Downstream of Valencia, 1.6 miles upstream of Chiquita Canyon Road.
Whittier Narrows	CSDLAC	Rio Hondo/San Gabriel River	15	NA	RH-1	Downstream of Whittier Narrows WRP 150 feet upstream of the Whittier Narrows Dam
Los Coyotes	CSDLAC	San Gabriel River	37.5	0	SGR-2, SGR-R9W	Downstream of Los Coyotes at Willow
Long Beach	CSDLAC	Coyote Creek	25	10.3	CC-1	Downstream of Long Beach at foot bridge 200 yards downstream of discharge
San Jose Creek	CSDLAC	San Gabriel River/San Jose Creek	100	0	SGR-1, SGR-R4	Downstream of San Jose Creek WRP at Alondra
Pomona	CSDLAC	San Jose Creek	15	0	SJC-1	Downstream of Pomona WRP at San Jose St.

NA Flow information is not available, but is likely to be minimal.

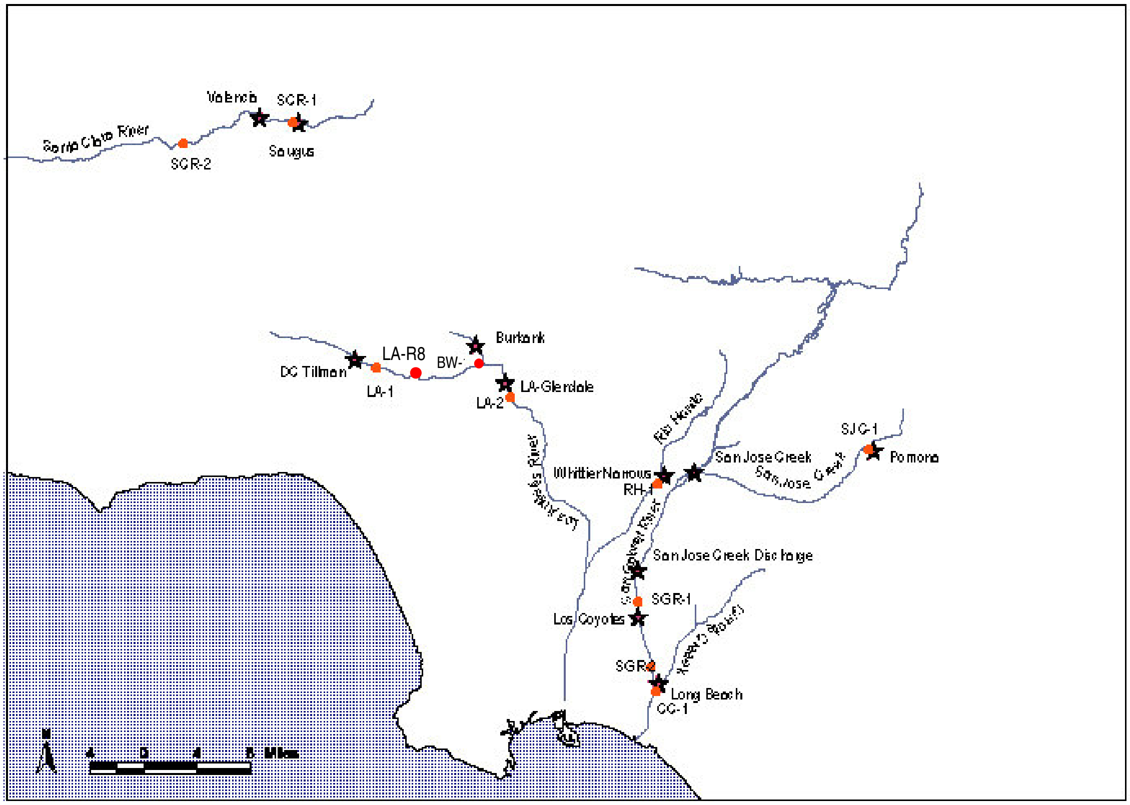

Figure ES- 1. Map of Sampling Locations

ANALYTICAL APPROACH

During the testing, it became clear that a WER greater than 1.0 for the sensitive invertebrate species, *Hyalella*, occurred in the waterbody, but a WER for a sensitive fish species, fathead minnow, was closer to 1. Consequently, an adjustment was made to the analytical approach, based on discussions with the Technical Advisory Committee (TAC) for the study, to take this fact into consideration. It was decided that to develop a SSO for ammonia, the WER calculated from the *Hyalella* data would be used to adjust the invertebrate data used to calculate the ammonia criteria whereas the fish data used in the criteria equation calculation would not be adjusted. After the adjustments for the invertebrate data, the criteria would be recalculated to determine the SSO. In these calculations, the objective is determined by the lower of 1) the temperature-adjusted Hyalella GMCV and 2) the lowest fish GMCV. This approach results in a SSO that is protective of both invertebrate and fish species.

Additionally, the TAC requested that the pH relationship for *Hyalella* be examined to determine whether or not it matched the pH relationship developed in the USEPA's 1999 Ammonia Criteria (criteria). The pH relationship is a critical part of the study because it is used to adjust the results from the laboratory dilution water tests to equivalent results at the same pH as the site water (before the WER is calculated). A separate pH study was conducted and the results of that study as well as the results from all of the laboratory dilution water tests were compared to the criteria pH relationship to determine if differences existed that justified the development of a separate pH relationship for *Hyalella*. The comparison demonstrated that, at least for the average pH values found in the waterbodies in this study (7.34 to 8.05), the *Hyalella* pH relationship does not appear to be significantly different from the criteria pH relationship. Additionally, the use of a pH relationship developed based on the study results in WERs that are much higher than the WERs calculated using the EPA pH relationship (See Table 9 in the report). So the use of the EPA pH relationship is a conservative approach to developing the WERs and SSOs for the study. As a result, a separate pH relationship was not used to calculate the WERs and SSOs for the study.

In addition, regression analyses were performed based on the results of the study to determine if any significant relationships could be ascertained between water quality constituents and the resulting toxicity in the waterbody. The analysis of the water quality constituents demonstrated that the ions tested (sodium, potassium, calcium, chloride, and TDS) have a statistically significant correlation to the toxicity of ammonia. However, the ions also have a more significant correlation to each other. Consequently, it is difficult to determine which specific ion or combination of ions has the greatest impact on reducing the toxicity of ammonia. A number of regression analyses were performed with different ions separately and in combination with other ions and the results demonstrated that sodium and chloride have similar relationships to toxicity, but sodium and TDS have the strongest relationship with the WER. None of the relationships had a high enough r-squared (R^2) to be predictive (all R2 values were 0.4 or less). A site-by-site analysis was also conducted to see if some of the variability could be reduced and more significant relationships determined. The analysis showed that different ions were the most significant influence in different waterbodies, but for the most part, increasing ion concentrations resulted in less toxicity and increasing WERs. The demonstration of these relationships shows that ions do appear to be the major site-specific driver in reducing ammonia toxicity. This phenomenon has been identified in other studies, though the exact mechanism(s) for the reduced toxicity have not been identified. The prevailing theory is that the ions in the water increase the ability of the organism to excrete ammonia and potentially reduce the uptake of ammonium ions by the organism (Borgmann, 1997). Other water quality constituents, such as BOD and TSS, did not demonstrate significant relationships to ammonia toxicity.

Several different SSO values were calculated based on the data collected. The first was a site-by-site SSO. Secondly, WERs and SSOs were calculated on a watershed basis. Finally, one WER was calculated based on all the data. All of the WER values were calculated based on the procedures presented in the *Interim Guidance on the Development of WERs for Metals* (USEPA, 1994). After the SSO values were calculated, the results were compared to the toxicity thresholds for any rare, endangered, threatened, or locally important species present in the waterbody to ensure that the results were protective of those species.

STUDY RESULTS AND PROPOSED WERS AND SSOS

The acute *Hyalella* tests resulted in WERs ranging from 1.395 to 2.303. The chronic *Hyalella* tests demonstrated much higher WERs, ranging from 7.025 to 44.59. Therefore, it was determined that it was conservative to use the acute tests to calculate the final WERs. The fathead minnow tests, as discussed previously, all had WERs around 1, ranging from 0.937 to 1.714. The WER guidance suggests the use of the lowest wet weather WER or the adjusted geometric mean of the dry weather results. As discussed in detail in the main report, the wet weather WERs were found to be extremely variable at a given site depending on the choice of values used to calculate the wet weather WER (e.g. upstream flow, ammonia concentration). For this reason, it was not considered appropriate to use a single, variable wet weather value instead of the adjusted geometric mean of the dry weather values. Therefore, the final WER is equal to the adjusted geometric mean of the acute *Hyalella* dry weather samples at all sites. Table ES- 2 presents the recommended final WERs for the study based on the acute *Hyalella* testing calculated for all of the scenarios discussed in the approach. The actual toxicity results are presented in the Results tables in Appendix 2-Summary of Study Results. Based on the results of the analysis, the recommended approach is to use the site-specific WERs to calculate site-specific SSOs.

Table ES- 2. Final WERs

Site	Recommended Final WER
LA1	1.966
LA2	1.967
BW1	1.400
SGR1	1.637
SGR2	2.303
CC1	2.038
SJC1	1.395
RH1	2.094
SCR1	2.233
SCR2	2.206
LA River	1.783
San Gabriel River	2.032
Santa Clara River	2.282
All Sites	1.956

To calculate the SSOs for a waterbody, a new criteria equation was developed for each of the scenarios. Each equation was calculated based on EPA guidance for determining aquatic life criteria. The SSOs are all equal to the pH relationship multiplied by the lower of 1) the *Hyalella* value adjusted by the WER or 2) the lowest fish value. This ensures that the SSOs are protective of both fish and invertebrates. The proposed SSO criteria equations based on the final WERs are shown in Table ES- 3.

Table ES- 3. Proposed Chronic Site-Specific Objectives

Site	Equation
LA1	**ELS Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.854 * MIN(2.85, 2.85 * 10^{0.028*(25-T)})$ **ELS Not Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.854 * 2.85 * 10^{0.028*(25-Max(T,7))}$
LA2	**ELS Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.854 * MIN(2.85, 2.85 * 10^{0.028*(25-T)})$ **ELS Not Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.854 * 2.85 * 10^{0.028*(25-Max(T,7))}$
BW1	**ELS Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.92 * MIN(2.85, 2.03 * 10^{0.028*(25-T)})$ **ELS Not Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.92 * 2.03 * 10^{0.028*(25-Max(T,7))}$
SGR1	**ELS Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.89 * MIN(2.85, 2.37 * 10^{0.028*(25-T)})$ **ELS Not Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.89 * 2.37 * 10^{0.028*(25-Max(T,7))}$
SGR2	**ELS Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.854 * MIN(2.85, 3.34 * 10^{0.028*(25-T)})$ **ELS Not Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.854 * 3.34 * 10^{0.028*(25-Max(T,7))}$

Table ES-3 cont'd. Proposed Site-Specific Objective Equations for Ammonia by Site

SCR1	**ELS Present** $CCC = \left(\frac{0.0676}{1 + 10^{7.688-pH}} + \frac{2.912}{1 + 10^{pH-7.688}} \right) * 0.854 * MIN(2.85, 3.24 * 10^{0.028*(25-T)})$ **ELS Not Present** $CCC = \left(\frac{0.0676}{1 + 10^{7.688-pH}} + \frac{2.912}{1 + 10^{pH-7.688}} \right) * 0.854 * 3.24 * 10^{0.028*(25-Max(T,7))}$
SCR2	**ELS Present** $CCC = \left(\frac{0.0676}{1 + 10^{7.688-pH}} + \frac{2.912}{1 + 10^{pH-7.688}} \right) * 0.854 * MIN(2.85, 3.20 * 10^{0.028*(25-T)})$ **ELS Not Present** $CCC = \left(\frac{0.0676}{1 + 10^{7.688-pH}} + \frac{2.912}{1 + 10^{pH-7.688}} \right) * 0.854 * 3.20 * 10^{0.028*(25-Max(T,7))}$
SJC1	**ELS Present** $CCC = \left(\frac{0.0676}{1 + 10^{7.688-pH}} + \frac{2.912}{1 + 10^{pH-7.688}} \right) * 0.92 * MIN(2.85, 2.02 * 10^{0.028*(25-T)})$ **ELS Not Present** $CCC = \left(\frac{0.0676}{1 + 10^{7.688-pH}} + \frac{2.912}{1 + 10^{pH-7.688}} \right) * 0.92 * 2.02 * 10^{0.028*(25-Max(T,7))}$
RH1	**ELS Present** $CCC = \left(\frac{0.0676}{1 + 10^{7.688-pH}} + \frac{2.912}{1 + 10^{pH-7.688}} \right) * 0.854 * MIN(2.85, 3.04 * 10^{0.028*(25-T)})$ **ELS Not Present** $CCC = \left(\frac{0.0676}{1 + 10^{7.688-pH}} + \frac{2.912}{1 + 10^{pH-7.688}} \right) * 0.854 * 3.04 * 10^{0.028*(25-Max(T,7))}$
CC1	**ELS Present** $CCC = \left(\frac{0.0676}{1 + 10^{7.688-pH}} + \frac{2.912}{1 + 10^{pH-7.688}} \right) * 0.854 * MIN(2.85, 2.96 * 10^{0.028*(25-T)})$ **ELS Not Present** $CCC = \left(\frac{0.0676}{1 + 10^{7.688-pH}} + \frac{2.912}{1 + 10^{pH-7.688}} \right) * 0.854 * 2.96 * 10^{0.028*(25-Max(T,7))}$

Table ES- 4 provides example objectives based on the site-specific equations listed above for different pHs. The table allows comparison of the site-specific objectives determined in this study.

Table ES- 4. Example Site Specific Objectives (Total Ammonia in mg-N/L) at Different pHs

		pH							
	Temperature	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**
LA1	20	9.6	9.2	8.2	6.0	3.4	1.5	0.67	0.37
LA2	20	9.6	9.2	8.2	6.0	3.4	1.5	0.67	0.37
BW1	20	7.4	7.1	6.3	4.6	2.6	1.2	0.52	0.29
SGR1	20	8.3	8.0	7.1	5.2	2.9	1.3	0.58	0.32
SGR2	20	11.2	10.8	9.6	7.1	3.9	1.8	0.79	0.44
CC1	20	9.9	9.5	8.5	6.2	3.5	1.6	0.70	0.39
SJC1	20	7.4	7.1	6.3	4.6	2.6	1.2	0.52	0.29
RH1	20	10.2	9.8	8.7	6.4	3.6	1.6	0.72	0.40
SCR1	20	10.9	10.5	9.3	6.8	3.8	1.7	0.76	0.42
SCR2	20	10.8	10.3	9.2	6.8	3.8	1.7	0.75	0.42

Introduction and Background

Starting in 1999, the County Sanitation Districts of Los Angeles County (CSDLAC), City of Los Angeles, and City of Burbank began a study to investigate the development of site-specific objectives (SSOs) for ammonia using a water effects ratio (WER). This report describes the results of the study and the analysis conducted to determine the WERs and SSOs for the waterbodies included in the study.

Ambient water quality criteria are set at the national level by the United States Environmental Protection Agency to be protective of conditions throughout the United States. Because of the variety of waterbodies and differing conditions throughout the country, the criteria developed on the national level might be over- or under-protective for some waterbodies. Beyond the headwaters, the waterbodies in Los Angeles County are typically effluent-dominated waterbodies running through concrete-lined channels or significantly altered watercourses. Characteristics of these waterbodies, such as high hardness and ionic composition, vary from conditions in other more “natural” waterbodies that contain flow other than urban runoff and publicly owned treatment works (POTWs) discharges. The objective of this study is to develop site-specific chronic objectives for ammonia in Los Angeles County waterbodies that are sufficiently protective of the aquatic habitat in these waterbodies. Site-specific acute objectives are not being proposed as a part of this study.

In 1999, the USEPA issued an update to the 1984 Ambient Water Quality Criteria for Ammonia. In both of the criteria documents, the USEPA acknowledged that ammonia toxicity may be dependent on the ionic composition of the exposure water, but the effects and understanding of these effects were insufficient to allow inclusion of them in the national criteria derivation. The 1999 Ammonia Criteria update states that these effects will "have to be addressed using water-effect ratios or other site-specific approaches" (USEPA, 1999). Studies cited in the 1999 Ammonia Criteria update include several studies done to investigate the impacts of the ionic composition of the exposure water on the toxicity of ammonia to a number of species, including Atlantic salmon, lake trout, rainbow trout, *Ceriodaphnia dubia*, and *Hyalella azteca*. The results of these studies indicate that the toxicity of ammonia may be reduced in waterbodies similar to those found in Southern California with high hardness and elevated concentrations of certain ions (calcium, sodium, and potassium). Because the waterbodies in Los Angeles County are primarily effluent-dominated, the hardness and ionic concentrations in these waterbodies are much higher than the concentrations found in the laboratory dilution water used in the studies that were the basis for the ammonia criteria. For this reason, there is a potential to develop a WER for ammonia in these waterbodies.

TEST SPECIES SELECTION

In the 1999 Ammonia Criteria update, the chronic criteria were developed based on a limited number of chronic toxicity studies. The most sensitive species used in the development of the criteria was *Hyalella azteca* (see 1999 Update, p. 76). The chronic study used in the development of the criteria was conducted by Uwe Borgmann in 1994. Borgmann also conducted acute toxicity tests on *Hyalella* that indicate that hardness and concentrations of certain ions may have a significant impact on the toxicity of ammonia to *Hyalella*.

The magnitude of a WER is likely to depend on the sensitivity of the test used to determine the WER. More sensitive tests are expected to result in higher WERs and less sensitive tests will result in WERs closer to 1 (USEPA, 1994). The WER guidance states that there is no reason to believe that different species with equally sensitive endpoints will result in different WERs. It is possible that the mode of action might differ from species to species and therefore the magnitude of the WER may vary. However, there are no data that support any conclusions about the existence or magnitude of such differences (USEPA, 1994).

Based on these requirements in the WER guidance, *Hyalella azteca* was chosen as the primary test species for the study. As discussed previously, this species is the most sensitive aquatic species used in the development of the chronic criteria in the 1999 Ammonia Criteria update. The endpoint of the *Hyalella* chronic toxicity test is close to, but not lower than, the chronic criteria for these waterbodies at the pH values observed in the waterbodies. The *Hyalella* acute toxicity endpoint value is higher than the acute criteria for these waterbodies. Additionally, initial tests have demonstrated that the conditions in the Los Angeles and San Gabriel Rivers significantly affect the toxicity of ammonia to this species. For these reasons, *Hyalella* is an appropriate species to use in the development of a WER for these waterbodies.

The WER guidance requires that at least one test be conducted with a secondary species to confirm the results with the primary species. Based on a review of the 1999 criteria document and other studies that have been conducted, the recommended secondary species for waterbodies designated as WARM at the discharge point is the fathead minnow (*Pimephales promelas*). The fathead minnow is the 4th most sensitive species used in the development of the chronic criteria in the 1999 Ammonia criteria update. It is also one of the species used by all of the dischargers participating in the study in their Whole Effluent Toxicity (WET) testing of effluent. As a result, determining the level of ammonia toxicity to this species in the rivers to which the POTWs discharge will correspond with the requirements to prevent chronic toxicity in effluent discharges to the river. Studies have not been conducted on this species to determine whether or not the conditions in the waterbodies in Los Angeles County have an impact on the toxicity of ammonia to this species.

For the purposes of this study, acute *Hyalella* studies are the basis of the development of the chronic WER. As discussed in the Calculation of the Final WER, the acute toxicity tests resulted in a lower WER than the chronic studies. The resulting SSO is therefore very conservative. Additionally, the shorter and less costly acute studies allowed more studies to be conducted. Finally, the acute toxicity test for *Hyalella* is a more frequently used and established test than the chronic toxicity test so there are more data from other laboratories to compare to the monitoring results. The WER guidance specifically outlines that the endpoint of the test is the determining factor for selecting the test, not whether or not the test is chronic or acute. As a result, according to the guidance, a WER developed using acute toxicity tests may be applied to a chronic criterion and vice versa as long as the endpoint of the primary test is not lower than the criteria being adjusted.

Since the WER is dependent on the sensitivity of the test and not the type of test (i.e. acute or chronic) either type of test may be used to adjust the chronic criterion. However, because *Hyalella* acute tests were not included in the development of the acute criteria, the ability of the proposed SSO to protect invertebrates that may be more acutely sensitive than *Hyalella* was assessed.

The 1994 Interim WER Guidance states on page 21 that "less sensitive toxicity tests usually give smaller WERs than more sensitive tests." The comparison between the more sensitive chronic *Hyalella* tests and the less sensitive acute *Hyalella* tests conducted for this study confirmed that statement by demonstrating that the chronic tests had much higher WERs than the acute tests. Based on this premise, the use of *Hyalella* rather than a more acutely sensitive species should result in a more conservative WER and be protective of the other invertebrates.

Although acute tests on *Hyalella* were not included in the calculation of the acute criterion, a study by Ankley (Ankley, 1995) was reviewed for implications on the criterion and discussed as being an appropriate study within the 1999 update. However, the study was not considered to have an impact on the acute criterion calculation and was therefore not added to the dataset. The Ankley study looked at a number of different pHs and water types (soft water, moderately hard water, and hard water). The range of LC50s for these studies was from 3.9 to 83.9 mg/L-N normalized to a pH of 8.0. Based on these results, the GMAV for *Hyalella* would be 25.3 mg/L-N total ammonia. This GMAV makes *Hyalella* the most acutely sensitive invertebrate tested for the acute criteria as well as the chronic criteria and the 7th most acutely sensitive species overall. Additionally, under soft water and low pH conditions, *Hyalella* is the most acutely sensitive species. Therefore, the use of acute tests for *Hyalella* is protective of invertebrates tested for both the acute and chronic criteria development, though adjustments to the chronic criterion only are being proposed in this study. Although *Hyalella* is not more acutely sensitive than fish species, the protection of fish was addressed through the SSO calculation process and is described in the Site-Specific Objectives section.

STUDY SUMMARY

Ten sampling locations on eight waterbodies were sampled downstream of ten wastewater treatment plants (POTWs). At all but one station, four samples were collected for the primary test species, *Hyalella azteca*, and one sample was collected for the secondary species, *Pimephales promelas* or fathead minnow. The primary test for *Hyalella* was an acute, 4-day test. A 21-day chronic test for *Hyalella* was also conducted at five of the ten locations to ensure the acute test results were protective of chronic conditions. Due to some Quality Assurance/Quality Control (QA/QC) problems with the data, not all of the collected samples were used in the analysis. Table 2 summarizes all of the samples collected and highlights the tests that were not used in the WER and SSO calculations. The following table (Table 1) summarizes the ten POTWs and associated sampling locations included in the study. For a complete description of the study, sampling procedures, and discharge characteristics, please refer to the monitoring plan in Appendix 1-*Ammonia Water Effects Ratio and Site-Specific Objective Workplan for Los Angeles County Waterbodies*.

Table 1. POTW Characteristics and Associated Sampling Locations

Name	Agency	Main Receiving Water	Design / Permitted Flow (mgd)	Typical Dry Weather Upstream Flow (mgd)	Sampling Location ID	Description
DC Tillman	City of Los Angeles	Los Angeles River	80	NA	LA-1, LA-R8	Downstream of DC Tillman at Van Nuys Blvd. and Coldwater Canyon
LA-Glendale	City of Los Angeles	Los Angeles River	20	51	LA-2, LA-R7	Downstream of LA Glendale at Los Feliz
Burbank WWTP	City of Burbank	Burbank Western Wash/Los Angeles River	9	NA	BW-1	Downstream of Burbank at Riverside Dr.
Saugus	CSDLAC	Santa Clara River	6.5	0	SCR-1	Downstream of Saugus- 25 feet downstream of discharge
Valencia	CSDLAC	Santa Clara River	12.6	5.4	SCR-2	Downstream of Valencia, 1.6 miles upstream of Chiquita Canyon Road.
Whittier Narrows	CSDLAC	Rio Hondo/San Gabriel River	15	NA	RH-1	Downstream of Whittier Narrows WRP 150 feet upstream of the Whittier Narrows Dam
Los Coyotes	CSDLAC	San Gabriel River	37.5	0	SGR-2, SGR-R9W	Downstream of Los Coyotes at Willow
Long Beach	CSDLAC	Coyote Creek	25	10.3	CC-1	Downstream of Long Beach at foot bridge 200 yards downstream of discharge
San Jose Creek	CSDLAC	San Gabriel River/San Jose Creek	100	0	SGR-1, SGR-R4	Downstream of San Jose Creek WRP at Alondra
Pomona	CSDLAC	San Jose Creek	15	0	SJC-1	Downstream of Pomona WRP at San Jose St.

NA Flow information is not available, but the flow is likely to be minimal.

Sampling Schedule

Due to field conditions and unexpected sampling complications, a number of changes were made to the sampling schedule presented in the work plan (Appendix 1). The following table (Table 2) summarizes the actual sampling schedule and all samples collected for the study. Some of the sample results were not used in the analysis because of sampling problems, but they are included in strikeout text for reference in this table. The problems with specific tests are noted in the table footnotes.

Table 2. Sampling Schedule

Date	BW1	LA1	LA2	CC1	RH1	SCR1	SCR2	SGR1	SGR2	SJC1
1/31/02		HA	HA			HA	HA			
3/4/02	HA			HA				HA	HA	HA
4/1/02		~~F1~~	HC, ~~F7~~		HA		HA			
4/9/02		~~F1~~	~~F1~~							
4/16/02				HA					HA	
4/29/02	~~F1~~						~~F15~~ (1)		~~F15~~ (1), ~~F7~~ (1)	
5/15/02 (2)	HA	~~HA~~ (5)	~~HA~~(5)							
6/4/02					HA	HA	HA			
6/12/02								HA		HA
6/18/02				HA					HA	
6/25/02		HA	HA							
7/9/02				F	F		F			F
7/16/02	HA					HA	HA			
7/23/02		F	F						F	
8/6/02	~~HC~~(6)						~~HC~~(6)			
8/20/02 (3)	~~F7~~					~~F7~~		~~F7~~		
8/27/02					~~HA~~(7)					~~HA~~(7)
9/10/02				HC	HC				HC	
9/24/02 (4)	~~F~~(8)					~~F~~(8)		~~F~~(8)		
12/17/02 (Wet)	HA		HA	HA					HA	HA
2/12/03 (Wet)		HA			HA	HA	HA	HA		

HA-Hyalella acute test.
HC-Hyalella chronic test
F-Complete Fathead 28 day test
F1-Fathead 1-day test. High mortality in site water resulted in early termination of test.
F7-Fathead 7-day test. High mortality in site water resulted in early termination of test.
F15-Fathead 15-day test
1-These tests were run as experiments to assess the sensitivities of juveniles vs. larval fathead minnows and mechanisms for running the fathead tests successfully without high initial mortality.
2-The renewal concentrations for the tests run on this date were switched for the 150 mg/L and 250 mg/L concentrations. The laboratory reported the results and felt that they were able to take into account the effect of this switch during the statistical analysis of the results.
3-Problems with pH control, test rerun on 9/24/02.
4-MHW sample contained parasite, no results.
5-Dissolved oxygen levels dropped below required minimum levels for an extended period of time so the tests were rejected for QA/QC reasons.
6-Control results were below acceptable levels and dissolved oxygen levels dropped below required minimum levels for an extended period of time so the tests were rejected for QA/QC reasons
7-Control results were below acceptable levels so the tests were rejected for QA/QC reasons
8-A parasite was found in the laboratory dilution water sample so the tests were rejected for QA/QC reasons.

Only three *Hyalella* acute samples were collected at SGR1 due to a malfunctioning valve. During the summer, much of the water from the San Jose Creek WRP is diverted to spreading grounds for reclamation purposes. In order to maintain consistent samples throughout the monitoring events, the amount of flow diverted to the spreading grounds had to be adjusted during sampling events. For a period of time during the monitoring events, the valve that adjusted the flow volumes was broken and could not be used to reduce the diversion volumes. Because of the requirement that samples be collected at least three weeks apart, an additional sample could not be collected during the dry season to replace the sample that could not be collected due to the malfunctioning valve.

Prior to this study, ten acute *Hyalella* initial samples were collected on the Los Angeles River and San Gabriel River and one chronic *Hyalella* test was collected on the San Gabriel River to assess the possibility for developing a WER on these waterbodies. Two samples were collected at each of five sites at different locations in the river (i.e. mid-stream and edge) to determine the spatial variability of the WER across the channel or at the same location to determine the reproducibility of the results. The data from this initial study that were collected at the same sites as the current study or at nearby sites within the same reach are included in this analysis. The following table lists the initial study sample locations. All acute samples were collected on October 4, 2000. A chronic sample was collected on the San Gabriel River at site SGR-R9W during June 2001. The chronic study collected at SGR-R9W was a 42-day test with both survival and reproductive endpoints.

Table 3. Sampling Locations for Initial Study

Location ID	Receiving Water	Description	Relationship to WER Sampling Locations	Date of Sample Collection
LA-R8	Los Angeles River	Downstream of DC Tillman at Coldwater Canyon	About 2 miles downstream LA1	10/4/00
LA-R4*	Los Angeles River	Upstream of LA-Glendale at Riverside Dr.	About 4 miles upstream of LA2	10/4/00
LA-R7	Los Angeles River	Downstream of LA Glendale at Los Feliz	Just downstream LA2	10/4/00
SGR-R4	San Gabriel River	Downstream of San Jose Creek WRP and upstream of Los Coyotes at Alondra Blvd.	Just downstream of SGR1	10/4/00
SGR-R9W	San Gabriel River	Downstream of Los Coyotes at Willow	SGR2	10/4/00

Note: *Data from LA-R4 is not included in calculations of the final WERs and SSOs presented in this report.

SAMPLING AND ANALYSIS METHODS

Samples were collected and analyzed based on the methods provided in the work plan for the study (See Appendix 1). Additionally, the complete details of all of the analytical work are included in the laboratory results provided by the laboratory and available for review upon request (16 binders of data).

Samples were collected as grab samples using an intermediate container method or by pumping into 5-gallon containers. At the laboratory, the necessary test volumes were obtained by compositing the individual sample bottles. Sample aliquots were then taken from the composited water and sent to the analytical laboratory EnviroMatrix Analytical, Inc. for analysis of the water quality constituents other than ammonia, pH, temperature, and dissolved oxygen.

Laboratory dilution water was composed of synthetic moderately hard water prepared in advance of the testing. One batch of water was prepared and used for all renewal samples needed during the testing process. The following tables summarize the key aspects of the sampling and analysis methods for the study.

Table 4. Analysis Methods

Constituent	Method of Analysis
Acute *Hyalella*	EPA/600/R-99/064
Chronic *Hyalella*	EPA/600/R-99/064
Chronic Fathead Minnow	EPA/600/4-91/002
Hardness	EPA 130.2
Alkalinity	EPA 310.1
Total Chlorine Residual	EPA 330.5
Turbidity	EPA 180.1
Chloride	SMEWW 4500 CL C
TOC	SMEWW 5310 B
TSS	SMEWW 2540 D
TDS	SMEWW 2540 C
Settleable Solids	SMEWW 2540 F
Sulfate	SMEWW 4500 SO4 E
BOD	SMEWW 5210 B
Calcium	EPA 3010/6010
Potassium	EPA 3010/6010
Sodium	EPA 3010/6010
Dissolved Oxygen	EPA 360.1
Conductivity	EPA 120.1
pH	EPA 150.1
Ammonia	EPA 350.3

Table 5. Analysis Protocols Summary

Analysis Component	Method Used
pH Buffers used for testing and pH study	Aeration, NaOH and HCl depending on the pH adjustment needed
Bottles	2.5 gallon glass pickle jars and 5 gallon glass carboys
Source of Organisms	Aquatic Indicators and in-house cultures for *Hyalella*, Aquatic Biosystems for fathead minnow
Age of Organisms	6-13 days *Hyalella*, Less than 24 hours old fathead minnow
Test Chamber	500 mL glass jars containing 250 mL water
Number of organisms	10 animals per replicate and surrogate
Dilutions	Four replicates and a surrogate with 7 dilutions to start, reduced to 5 dilutions after ranges established
Feeding methods	Acute *Hyalella* fed 1 mL of Wheat Grass slurry or YCT on Day 2, Chronic fed daily, Fathead fed newly hatched *Artemia* two times daily
Ammonia measurements	Two cross-calibrated instruments on surrogate containers
Renewal method	Acute samples renewed on Day 2, Chronic samples renewed daily
Dilution preparation method	Spiked samples with increasing amounts of ammonium chloride
Data analysis method and software used	ToxCalc v5.0.23
Photoperiod	16 hours light, 8 hours darkness

Detailed discussions of individual events and any protocol deviations are included in the laboratory results for the study.

Study Results

The results of the study are summarized in the tables in Appendix 2-Summary of Study Results. The complete laboratory reports are contained in sixteen binders of results that can be supplied upon request. These binders contain all of the laboratory records for the study and present both total and un-ionized ammonia toxicity results for each sample. In addition to the toxicity results, all of the results of the water quality analyses are included in these binders as well. Additionally, an electronic database of the results of the study has been developed and is available for review upon request from Larry Walker Associates.

QA/QC Analysis and Review

The work plan for the study (Appendix 1) contains a number of quality assurance/quality control (QA/QC) requirements for the sample collection and analysis. This section summarizes the QA/QC issues that occurred during the testing, steps taken to resolve the issues, and any tests that were considered unacceptable for analysis based on the results of the review. For details on the QA/QC requirements for the study, see Section 4 of the workplan in Appendix 1.

TEST ACCEPTABILITY

All tests were reviewed and a summary of all the QA/QC requirements in the WER is included as Appendix 3 – QA/QC Requirements. Although a number of deviations from the testing protocol were determined, only a few were considered to have a significant impact on the test results. Listed below are the two criteria used to determine if a test was unacceptable for the purposes of the study:

1. Survival in the laboratory dilution water control test was below the acceptable level for the test.
2. Dissolved oxygen levels in the test were below the minimum required value (3.5 mg/L for *Hyalella* and 4.0 for fathead minnow) for more than 10% of samples collected during the testing period (approximately more than 1 day in all of the dilutions in the acute tests).

In some cases, control survival in the site water was below the required survival rate. These tests were still considered acceptable as long as the survival rate in the laboratory dilution water control was acceptable, because the control samples in site water all contained some ammonia that might have impacted the survival of the test organisms.

These two criteria were used to eliminate unacceptable test results from the WER analysis because the EPA ammonia criteria documents used both the control survival and the dissolved oxygen levels to determine whether or not a particular study would be included in the calculation of the national ammonia criteria. Additionally, it was clear from the data review that these two issues had impacted the results of at least some of the tests that failed the criteria.

Although the two criteria discussed above were the only ones used to reject test results, the QA/QC review also examined the other QA/QC criteria identified in the WER guidance. The QA/QC criteria in the guidance is meant to provide a framework for conducting the tests in the most consistent manner possible and provide a mechanism for assessing if any toxicity tests are not appropriate for data analysis because of the occurrence of major problems (the two conditions identified above) or the sum total of a large number of minor issues. Because of the difficulty in conducting tests with living organisms in site water and the natural variability that occurs during any type of water quality testing, some other less than ideal conditions occurred in some of the tests. However, in the context of the results and the rest of the conditions during the testing, none of these variations was considered to have a significant impact on the test results and no tests were rejected as a result of the variations. The following issues were the ones most commonly identified during the QA/QC review:

- Temperature deviations were found in most tests, but the deviations were just outside the acceptable range in most cases.
- Turbidity was not run on any of the samples collected after 3/4/02. Turbidity was inadvertently left off of the Chain-of-Custody during the April monitoring events and subsequent to those events, the lab requested that the COC just state "water chemistry" rather than detailing out all of the constituents. The lab apparently then based the water chemistry analysis on the inaccurate April COCs and turbidity analyses were not completed after the first two events.
- There were occasional inversions in the data (a lower concentration dilution had a lower survival than a higher concentration dilution), especially in site water, and occasional non-normal distributions.
- The 36-hour holding time was exceeded in a few samples because the laboratory was not able to set up all of the tests as quickly as planned.

The QA/QC review resulted in the decision to not use the results from a few of the collected samples. Because an additional sample was collected at each site, the number of samples per site was still sufficient to calculate the WER and SSO values. Following is a discussion of the specific QA/QC issues for each type of test, as well as the tests that were considered unacceptable for the purposes of the study.

HYALELLA

Four site water and one laboratory water acute *Hyalella* tests were rejected from this study. One set of tests was rejected because the survival in the control was unacceptable. Two other tests were rejected because the dissolved oxygen fell below the minimum level for over 10% of the testing. The rejected samples are explained below:

- LA1 from 5/15/02-The dissolved oxygen levels were below the minimum level in 20% of the measurements.
- LA2 from 5/15/02-The dissolved oxygen levels were below the minimum level in 40% of the measurements.
- 8/27/02 tests because the laboratory dilution water control survival was only 45%. The samples from SJC1 and RH1 for this sample date were rejected because of the control survival and the fact that no acceptable side-by-side laboratory dilution water test was available for WER calculations.

For each of these tests, the QA/QC problems appeared to have had a significant impact on the test results. For the LA1, LA2 and 8/27/02 tests, the toxicity results were significantly lower than the results obtained during other events. For the laboratory dilution water test on 8/27/02, the result was greater than a factor of 2 lower than the average of all of the other tests collected during the study, and therefore could have been considered unacceptable from that perspective as well. None of the other tests that were accepted for use in the study demonstrated significant deviations from the other test results.

For the chronic *Hyalella* studies, the control survival in the laboratory dilution water test conducted on 8/6/02 was slightly below the acceptable level (80%) at 77.5%. Additionally, both the site water tests for this date (SCR2 and BW1) had dissolved oxygen levels below the minimum value for more than 10% of the testing period. Therefore, the set of tests from 8/6/02 was considered unacceptable and was rejected from this study. In the June 2001 initial chronic test, the control survival in the laboratory dilution water was below the acceptable level and a reproductive endpoint could not be determined because the majority of the organisms died before the 42-day period was completed. Therefore, a chronic WER could not be calculated and this test was rejected from the analysis.

FATHEAD MINNOW TESTS

A number of problems occurred in the initial toxicity tests for fathead minnows. These initial tests resulted in very high mortality in the first 24 hours of the test at levels over 10 mg/L of total ammonia. The tests were being run at a target pH of 8.0, but the pH in some cases was as high as 8.5. The testing protocols were changed so that the tests were run at a lower pH and lower temperature. The protocol changes allowed the testing to proceed successfully. Therefore, a number of short-term tests were run in April and May 2002 to determine a successful procedure to run the test. None of these tests were conducted for the full 28 days and are therefore not considered acceptable for this study.

In July through September 2002, complete 28-day tests were conducted at all of the sites. One set of tests (9/24/02) was unacceptable for use in this study because the laboratory dilution water fathead minnows were found to have a parasite. For that reason, no results were obtained in the laboratory dilution water for the 9/24/02sample date. Consequently, the site water samples collected on that date (BW1, SGR1, and SCR1) do not have an acceptable laboratory dilution water test available for calculating a WER and could not be used for this study.

LABORATORY DILUTION WATER TEST ACCEPTABILITY

In addition to the QA/QC requirements, the laboratory dilution water results were compared to laboratory dilution water tests from other laboratories/studies to determine if the tests were acceptable. In addition, the test results in lab water from this study were compared to the average result for all of the laboratory water tests to determine if any were outside of the range of the other tests.

In order for the tests to be considered acceptable, the results in laboratory water need to be within a factor of 2 of the results in laboratory water from other studies. Ankley et. al, 1995, was used for the acute *Hyalella* comparison. For the chronic *Hyalella* and chronic fathead minnow tests, the results from the criteria document were used for comparison. (The results from the criteria document for the *Hyalella* chronic test are not directly comparable to the tests that were run for this study because the chronic test cited in the criteria document was a 42-day test with a reproductive endpoint, and this study conducted a 21-day test with a growth endpoint. However, since the chronic studies are not used for the calculation of the SSOs for the study, this comparison does not impact the study results. Therefore, the information on chronic test results is shown for informational purposes only).

One of the difficulties with the acute comparisons was that the other lab tests were run in waters with different ion compositions and different hardness. The results of this study demonstrate that ionic concentrations and hardness can impact the toxicity of ammonia to *Hyalella*. Consequently, the waters with compositions that were considered most similar to the laboratory dilution waters for this study (moderately hard water, rather than soft or hard water, with the pH closest to 8.0) were used. In addition, all of the results were normalized to pH 8 for comparison purposes. Table 6 and Table 7 summarize the results from this study as compared to the other laboratory results. In addition to comparing the study results to results from the Ankley study and the criteria document, the average of the results from this study was also compared to determine if all of the study results were within a factor of 2 of the average.

Table 6. Acute Laboratory Dilution Water Test Comparison

Event Date	Test Type	MHW Adjusted to pH 8 in mg-N/L	Factor of Difference for Other Lab Studies [1]	Factor of Difference for Average WER Study Result [2]
Result at pH 8 in mg-N/L			71 [3]	56.3 [4]
1/31/02	Hyalella Acute	71.8	1.0	1.3
3/4/02	Hyalella Acute	70.5	1.0	1.3
4/1/02	Hyalella Acute	47.0	1.5	1.2
4/16/02	Hyalella Acute	65.6	1.1	1.2
5/15/02	Hyalella Acute	61.6	1.2	1.1
6/4/02	Hyalella Acute	64.8	1.1	1.2
6/12/02	Hyalella Acute	38.6	1.8	1.5
6/18/02	Hyalella Acute	48.2	1.5	1.2
6/25/02	Hyalella Acute	52.3	1.4	1.1
7/16/02	Hyalella Acute	45.3	1.6	1.2
12/18/02	Hyalella Acute	88.9	1.3	1.6
2/12/03	Hyalella Acute	40.3	1.8	1.4
10/4/00	Hyalella Acute	37.4	1.9	1.5

1. The results in this column show the factor of difference between the results published by Ankley, et. al, 1995 and the results of the laboratory dilution water test associated with each sampling event.
2. The results in this column show the factor of difference between the average of the laboratory dilution water study results for this WER study and the results of the individual laboratory dilution water test associated with each sampling event.
3. The result from Ankley, et. al, 1995 adjusted to pH 8.
4. The average of the laboratory dilution water samples collected during this WER study.

Table 7. Chronic Laboratory Dilution Water Test Comparison

Event Date	Test Type	MHW Adjusted to pH 8, Temp 25 in mg-N/L	Factor of Difference for Other *Hyalella* Chronic Lab Studies [1]	Factor of Difference for Other Fathead Minnow Chronic Lab Studies [1]
Result at pH 8, Temp. 25 in mg-N/L			1.45[3]	3.09[4]
7/9/02	Fathead Chronic	6.2		2.0
7/23/02	Fathead Chronic	7.9		2.6
4/1/02	Hyalella Chronic	2.94	2.0	
9/10/02	Hyalella Chronic	0.52	2.8	

1. The results in this column show the factor of difference between the results for the *Hyalella* chronic study presented in the 1999 ammonia criteria and the results of the laboratory dilution water test associated with each sampling event.
2. The results in this column show the factor of difference between the results for the fathead minnow chronic study presented in the 1999 ammonia criteria and the results of the laboratory dilution water test associated with each sampling event.
3. The chronic *Hyalella* GMCV from the 1999 ammonia criteria document.
4. The chronic fathead minnow GMCV from the 1999 ammonia criteria document

In all cases, the *Hyalella* acute tests were less than a factor of 2 different from the Ankley results and from the average of the other tests in the study. Therefore, they were all considered acceptable for calculating WERs. The fathead chronic results were higher than the criteria document results by a factor of 2. One of the *Hyalella* chronic results was a factor of 2 higher than the criteria document results and one was almost a factor of 3 lower. The observed differences might be attributable to the differences in the test endpoint and duration and do not directly indicate that the tests are problematic. In most cases, the study results are higher than the other laboratory results that they are compared to, so the WERs determined in this study would be lower than predicted by the other laboratory results. One of the *Hyalella* chronic studies is lower than the other study result, but the other chronic *Hyalella* test is higher. Both result in WERs that are at least three times higher than the acute WERs (see Table 12) so the discrepancies do not dispute the fact that the use of acute WERs is conservative for this study. Additionally, none of the chronic tests were used in the WER calculations, therefore, the discrepancies in these results are not considered problematic for the study.

WER CALCULATIONS AND ACCEPTABILITY

The final step in reviewing the data was to compare the water quality during the sampling to typical conditions at the sites. Table 8 summarizes the average water quality during the testing (in the rows marked "WER Study") as compared to average conditions at the POTW receiving water monitoring location (where historic monitoring data were available) nearest to the sampling location (marked "Typical Conditions"). The results were almost all within the range reflective of the typical conditions (i.e. mean plus or minus two standard deviations) and were considered acceptable. The "Typical Conditions" rows show the mean of the historic data with the mean plus or minus two standard deviations in parentheses so that the out of range values could be determined. The few cases where the results were out of range are highlighted in bold and italics in Table 8.

Table 8. Water Quality Conditions During Testing as Compared to "Typical" Conditions (1)

Discharger	Station		Average Ammonia (mg/L-N)	Average Hardness (mg/L)	Average Alkalinity (mg/L)	Average DO (mg/L)	Average TDS (mg/L)	Average Chloride (mg/L)	Average Conductivity (mg/L)	Average pH
Pomona	WER Study (SJC1)		7.5	189	207	9.1	487	117	863	7.6
	Typical Conditions (RA)	Average	5.0				400	86		7.38
		Range [2]	0.78-9.18				2-798	20-152		6.14-8.62
		Number Samples	11				6	9		191
Saugus	WER Study (SCR1)		10.1	198	190	8.7	695	***196***	1233	7.4
	Typical Conditions (RB)	Average	9.7	259	253		712	125	1226	7.34
		Range [2]	1.88-17.48	185-333	159-347		590-834	***83-167***	986-1466	6.86-7.82
		Number Samples	95	86	56		16	15	50	310
Valencia	WER Study (SCR2)		6.7	333	246	8.8	743	122	1279	7.8
	Typical Conditions (RE)	Average	4.1	379	257		775	96	1285	
		Range [2]	0-9.53	311-447	205-309		477-1073	56-136	1073-1497	
		Number Samples	90	83	53		16	15	47	
San Jose Creek	WER Study (SGR1)		4.4	***181***	159	8.7	498	114	816	7.5
	Typical Conditions (R3)	Average		200						
		Range [2]		198-202						
		Number Samples		4						
Los Coyotes	WER Study (SGR2)		4.2	***242***	198	9.8	664	175	1184	8.3
	Typical Conditions (R5)	Average	5.6	224						
		Range [2]	1.42-9.82	218-230	219-230					
		Number Samples	6	2						
Long Beach	WER Study (CC1)		2.4	243	***228***	9.2	709	145	1202	8.3
	Typical Conditions (RA)	Average	5.0	268	***146***	12.8	856	151	1497	7.65
		Range [2]	0.6-9.4	202-334	(4)	0-26.6	468-1244	93-209	0-3073	6.91-8.39
		Number Samples	59	2	1	54	55	48	55	369
Whittier Narrows [3]	WER Study (RH1)		0.7	***177***	170	8.3	449	85	***715*** [5]	7.8
	Typical Conditions (RA)	Average	5.6	227	120		469	79	997	7.52
		Range [2]	0-15.8	181-273	0-286		417-521	73.2-84.8	925-1069	6.62-8.42
		Number Samples	6	17	4		3	3	3	84
LA Glendale	WER Study (LA2)		7.8	251	199	8.5	563	***134***	1019	7.8
	Typical Conditions (R7)	Average	6.5	284		9.3	644	107	1034	7.61
		Range [2]	2.5-10.5	216-352		6.45-12.05	546-742	83-131	838-1230	7.41-7.81
		Number Samples	9	9		27	9	9	9	27
DC Tillman	WER Study (LA1)		11.5	174	188	8.3	511	***147***	1010	7.9
	Typical Conditions (R8)	Average	8.8	282		11.0	689	109	1079	8.05
		Range [2]	4.03-13.63	132-432		6.0-16.0	471-907	88.4-129.6	771-1387	7.51-8.62
		Number Samples	9	9		27	9	9	9	27
Burbank	WER Study (BW1)		14.2	212	217	10.7	522	124	981	8.3
	Typical Conditions (R5)	Average	16.0	270			599	123	1166	8.0
		Range [2]	6.2-25.8	196-344			333-865	63-183	928-1404	7.48-8.52
		Number Samples	4	4			4	4	4	98

1. The stations that start with R (i.e. RA, RB, RE, etc.) are the "typical" conditions used for comparison in this analysis. These represent the POTW receiving water monitoring location that is closest to the chosen sampling location. The average conditions were taken from the work plan for this study. For the most part, the averages and standard deviations are based on data from 1996 to 2000. In some cases there are only a few data points during this period of time.
2. The range is equal to the mean plus or minus two standard deviations and is used to assess whether the sampling results are within the range of typical conditions.
3. Whittier Narrows began nitrification and denitrification between when the "typical" condition measurements were collected and this study. This is the likely reason for some of the differences in water quality downstream of this plant.
4. Only one data point was available for alkalinity so it is not possible to determine the range of typical conditions for this constituent.
5. The average conductivity at RH1 was significantly decreased by the wet weather event. If the average of just the dry events is calculated, the value is 810.

Chloride appears to be the one constituent that was present in higher concentrations than previously observed at more than one site. This is likely due to the fact that the water supply for this area has been higher in chlorides recently, as compared to past years, because of the drier than normal conditions over the past few years. Chloride is one of the constituents that may have an impact on toxicity. However, the overall ion composition (TDS) has the strongest influence, and it is not possible to separate out the impact of specific ions (See Data Analysis Section). Both TDS and conductivity are in the range of typical values at the three sites. Given that TDS is within typical values, it is unlikely that the higher chloride concentrations observed during monitoring had significant impacts on the observed toxicity.

QA/QC SUMMARY

The QA/QC analysis demonstrated that, except for 10 rejected site water sample results, the majority of the tests collected were acceptable for the analysis, the results of the acute *Hyalella* laboratory water results compared well with other laboratory studies, and the samples were collected during typical conditions. The rejected results do not prevent the development of WERs and SSOs for the waterbodies in the study.

Data Analysis

PH RELATIONSHIP CALCULATION

The toxicity of ammonia to aquatic organisms is partially dependent on the pH of the water. During the toxicity testing for this study, the pH of the laboratory dilution water was often different from the pH of the site waters being tested concurrently with the lab water, primarily because only one laboratory dilution water sample was run with multiple site waters that had different compositions. The composition of the water impacts the pH of the water and the ability of the water to remain at a given pH throughout the test (buffering capacity). Every effort was made to run the tests at the same pH. However, depending on the water composition, the pH of the sample often "drifted" higher or lower during the testing period and resulted in some differences in pH values across the tests. To be able to compare the toxicity in laboratory water to site water, the results of the two tests need to be adjusted to the same pH. The 1999 ammonia criteria document contains a pH relationship that could be used for the adjustment. However, the TAC for the study requested that the pH relationship for *Hyalella* be examined to determine whether or not it matched the pH relationship developed in the 1999 ammonia criteria document.

To address the TAC's concern, a separate pH investigation was conducted and the results of the investigation (as well as the results from all of the laboratory dilution water tests) were compared to the pH relationship identified in the 1999 ammonia criteria document to determine if differences existed that justified the development of a separate pH relationship for *Hyalella*. The first pH investigation was conducted in October 2002. This investigation targeted pH values that were 0.2 units apart from each other over the range of 7.6 to 8.6. Throughout the investigation it was difficult to control the pH well enough to see differences between the targeted pH ranges. Additionally, control survival during the tests was significantly less than the acceptable acute *Hyalella* survival of 90% for most of the tests. Consequently, this investigation was not considered to be acceptable and another investigation was run in January 2003. For this pH investigation, a smaller number of tests were run which targeted pH values of 6.5, 7.5, and 8.5. The control results from these tests were adequate and the wider range of target pHs allowed differences between the individual results to be assessed. However, the small number of tests run made it difficult to make significant conclusions based on these tests alone. Therefore, both the pH investigation results and all of the results from the laboratory dilution water tests run during the study were considered in assessing the pH relationship for *Hyalella*.

As a first step, the results of the pH investigation were compared to the pH relationship equation from the 1999 ammonia criteria document. The results from the pH investigation demonstrated a linear relationship for the pH values tested during the investigation (actual pHs of 7.1-8.0 based on the targets of 6.5, 7.5, and 8.5). The pH relationship in the 1999 ammonia criteria document is approximately linear for these pHs. The slopes of the two lines are similar, though the one developed through the special pH investigation is slightly less steep than the 1999 ammonia criteria document relationship. The pH investigation also demonstrated greater toxicity (lower LC50s[1]) than would have been predicted by the criteria equation, but at the higher pHs usually seen in the river, the predicted toxicity is much more similar. The following graph (Figure 1) compares the investigation to the criteria equation. In Figure 1, the solid line is a linear regression of the pH relationship from the criteria document for the pH range of 7.1-8.0, while the dotted line is the linear regression of the relationship measured in the pH investigation. The triangles represent the actual values measured in the pH investigation and the squares show the actual values of the pH relationship from the criteria document.

[1] LC50 is the concentration of ammonia that caused 50% of the test organisms to die during the testing period.

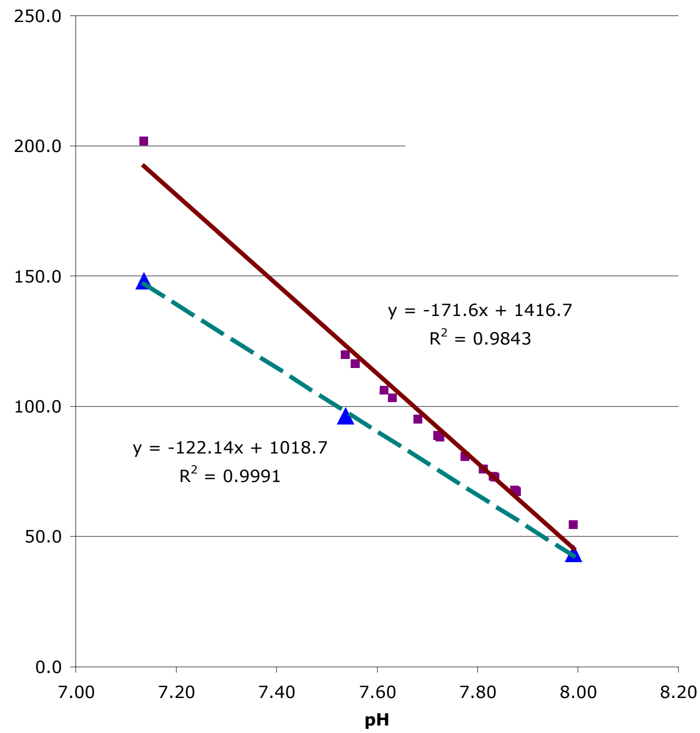

Figure 1. Comparison of pH Study Regression Line to Criteria pH Equation Regression Line

The slopes and intercepts of the two lines were compared based on the 95% confidence interval to determine if significant differences existed between the two lines. For the criteria predicted regression equation, the slope is equal to –171.6 ± 12.7 (-184.3 to –158.9) and the pH investigation slope is equal to –122.1 ± 44.4 (-166.5 to -77.7). The confidence intervals for the slopes of the two lines overlap, indicating that significant differences may not exist between the two slopes. The same is true of the intercepts for the two lines. For the criteria predicted regression equation, the intercept is equal to 1416.7 ± 97.7 (1319 to 1514.4). The intercept for the pH investigation is equal to 1018.7 ± 336 (682.7 to 1354.7). Therefore, differences between the two pH relationships (for the pHs of concern in this study) can not be determined with sufficient confidence to warrant the development of a separate relationship for this study.

Secondly, the actual results of all of the laboratory dilution water tests were combined with the pH investigation results and compared to the predicted LC50 value based on the criteria relationship. For each of the laboratory dilution water results, the LC50 value predicted by the criteria equation for that pH was calculated. The actual LC50 results were then compared to the predicted values to determine if significant differences existed. The following graph (Figure 2) shows the comparison of the actual results to those predicted by the criteria equation. In the figure, the squares represent the laboratory dilution water sample results collected during the study and the triangles are the results from the pH relationship study. The solid line is the criteria equation pH relationship with the 95% confidence intervals around the relationship represented by the dotted lines in the figure.

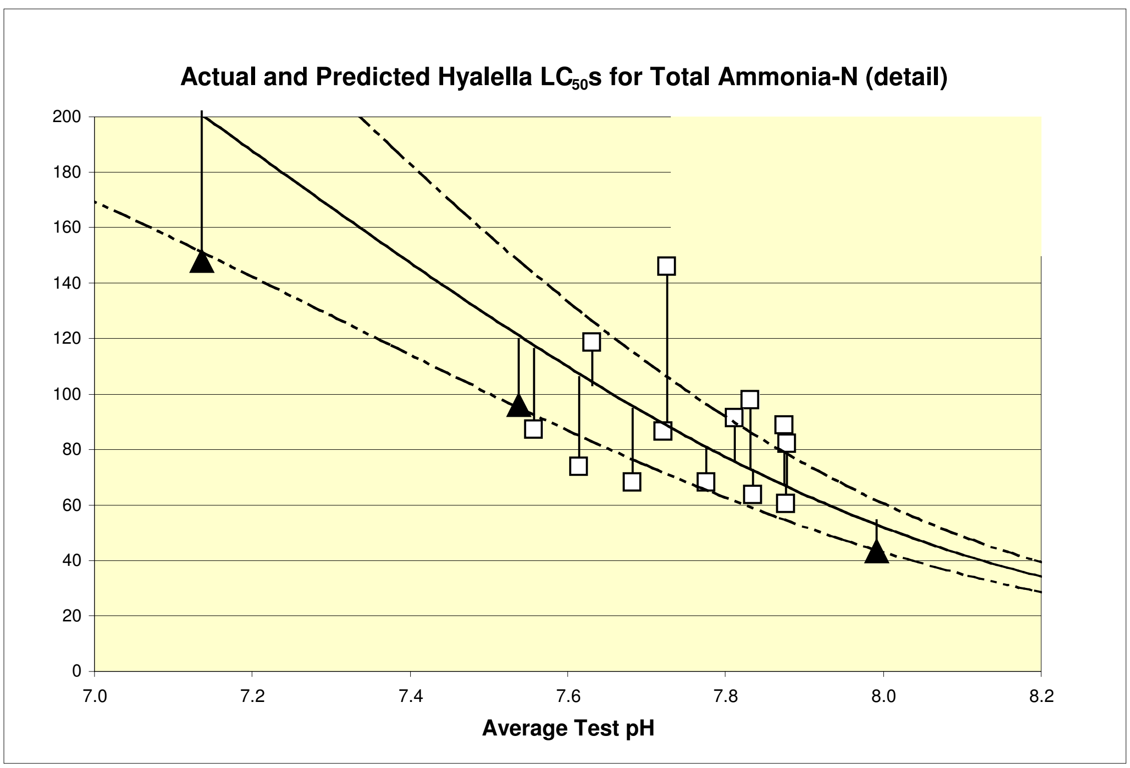

Figure 2. Comparison of Laboratory Dilution Water Results to LC50s Predicted by Criteria pH Equation

As shown in Figures 1 and 2, the pH relationship measured in this study is not different enough from the relationship in the USEPA criteria document to justify development of a separate relationship for this study. As such, a unique pH relationship for ammonia toxicity to *Hyalella* is not justified to establish SSOs in this study. Over half of the data points from this study fall within the 95% confidence interval for the EPA pH relationship, and (except for one datapoint) the remaining values are just outside of the confidence interval range. The variability of the study results is clustered around the criteria equation line and no clearly different relationship can be determined based on this data. The study results (7.1 to 8.0) cover the average pH values found in most of the waterbodies being studied (7.34 to 8.05). It is possible that outside of this range, the pH relationship may vary more significantly from the criteria relationship. However, the collected data are more similar to the EPA pH relationship at higher pHs that are of more concern in these waterbodies than the lower pH range. None of the information collected indicates that significant variation from the EPA pH relationship would occur at higher pHs where data were not collected. Therefore, the comparison demonstrated that, for the average pHs found in the waterbodies in this study (7.34 to 8.05), the pH relationship for *Hyalella* does not appear to be significantly different and, therefore, no separate pH relationship was warranted for development of WERs in this study.

In addition, the use of the pH relationship developed specifically for this study would result in higher WERs than those developed using the pH relationship from the criteria document. Table 9 provides some examples of the WERs found using the EPA pH relationship as compared to the study specific pH relationship. Based on the pH study conducted, a linear relationship provides a good fit to the available data and is the best relationship that could be determined based on the available data. .

Table 9. Comparison of WERs Based on EPA and Study-Specific pH Relationships

Site	EPA pH relationship based WERs	Study specific pH relationship based WERs
LA1	2.457	3.798
LA2	2.176	3.939
BW1	1.603	2.257
SGR1	1.407	1.425
SGR2	1.929	2.148
CC1	1.822	3.972
SJC1	1.350	1.767
RH1	2.076	2.549
SCR1	2.787	6.221
SCR2	2.296	4.476

As shown in the table, the study-specific pH relationship WERs are much higher and much more variable than the EPA pH relationship WERs. Consequently, the use of the EPA pH relationship is a conservative approach to determining the WERs for the study.

OTHER CONSTITUENT RELATIONSHIPS

The premise of this study was that ionic constituents present in waterbodies in Southern California reduced the toxicity of ammonia to *Hyalella* and potentially to other aquatic organisms. In addition to calculating a SSO based on these conditions, the water quality constituents were compared to the toxicity results and the WER results to assess if any clear relationships existed. To conduct this assessment, two approaches were taken. First, correlation coefficients were developed to determine if any water quality constituents were strongly correlated with the toxicity results and also to assess which water quality constituents were related to each other. Secondly, a stepwise, multiple regression analysis was used to determine if an equation could be developed to predict the toxicity based on water quality parameters. In order to conduct these analyses, all of the results of the study were adjusted to a pH of 8.0 using the criteria pH relationship equation.

CORRELATION COEFFICIENT ANALYSIS

Table 10 and Table 11 are correlation matrices containing all of the water quality constituents that could impact toxicity as compared to the toxicity results, the WER, and each other. Both the logarithm of the concentration and the concentration itself were compared and it was determined that the log of the concentrations were much more strongly correlated than the direct concentration and is shown in the tables. The tables show the correlation coefficients for each comparison. As the correlation coefficients get closer to ±1.0, the stronger the relationship between the two variables. Coefficients over ±0.6 are considered significant correlations that might impact the regression analysis.

Table 10. Correlation Matrix of Toxicity Results to Water Quality Constituents

Correlation Matrix
Row exclusion: WQ Stat analysis

	LC50 pH 8	ln(Sodium)	ln(Potassium)	ln(Calcium)	ln(BOD)	ln(Sulfate)	ln(TDS)	ln(Chloride)	ln(Hardness)
LC50 pH 8	1.000	.561	.435	.373	-.162	.463	.551	.560	.425
ln(Sodium)	.561	1.000	.771	.711	-.448	.809	.953	.968	.772
ln(Potassium)	.435	.771	1.000	.418	-.235	.523	.697	.828	.578
ln(Calcium)	.373	.711	.418	1.000	-.248	.877	.727	.626	.938
ln(BOD)	-.162	-.448	-.235	-.248	1.000	-.389	-.305	-.396	-.248
ln(Sulfate)	.463	.809	.523	.877	-.389	1.000	.781	.714	.919
ln(TDS)	.551	.953	.697	.727	-.305	.781	1.000	.936	.777
ln(Chloride)	.560	.968	.828	.626	-.396	.714	.936	1.000	.703
ln(Hardness)	.425	.772	.578	.938	-.248	.919	.777	.703	1.000

35 observations were used in this computation.
4 cases were omitted due to missing values.

Table 11. Correlation Matrix of WERs to Water Quality Constituents

Correlation Matrix
Row exclusion: WQ Stat analysis

	WER	ln(Sodium)	ln(Potassium)	ln(Calcium)	ln(BOD)	ln(Sulfate)	ln(TDS)	ln(Chloride)	ln(Hardness)
WER	1.000	.640	.438	.509	-.343	.604	.631	.592	.577
ln(Sodium)	.640	1.000	.771	.711	-.448	.809	.953	.968	.772
ln(Potassium)	.438	.771	1.000	.418	-.235	.523	.697	.828	.578
ln(Calcium)	.509	.711	.418	1.000	-.248	.877	.727	.626	.938
ln(BOD)	-.343	-.448	-.235	-.248	1.000	-.389	-.305	-.396	-.248
ln(Sulfate)	.604	.809	.523	.877	-.389	1.000	.781	.714	.919
ln(TDS)	.631	.953	.697	.727	-.305	.781	1.000	.936	.777
ln(Chloride)	.592	.968	.828	.626	-.396	.714	.936	1.000	.703
ln(Hardness)	.577	.772	.578	.938	-.248	.919	.777	.703	1.000

35 observations were used in this computation.
4 cases were omitted due to missing values.

As shown in the matrix, all of the ions that were analyzed for the study showed very similar correlations to the ammonia toxicity result and the WER. The results also show that the ions are all very strongly correlated to each other. This poses a potential problem for regression analysis because the correlations between the constituents can make it difficult to distinguish impacts from an individual constituent.

MULTIPLE REGRESSION ANALYSIS

To address the significant correlation between the constituents, both forward and backward stepwise techniques were used to determine if the significant correlations between constituents determined above impacted the results of the analysis. Scatter plots were developed for some of the constituents to see if anything other than a linear relationship was indicated by the data. Based on the plots and the significant correlation coefficients (which assume a linear relationship), linear regression was used for this analysis. It is possible that other, more complex, relationships exist between toxicity and water quality, but these were not assessed for this study and there was nothing that indicated another relationship was likely.

The correlations between the ions did have an impact on the regression analysis. The forward and backward analyses resulted in different equations when all of the ions were included. This indicates that the correlations between the individual constituents impacted the ability to conduct the analysis. A variety of combinations of constituents were used to try to remove the interference, but the results were generally the same. For toxicity, the forward regression resulted in sodium being the only factor impacting toxicity, while chloride showed up as the factor using the backwards regression analysis. However, both only had R^2 value for the predicted equations of 0.29. When a simple regression was conducted on the individual ions, sodium demonstrated the strongest relationship to toxicity (R^2 of 0.316), but chloride showed a similar strength relationship (R^2 of 0.314).

Stepwise regression was also used to examine the relationship of the WER to the water quality constituents. Similar results were found for the relationship to the WER, except that TDS was the determining factor in the backwards regression analysis rather than chloride. Slightly higher R^2 values were found for the relationship to the WER (0.38 for TDS and 0.391 for sodium). A simple regression analysis determined that both sodium and TDS produced equations with the exact same R^2 value (0.393). The relationships to the WER were slightly stronger than the relationships found for toxicity, but the analysis did not allow a distinction between the impacts of TDS as compared to sodium.

Finally, a multiple regression analysis was run using all of the ions and just the two that were determined from the stepwise analysis. The results of this analysis showed that the two ions alone (sodium and chloride for toxicity and sodium and TDS for the WER) have a better relationship to toxicity and the WER than all of the ions combined, but the combination produces lower R^2 values than either of the two constituents on their own.

Principal Components Analysis

Because the correlations between the ions made the regression analysis difficult to interpret, a principal components analysis was conducted to try to reduce the impact of the correlations. A principal components analysis takes all of the constituents and determines whether the relationships between them can be used to create a number of factors that represent the correlated constituents. So, rather than comparing eight or nine constituents to the toxicity and WER results, one or two factors that represent the constituents can be used in the comparison.

The analysis resulted in the development of two factors, one primarily accounting for the ions and the other representing the other constituents (i.e. BOD, TOC, hardness, etc.). A stepwise multiple regression analysis was run using these two factors to see if a better fit to the data could be found. The results of the analysis confirmed that the ions are the major factor influencing the toxicity. The other factor did not show up in the final stepwise regression equation, indicating that it was not significant. However, the predictive ability of the factor representing ions was not any better than the previous analysis. Therefore, it was determined that the use of the factor did not provide any additional information to the analysis. The use of an individual ion, such as chloride, sodium, or TDS, appears to provide the best relationship for predicting toxicity or a WER.

Site-by-Site Analysis

As a final step in the analysis of the water quality constituents, regression analyses were conducted on a site-by-site basis using the toxicity results and the WERs. The purpose of this analysis was to determine if some of the variability that could not be accounted for in the previous analysis was reduced when the results were examined on a site-by-site basis. The results of the site by site analysis indicate that different ions had the strongest impact on the toxicity and WER results in different waterbodies. Except for SCR2, all of the sites showed a relationship to at least one ion that had a R^2 value greater than 0.6. However, there was no consistency among the sites as to which ion had the strongest relationship and often the strongest relationship was different for the toxicity values at the site as compared to the WER results. For RH1 and SGR1, R^2 values over 0.9 were found in the regression relationship for almost every ion. Multiple regression analysis could not be performed because there were not enough samples at each location to compare with the multitude of constituents measured. Consequently, the site-by-site analysis clearly demonstrates that relationships exist between toxicity and ammonia WERs and ions in the water. However, the ions having the most significant impact varies by site. This variability in the ion relationships between sites might be the reason that a clear relationship for specific ions could not be determined when all of the data were analyzed together.

In almost all cases, when significant correlations occurred between a constituent and LC50 or WER values (both on a site-by-site basis and cumulatively), increasing ion concentrations resulted in higher LC50s and WERs. The only exceptions to this trend were BW-1, CC-1, and SJC-1. At each of these sites, higher concentrations of ions generally result in lower WERs. No reasons for the differences at these sites could be found, and the ion concentrations and WERs found at these sites are similar to those found at the other sites.

Analysis Conclusions

Based on these analyses, it is clear than ions play a role in determining the toxicity of ammonia, but the results of this study can not be used to develop a clear, predictive relationship. Other studies that have been conducted have found similar results, that higher ion concentrations reduce the toxicity of ammonia to invertebrates. The prevailing theory is that the ions in the water increase the ability of the organism to excrete ammonia and potentially reduce the uptake of ammonium ions by the organism (Borgmann, 1997). Other water quality constituents, such as BOD and TSS, did not demonstrate significant relationships to ammonia toxicity.

The site-by-site analysis indicates that ions may play different roles in the individual waterbodies in Southern California, though the reasons for this are unclear. However, the general trend demonstrated in all of the analyses was that increasing concentrations of ions resulted in less toxicity and higher WERs as was expected based on the review of available studies on this topic.

The statistical analysis results are included as Appendix 5-Water Quality Statistical Analysis to this report.

Calculation of Final WER (fWER)

The calculation of the final WER for the study is based on the process outlined in the WER guidance document and summarized in the work plan for this study. The process involves calculating WERs for each of the dry weather events and taking the adjusted geometric mean of those WERs. That result is then compared to the WER calculated for wet weather events (hWER) to determine the final WER (fWER). The following summarizes the calculation process.

1. Adjust the pH of the laboratory water to the pH of the site water sample using the acute criteria equation (for chronic tests use the chronic criteria equation and also adjust temperature for invertebrates).
2. Calculate WER for each sample collected by dividing site water LC50 by adjusted lab water LC50.
3. Calculate adjusted geometric mean WER from dry weather samples.
4. Calculate hWER for wet weather samples.
5. Compare dry weather WER to hWER to determine fWER.

STEPS 1 AND 2: CALCULATION OF DRY WEATHER WERS FOR EACH SAMPLING EVENT

For each site and sampling event, the laboratory dilution water sample result was pH adjusted to match the corresponding site water pH. Then, dry weather WERs were calculated by dividing the site water result by the adjusted laboratory dilution water result. Table 12 summarizes all of the individual dry weather WERs calculated for this study, including the chronic fathead minnow and *Hyalella* results. For each site, two to three dry weather WERs were calculated based on the acute *Hyalella* samples (Acute *Hyalella* WER 1, Acute *Hyalella* WER 2, Acute *Hyalella* WER 3). Additionally, at some sites, another acute *Hyalella* WER was available from the initial study in October 2000 (Initial Study WER). As shown in Table 12, the chronic *Hyalella* tests resulted in WERs that were significantly higher than the acute WERs. The results confirm that the use of acute WERs for the study is quite conservative.

Table 12. Individual WER Results for Dry Weather Samples

Site	Acute *Hyalella* WER 1	Acute *Hyalella* WER 2	Acute *Hyalella* WER 3	Initial Study WER	Chronic Fathead WER	Chronic *Hyalella* WER
LA1	2.457		1.655	2.357	1.222	
LA2	2.176		1.723	2.467	0.937	7.025
BW1	1.603	1.446	1.316			
SGR1	1.407	1.473		3.950		
SGR2	1.929	1.849	3.202	3.726	1.476	35.88
CC1	1.822	2.099	2.791		1.405	30.70
SJC1	1.350	1.767			1.714	
RH1	2.076	2.226			1.304	44.59
SCR1	2.787	2.669	1.884			
SCR2	2.296	2.009	2.760		1.393	

To assess the variability of the WERs by site, a box plot (Figure 3) was created that shows the quartiles of the acute *Hyalella* WERs determined at each site and the median value (as shown by the middle line in each box) at each site. The plot shows that for most sites, the range of WERs determined was quite small and almost all of the sites had WERs that were statistically in the same range as the other sites. Only BW-1, SGR-1, and SJC-1 had median WERs that were outside the range of the other sites, while SGR-1 and SGR-2 demonstrated the largest range in WERs. The larger range at these sites is due to the higher WERs from the initial study. The likely reason for these higher WERs is the higher TDS values at these sites during the initial study period.

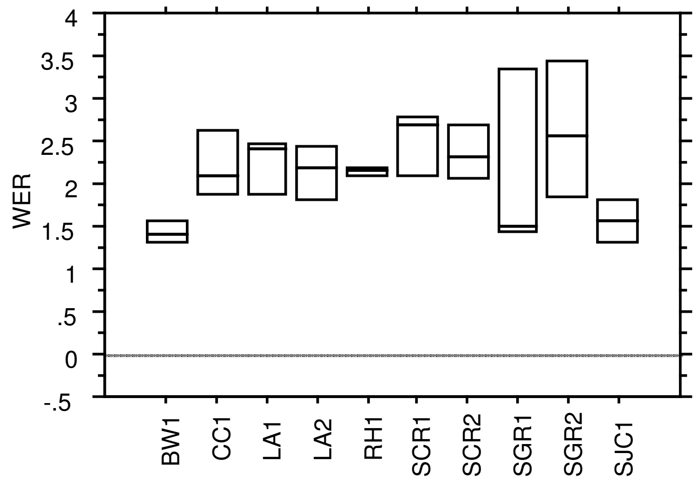

Figure 3. Box Plot of Dry Weather WERs Calculated for Each Site (WER1, WER2, WER3, and Initial Study WER)

Assessment of Initial Study Data

The initial study data were collected and analyzed using the same methods as those used to collect the remainder of the samples during the study and were subjected to the same QA/QC review. Additionally, the initial study data were accepted as the basis for developing a WER for these waterbodies and the results and sampling plan were included in the workplan for the study. Consequently, there is no reason to believe that these samples do not provide accurate information about the WER for the sampled waterbodies. However, because the samples were collected during a different time frame, the water quality and results were reviewed to determine whether or not there was any scientific reason to not use the initial study data to calculate the WERs.

First, the analytical methods, sampling methods, and QA/QC were carefully scrutinized to determine if any differences existed that would indicate that the initial study data were not valid when compared to the rest of the data. Following are the results of the analysis:

- Samples were collected at the same site for LA2, SGR1, and SGR2, but not LA1. The initial study site close to LA1 was LA-R8. LA-R8 is downstream of the Tillman POTW as is LA1 and is downstream of LA1, but is within the same reach as LA1. One additional site was sampled during the initial study, but is not included in the final analysis (LA-R4). Only those data collected from the same sites as the rest of the samples or a nearby site within the same reach will be used in the analysis.

- Two samples were collected at each site. The lowest WER obtained during the sampling will be used for the analysis.
- The same bottles and cleaning methods were used for the sampling. All of the samples collected during the initial study were collected using a clean intermediate container grab collection method. The remaining samples were collected using a combination of the intermediate container method and a pump with specially cleaned tubing. Additional sample volume was needed during later sampling events to ensure the ability to run the sample even if bottle breakage or other sample loss occurred. This resulted in the need to pump some of the samples to expedite sample collection time.
- The laboratory that conducted the testing for the initial study is the same laboratory that conducted the remainder of the tests.
- The laboratory used the same analytical methods for both sets of testing. The initial study samples were adjusted for pH using aeration. For the remainder of the study, pH adjustments were based on a combination of aeration and buffer addition.
- Dissolved oxygen, temperature, and pH were all measured on a daily basis during the testing.
- Daily observations were made of the condition of the *Hyalella*.
- Chloride, calcium, settleable solids, sulfate and BOD analyses were not run during the initial testing, but the remainder of the analytes tested during the rest of the study were analyzed.
- All QA/QC requirements for the testing were met (See Appendix 3).

Based on this review, the only substantial variation in the study protocol was the analysis of additional water quality parameters during the remainder of the study. Although the addition of the parameters allowed a more detailed analysis of the potential causes of the WER, the analysis does not impact the calculation of the WER. Therefore, for the samples collected at the same monitoring location or within the same reach as the rest of the study, no sampling or analysis method variations occurred that provide any reason to invalidate the initial study data.

Secondly, the water quality during the sampling period was examined to determine if any unusual conditions existed during the sampling. To assess this, the water quality during the initial sampling was compared to both the typical conditions discussed previously and the average conditions found during the work plan sampling. Table 13 summarizes those results.

Table 13. Water Quality Conditions During Initial Study as Compared to WER Study and "Typical" Conditions (1)

Discharger	Station		Average Ammonia (mg/L-N)	Average Hardness (mg/L)	Average Alkalinity (mg/L)	Average DO (mg/L)	Average TDS (mg/L)	Average Chloride (mg/L)	Average Conductivity (µS/cm)	Average pH
San Jose Creek	Initial Study (SGR-R4)		8.98	244	226		880			8.0
	WER Study (SGR1)		4.4	181	159	8.7	498	114	816	7.5
	Typical Conditions (R3)	Average		200						
		Range [2]		198-202						
		Number Samples		4						
Los Coyotes	Initial Study (SGR-R9W)		4.14	236	226		781			8.1
	WER Study (SGR2)		4.2	242	198	9.8	664	175	1184	8.3
	Typical Conditions (R5)	Average	5.6	224						
		Range [2]	1.42-9.82	218-230	219-230					
		Number Samples	6	2						
LA Glendale	Initial Study (LA-R7)		4.33	236	146		595			7.7
	WER Study (LA2)		7.8	251	199	8.5	563	134	1019	7.8
	Typical Conditions (R7)	Average	6.5	284		9.3	644	107	1034	7.61
		Range [2]	2.5-10.5	216-352		6.45-12.05	546-742	83-131	838-1230	7.41-7.81
		Number Samples	9	9		27	9	9	9	27
DC Tillman	Initial Study (LA-R8)		13.6	218	187		619		1100	7.5
	WER Study (LA1)		11.5	174	188	8.3	511	147	1010	7.9
	Typical Conditions (R8)	Average	8.8	282		11	689	109	1079	8.05
		Range [2]	4.03-13.63	132-432		6.0-16.0	471-907	88.4-129.6	771-1387	7.51-8.62
		Number Samples	9	9		27	9	9	9	27

1. The stations that start with R (i.e. RA, RB, RE, etc.) are the "typical" conditions used for comparison in this analysis. These represent the POTW receiving water monitoring location that is closest to the chosen sampling location. The average conditions were taken from the work plan for this study. For the most part, the averages and standard deviations are based on data from 1996 to 2000. In some cases there are only a few data points during this period of time.
2. The range is equal to the mean plus or minus two standard deviations and is used to assess whether the sampling results are within the range of typical conditions.

On the Los Angeles River, the water quality constituents were all within the range of the rest of the study and the typical conditions for the study. On the San Gabriel River, TDS was higher than the TDS measured during the remainder of the sampling events, but the remainder of the constituents were similar to the rest of the study. The higher TDS measurements could account for the higher WERs seen during the initial study on the San Gabriel River. The Los Angeles River initial study WERs are similar to the WERs found during the rest of the study.

Finally, the impact on the final WER (fWER) calculated from the individual results was assessed. Because the fWER is equivalent to an adjusted geometric mean, the final calculation is partially dependent on the sample size. Therefore, as the number of samples increase, so does the adjusted geometric mean even if the other components of the calculation (average and standard error for the samples) remain the same. Table 14 lists the different fWERs calculated based on adjusting the sample size with and without the initial study results.

Table 14. Analysis of Impact of Additional Sample Number on fWER with Initial Study Data

Site	Initial Study Data	No Initial Study, but extra sample	Initial study, but one less sample	No initial study
LA1	1.966	1.858	1.891	1.83
LA2	1.967	1.826	1.904	1.77
SGR1	1.637	1.423	1.475	1.41
SGR2	2.303	2.059	2.251	2.02

The results show that the sample size does impact the fWER calculation, though it is more significant in the San Gabriel River than in the Los Angeles River.

The review shows that the methods and analysis techniques did not vary between the two sets of sampling events. The TDS concentrations during the initial sampling event on the San Gabriel River were higher than the average observed during the remainder of the sampling events, but the remainder of the water quality constituents were similar. By including the initial study data, a wider range of conditions in the river is represented by the WER. Additionally, although the initial study WERs are higher than the remainder of the study results, at least a portion of the impacts on the fWERs is based on the additional sample, not the higher value of the WER. Because the methods and analysis techniques are similar between the two sampling periods, there is no scientifically valid reason to exclude the initial study data. Although the fWERs for these four sites are higher with the initial study data than without the data, there is no evidence that these results do not represent an actual condition in the waterbody that should be captured by the fWER. Because of the number of conservative assumptions that have been built into the calculation of the fWERs (acute studies rather than chronic studies, the use of the EPA pH relationship, the use of an adjusted geometric mean rather than an unadjusted geometric mean or median value), the use of the initial study data will not result in a fWER that is not protective of the waterbody. As a result, the initial study data with the exception of LA-R4 will be included in the calculation of the fWERs.

STEP 3: CALCULATE THE ADJUSTED GEOMETRIC MEAN OF THE DRY WEATHER *HYALELLA* ACUTE WERS

For each site, a number of dry weather WERs were calculated (as shown above). To determine one dry weather WER applicable to the site, the WER guidance recommends the use of the adjusted geometric mean of the dry weather results. Using the individual acute *Hyalella* WERs listed in Table 12, the adjusted geometric mean WER for dry weather for each site was determined using the following process (1994 guidance page 71):

- Take the natural logarithm of each of the WERs.
- Calculate the arithmetic mean of the logarithms ($\bar{x}$).
- Calculate the sample standard deviation of the logarithms (s): $s = \sqrt{\dfrac{(x-\bar{x})^2}{n-1}}$

- Calculate the standard error of the arithmetic mean (SE): $SE = \frac{s}{\sqrt{n}}$
- Calculate the adjusted geometric mean (A): $A = \exp(\bar{x} - (t_{0.7})(SE))$ where $t_{0.7}$ is the value of the Student's t statistic for a one-sided probability of 0.70 with n-1 degrees of freedom (df). The following table summarizes some typical values of $t_{0.7}$.

Degrees of Freedom	**$t_{0.7}$**
1	0.727
2	0.617
3	0.584
4	0.569
5	0.559
6	0.553
7	0.549
8	0.546
9	0.543
10	0.542
11	0.540
12	0.539

Table 15 summarizes the dry weather adjusted geometric means for each site along with the geometric mean and median value for comparison.

Table 15. Adjusted Geometric Mean, Geometric Mean, and Median WERs of Dry Weather *Hyalella* Acute Samples[1]

Site	**Adjusted Geometric Mean**	**Geometric Mean (not adjusted)**	**Median**
LA1	1.966	2.124	2.357
LA2	1.967	2.099	2.176
BW1	1.400	1.450	1.446
SGR1	1.637	2.015	1.473
SGR2	2.303	2.554	2.485
CC1	2.038	2.202	2.099
SJC1	1.395	1.544	1.544
RH1	2.094	2.150	2.150
SCR1	2.233	2.411	2.669
SCR2	2.206	2.335	2.296

1. Calculated from WER 1, WER 2, WER 3, and Initial Study WER.

STEP 4. CALCULATE THE WET WEATHER HWERS

For the wet weather samples, the WER guidance provides a calculation procedure to determine a wet weather WER that is protective of the different conditions that occur during wet weather and is also comparable to the dry weather WER results. The following equations are used to calculate the hWER for wet weather samples:

$$HCE = \frac{[(CCC)(WER)(eFLOW + uFLOW)] - [(uCONC)(uFLOW)]}{eFLOW}$$

$$hWER = \frac{(HCE)(eFLOWdf) + (uCONCdf)(uFLOWdf)}{(CCC)(eFLOWdf + uFLOWdf)}$$

where:

HCE	=	the highest concentration of ammonia in the effluent.
CCC	=	the chronic criteria to be adjusted.
eFLOW	=	the effluent flow at the time of sample collection
uFLOW	=	the upstream flow at the time of sample collection
uCONC	=	the concentration of ammonia in the upstream water
eFLOWdf	=	the effluent flow at design flow conditions
uCONCdf	=	the upstream concentration at design flow conditions
uFLOWdf	=	the upstream flow at design flow conditions

The calculation of the hWER for wet weather events is dependent on the effluent and river flow (eFLOW and uFLOW) at the time of sampling, the design flow of the effluent and river (eFLOWdf and uFLOWdf), the concentration of ammonia upstream of the discharge (uCONC), and the chronic criterion (CCC). In Southern California waterbodies, these values vary significantly during rain events. For example, a sudden downpour could cause the flow in the river to increase by a factor of two or more during the period of time while samples were being collected.

During sampling, estimates of flow were made during sample collection, but the magnitudes of the flows made it very difficult to make estimates with significant accuracy. Direct flow measurements during wet weather events were not possible because of safety concerns. Except for the Santa Clara River, these waterbodies are channelized high flow rivers with very high velocities and flow rates during wet weather. Sampling was conducted from a bridge or the side of the channel to prevent the sampling crew from being swept away in the flows. Portable flow measuring equipment was not available that was capable of measuring flows of the magnitude that occur during wet weather events. Additionally access to the entire width of the river is restricted in some cases during high flows, and it is not possible to get estimates from all portions of the river. For this reason, gauging stations near the sampling location were used to estimate the flow at the time of sample collection. However, the gauging stations did not always collect flow measurements during the sample collection time and were different distances from the sample locations. It is possible that the flows at the gauging stations could have been influenced by a shower in the vicinity of the station that did not impact the sample location. Consequently, though the flow measurements that were obtained are considered to be the best estimate of flows at the time of sampling, there is some error associated with these measurements and choices had to be made as to how to use the flow data in the calculations. For example, if the gauge recorded a flow an hour before the sampling period and two hours after the sampling period, should the actual measurement of flow closest to the sample collection be used or should an interpolation of the possible flow at the time of sample collection be used?

Another area of possible variability is the choice of the chronic objective to use in the comparison. The WER guidance is unclear as to whether the chronic objective that applies during defined design flow conditions (dry weather) or the chronic objective that was applicable during the wet weather event be used for the calculation. Because of the possible variations in the way in which the wet weather hWER could be calculated, different scenarios were reviewed to assess the impact of the assumptions on the calculation of the hWER. The following scenarios were assessed:

- Scenario 1: Use the criteria applicable to the design flow condition (dry weather) and the recorded gauge flow measurement from the time closest to the actual sample time.
- Scenario 2: Use the criteria applicable to the wet weather event sampled and the recorded gauge flow measurement from the time closest to the actual sample time.
- Scenario 3: Use the criteria applicable to the design flow condition (dry weather) and the flow measurement interpolated from the recorded gauge measurements for the actual sample time.
- Scenario 4: Use the criteria applicable to the wet weather event sampled and the flow measurement interpolated from the recorded gauge measurements for the actual sample time.

The following table (Table 16) shows the hWERs for each of the scenarios.

Table 16. Wet Weather hWER Variability Based on Flow and Criteria Assumptions

Site	Sample Date	Scenario 1	Scenario 2	Scenario 3	Scenario 4
LA1	2/13/03	8.0	8.0	9.4	9.4
LA2	12/17/02	1.6	1.7	1.1	1.4
BW1	12/17/02	6.3	4.3	13.7	13.7
SGR1	2/13/03	8.4	8.4	8.8	8.8
SGR2	12/17/02	4.7	4.7	4.8	4.8
CC1	12/17/02	8.5	8.6	8.6	8.7
SJC1*	12/17/02	3.9	3.9	N/A	N/A
RH1	2/13/03	5.1	5.1	3.9	3.9
SCR1*	2/13/03	12.5	12.5	N/A	N/A
SCR2*	2/13/03	9.9	9.8	N/A	N/A

*The only flow measurements available for these sites were mean daily discharge so the hWERs could not be calculated at different flows. Therefore, Scenario 3 and 4 are not applicable to these sites (N/A).

The assessment determined that the hWERs were sensitive to the choice of assumptions, especially the flow used to calculate the hWER. For example, at BW-1, the hWERs range from 4.3 to 13.7 depending on the assumptions used.

In addition, the hWER is partially dependent on the upstream ammonia concentration. Just as flows can change rapidly over time during the course of a wet weather event, so can the upstream ammonia concentration as discharges are diluted to different degrees by the river flow. Therefore, the hWER is very dependent on the exact conditions that occurred during sample collection. To demonstrate this, the flow measurements collected over the course of the storm at the gauge nearest to LA2 were used to determine the hWERs that would occur at that site during the course of the storm (assuming no change in upstream ammonia concentrations). The following graph shows the flows and the associated hWERs.

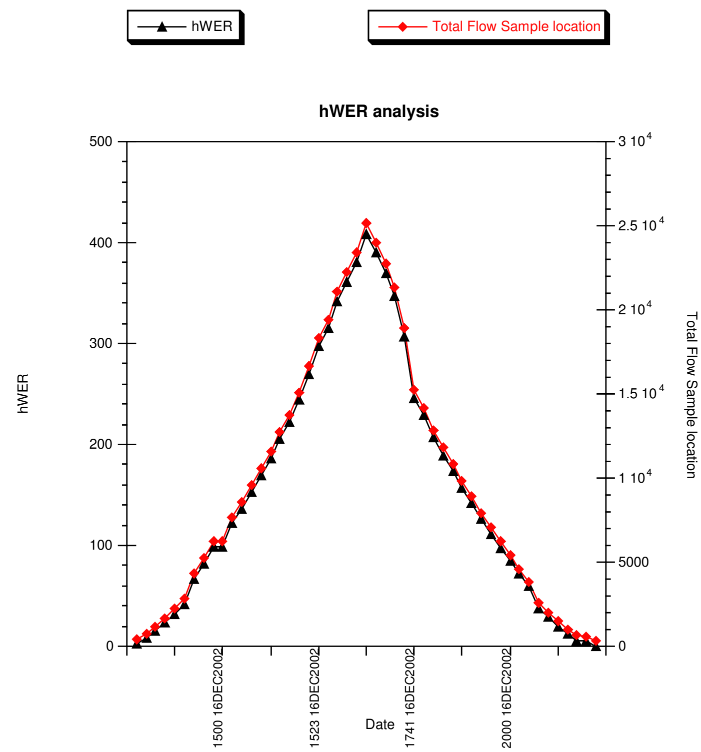

Figure 4. Estimated hWERs at LA2 during 12/17/02 Storm Event Based on Changing Flow Rates

Over the course of the storm, the hWERs were estimated to range from 1.0 to 409 depending on the flow and estimated upstream ammonia concentration. As shown in Figure 4, the hWERs track the flows almost exactly. Therefore, samples collected at the beginning or end of the storm have lower hWERs than those collected during the storm peak.

For calculation purposes, the measured flow closest to the sampling period and the chronic criteria calculated during the wet event were used to determine the hWER for the study. Table 17 summarizes the calculated hWER (in bold) and the information that was used to calculate the hWER during wet weather events.

Table 17. Flow and hWER Results for Wet Weather Samples

Site	Date	Wet Weather WER (hWER)	NH3 Criteria (CCC)	WER	Effluent flow (eFLOW)	Upstream flow (uFLOW)	Upstream NH3 Conc. (UCONC)	Design effluent flow (eFLOWdf)	Design upstream NH3 conc. (uCONCdf)	Design upstream flow (UFLOWdf)	Highest Effluent NH3 Conc. (HCE)
LA1	2/13/03	**8.027**	4.73	2.3	66	167	0.0	125	0.42	0.0	38.0
LA2	12/17/02	**1.740**	2.87	1.4	24	127	5.1	31	7.3	79.6	-0.8
BW1	12/17/02	**4.314**	2.35	2.0	9	10	0.0	14	0.0	0.0	10.2
SGR1	2/13/03	**8.378**	2.17	0.4	61	1345	0.0	156	0.46	0.0	18.2
SGR2	12/17/02	**4.736**	2.17	2.0	54	71	0.0	59	4.2	0.0	10.3
CC1	12/17/02	**8.622**	2.43	1.9	30	176	0.37	39	0.41	16.1	29.4
SJC1	12/17/02	**3.938**	2.78	1.6	9	12	0.0	23	0.0	0.0	11.0
RH1	2/13/03	**5.084**	3.09	1.0	15	61	0.0	23	0.0	0.0	15.7
SCR1	2/13/03	**12.54**	4.01	2.5	14	56	0.0	10	0.05	0.0	50.2
SCR2	2/13/03	**9.845**	3.58	2.5	20	94	0.0	20	0.53	8.4	50.1

HCE = the highest concentration of ammonia in the effluent.
CCC = the chronic criteria to be adjusted.
eFLOW = the effluent flow at the time of sample collection
uFLOW = the upstream flow at the time of sample collection
uCONC = the concentration of ammonia in the upstream water (if the value was not detected, the concentration was set equal to zero).
eFLOWdf = the effluent flow at design flow conditions
uCONCdf = the upstream concentration at design flow conditions
uFLOWdf = the upstream flow at design flow conditions

STEP 5. DETERMINE THE FWER

The WER guidance lays out a proposed procedure for determining the fWER from the calculated dry and wet weather WERs. Assuming that at least 19% of the data were collected during flows two to ten times higher than design flow of the waterbody, and the range of the WERs are not more than a factor of five apart, the fWER is the lower of the adjusted geometric mean of all the design flow (dry weather) WERs and the lowest hWER. Based on the analysis presented in this document and input from the TAC, two deviations from this procedure were used to determine the fWER for the study. The deviations are discussed in detail in the next two sections.

Use of Wet Weather hWER

The WER guidance procedure places a large emphasis on the wet weather sample and the results obtained during wet weather. During the calculation of the wet weather hWERs, it became clear that the determination of the hWER was significantly impacted by the assumptions used in calculating the hWER, especially the flow conditions (see Step 4 discussion above). Because the flow conditions are highly variable in Southern California, the use of a hWER based on a flow condition that could change dramatically over a very short period of time is difficult to justify. Consequently, the appropriateness of using the wet weather hWER versus the adjusted geometric mean of the dry weather WERs was evaluated.

As shown in the discussion of the wet weather hWER (Step 4 above), the hWER calculations generally result in wet weather hWERs that are significantly higher than the adjusted geometric mean of the dry weather WER. The one exception is LA2 where the hWER drives the fWER using the calculation conditions chosen. However, because the choice of calculation conditions causes such variability in the hWER, under other wet weather conditions, the hWER may not be the lowest value. Over the course of the storm at LA2, the hWER was estimated to range from 1.0 to 409 based on the changing flow conditions in the river.

Additionally, the chronic objectives are the only objectives being adjusted by the fWER. The chronic objective is based on a 30-day averaging period. Wet weather events in Southern California occur over a matter of hours to days, but generally do not last for weeks at a time. Therefore, the application of a hWER based on a short term condition to a 30-day chronic objective is not appropriate. Therefore, it was determined that the appropriate approach for this study was to use the adjusted geometric mean of the dry weather events as the fWER for all of the sites.

Use of Fathead Minnow Tests

The WER guidance uses the results of toxicity tests on a primary and secondary species to determine the fWER. However, as the results for this study were gathered, it became clear that the secondary species in this study (the fathead minnow) demonstrated lower WERs than the primary test species results (0.9-1.7). Because the fathead minnow is a less sensitive species than *Hyalella,* a lower WER was expected during the testing. Based on the differences in sensitivities between the two species (*Hyalella* is two times more sensitive to ammonia than fathead based on the 1999 Update) , the WERs found for fathead confirm the results of the *Hyalella* testing because they are approximately half of the WERs found for *Hyalella.* However, since the fathead minnow was less sensitive than *Hyalella* (i.e. ammonia toxicity was observed at a significantly higher concentration, above the range of the probable SSO value), the question was raised whether the fathead minnow WER value should be given equal weight in the final WER calculation. Consequently, the TAC recommended, and the study participants and the Regional Board agreed, that an alternative approach should be used for calculating ammonia SSOs would be undertaken for this particular study. Using the alternative approach would guarantee the protection of both invertebrates and fish in the waterbodies based on the results of the WER testing.

To calculate the 1999 EPA chronic criteria equation for ammonia, data that reflects the toxicity of ammonia to invertebrate species (such as *Hyalella*) and vertebrate species (such as the fathead minnow) were compiled. Then, the basic procedure outlined in the *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (USEPA, 1985b) was used to compare the invertebrate and vertebrate data and calculate the chronic criteria equation for ammonia. The TAC recommended that the *Hyalella* WER results be used to adjust the invertebrate data used to calculate the 1999 ammonia criteria. However, to be protective of the fish species, the data for the vertebrate species should not be changed based on this study. Then, the criteria would be recalculated based on the adjusted invertebrate data to determine a new criteria equation that would be protective of vertebrates and invertebrates alike. Based on this recommended approach from the TAC, the fWERs calculated for this study were based exclusively on the acute *Hyalella* results and only the invertebrate data was adjusted by these fWERs in the calculation of the recommended SSOs.

Although it was determined that the fish data would not be adjusted based on the results of the study, the fathead minnow data were examined to ensure that the data collected was adequate for the study. Tests were collected in the month of July and the month of September. However, the results collected in September could not be used because a parasite was found in the laboratory dilution water sample. Because the successful fathead minnow samples were all collected during one month, the *Hyalella* results were examined to determine if any significant differences occurred during different times of the year that might suggest the need for additional fathead monitoring. For the analysis, dry weather wet season and wet weather events were compared to the results from the dry season, dry weather events to assess if any differences existed.

At all but one site, the dry weather wet season sample was the highest or second to highest result for the site (in terms of total ammonia-N LC50s). In all cases, the pH of the dry weather wet season sample was similar to or slightly higher than the dry season samples. Additionally, the dry weather wet season sample results were generally similar to results from the dry season events. The dry weather wet season data fit well within the spread of the results of all of the data and does not appear to have significant differences from the dry season data. Also, as discussed in the analysis of the hWERs, the wet weather data demonstrate that at all but one site, the toxicity of ammonia is significantly reduced during wet weather events. Based on these two analyses, differences between dry weather samples during different seasons were not significant and wet weather samples demonstrated that toxicity was likely to be reduced during wet weather. Consequently, the lack of samples in any month other than July is not likely to have not accounted for a condition that would significantly alter the results obtained. Additionally, because the fathead minnow data were not used to adjust the criteria and determine SSOs, the small dataset does not impact the WER and SSO calculations.

FWER Analysis

Based on the two deviations described above, fWERs were calculated as the adjusted geometric mean of the dry weather acute *Hyalella* WERs. Due to the complexity of the study and number of samples collected, a number of fWERs were calculated and are presented in this report to illustrate how different approaches affect the study results. Then, based on the analysis, recommendations for the fWERs were determined. Different fWERs were calculated for each individual sampling station, each waterbody in the study and all sampling stations. Based on the individual WERs shown in Table 12, the following fWERs were calculated.

Table 18. fWERs for All Scenarios

Site	fWER
LA1	1.966
LA2	1.967
BW1	1.400
SGR1	1.637
SGR2	2.303
CC1	2.038
SJC1	1.395
RH1	2.094
SCR1	2.233
SCR2	2.206
LA River	1.783
San Gabriel River	2.032
Santa Clara River	2.282
Upper San Gabriel River	1.708
Lower San Gabriel River	2.134
Overall WER for Study	1.956

The watershed fWERs and overall fWER for the study were determined by using the individual dry weather WERs for the watershed and the whole study and calculating the adjusted geometric mean of all of the appropriate values. For example, for the Santa Clara River, the fWER is equal to the adjusted geometric mean of the three WERs from SCR1 and the three WERs from SCR2.

Site Analysis

The variability in fWERs between sites and watersheds is not very significant, ranging from 1.395 to 2.303. For the most part, the watershed fWERs and overall fWER for the study are all around 2. To determine whether or not the differences between the sites were significant, an analysis of variance (ANOVA) was conducted. This analysis basically compares the means of the WERs collected at each site, the variance of the WERs, and information about the entire dataset to determine if the results are statistically different at a 95% confidence level. The results demonstrated that all of the WERs were statistically similar at the 95% confidence level except BW1 and SGR2.

Based on these results, it would be possible to recommend one SSO that applied to all waterbodies that was equal to the overall watershed fWER for the study (1.956). However, because differences were seen between the Burbank Western Wash and the San Gabriel River, the chosen approach for this study was to use a site-by-site approach to account for the variability observed in the waterbodies and account for the possible differences in the ions causing the WER as demonstrated by the water quality analysis comparison.

Connection to Water Quality Analysis

Based on the results of the water quality constituent analysis described earlier, ions were found to be a factor contributing to the reduced toxicity and WERs found in the study. In particular, sodium and TDS were demonstrated to have the most significant relationship to the WER. To confirm this analysis, the final WERs were plotted against average site TDS and sodium concentrations to see if increasing ion concentrations resulted in higher WERs.

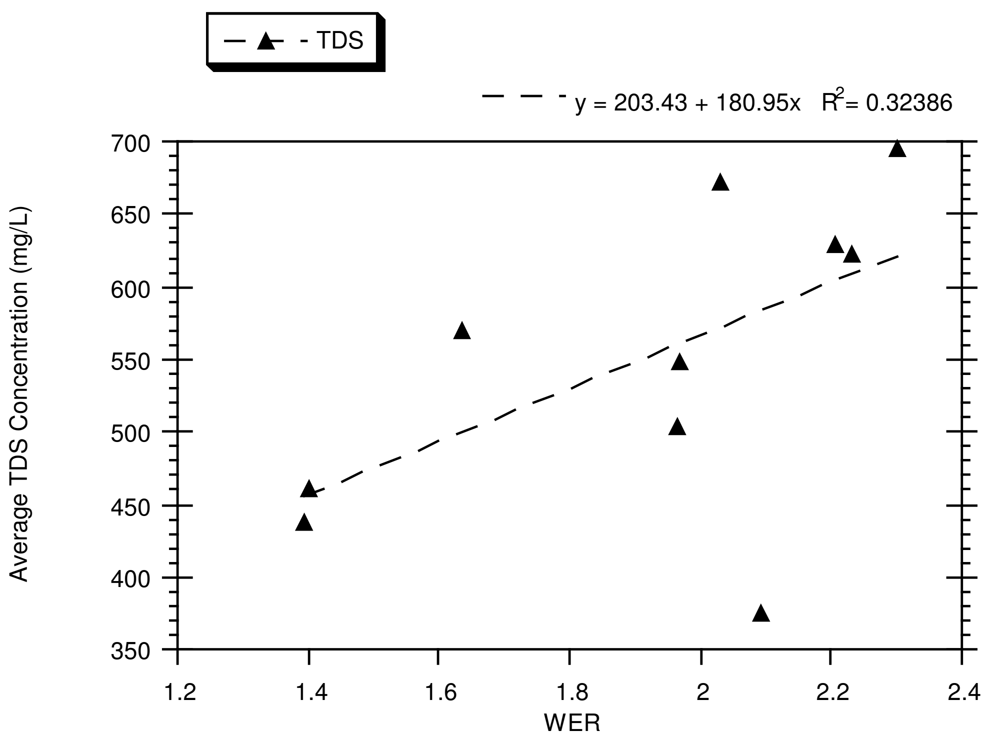

Figure 5. Comparison of Final WERs to Average TDS Concentrations at Each Site

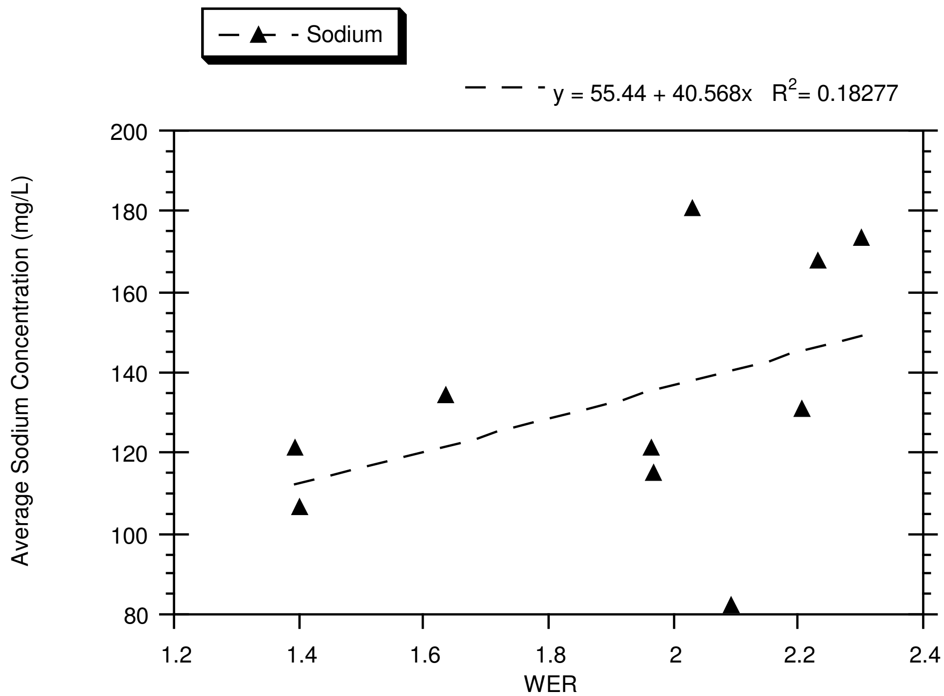

Figure 6. Comparison of Final WERs to Average Sodium Concentrations at Each Site

Both TDS and sodium confirmed the expected analysis results when compared to the final calculated WERs. At higher concentrations of sodium and TDS, higher WERs were found at almost every site.

Sensitivity of fWER Calculations

The laboratory that conducted all of the toxicity testing (MEC Analytical Systems, Inc.) during the study provided comprehensive reports and analysis of the data for review. As part of the reports, they calculated the toxicity results using both the average and maximum ammonia concentrations observed during testing and provided all of the pH and temperature information collected during the tests. Although the TAC recommended the use of the average conditions, because ammonia, pH, and temperature all varied to some degree during each testing period, the change in fWERs based on this variability was assessed. For the analysis, fWERs were calculated based on the maximum ammonia results and compared to the fWERs calculated above. The impact of pH variability was also examined. The maximum and minimum pH for each test was used to calculate the fWERs for comparison. Table 19 shows the results of this analysis.

Table 19. Variability of fWERs Based on Ammonia and pH Variability

Site	fWER by Site	fWER based on Maximum Ammonia Values	fWER based on Maximum pH Values	fWER based on Minimum pH Values
LA1	1.966	NC	2.555	1.473
LA2	1.967	NC	1.190	1.640
BW1	1.400	1.312	1.584	1.443
SGR1	1.637	1.434	1.491	1.623
SGR2	2.303	2.097	2.331	2.608
CC1	2.038	2.012	2.395	2.372
SJC1	1.395	1.473	0.353	1.360
RH1	2.094	2.027	2.216	2.042
SCR1	2.233	1.893	2.267	1.969
SCR2	2.206	2.057	2.625	2.192

NC-Could not calculate a fWER because the laboratory did not provide LC50s based on maximum ammonia concentrations for some of the sampling events.

Based on this analysis, the impact of maximum ammonia concentrations and maximum and minimum pH values is not significant in most cases. In almost all cases, the alternative calculations result in similar or higher fWERs. Under all of the scenarios, the bulk of the fWERs are approximately 2 and do not change significantly from the fWERs calculated using average conditions. The average conditions are the most appropriate values to use because they are the conditions to which the organisms are exposed over the course of the entire test. The extreme conditions represent a value to which the organisms were only exposed for a short period of time and the impact of the short term exposures cannot be extrapolated from the test results that represent the overall conditions during the test. Additionally, average ammonia, pH and temperature conditions during testing are the values used to determine the 1999 Ammonia Criteria that are being adjusted by the WER. Therefore, the fWERs calculated using the average concentrations were used for this study.

Recommended fWERs

Based on the analysis conducted above, the recommended fWERs for this study are the site-by-site fWERs calculated using average pH and ammonia results and including the initial study data from 4 of the 5 initial study sites. The recommended fWERs are listed in Table 20.

Table 20. Recommended fWERs for Study

Site	Recommended fWER by Site
LA1	1.966
LA2	1.967
BW1	1.400
SGR1	1.637
SGR2	2.303
CC1	2.038
SJC1	1.395
RH1	2.094
SCR1	2.233
SCR2	2.206

Site-Specific Objectives

The final step in the process is the calculation of a site-specific objective (SSO) for the sites based on the fWERs. The traditional approach to calculating a SSO is to multiply the fWER times the existing objective to obtain the SSO. Because of the alternative approach taken under this study (i.e. invertebrate and vertebrate data being adjusted independently within the chronic criteria equation calculation), the method for calculating the SSO is more complicated. The approach taken included a recalculation of the criteria using the 1999 Ammonia Criteria document and the *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (Guidelines) (USEPA, 1985b). In this section, a basic summary of the calculation process based on these two documents is presented along with the proposed SSOs calculated from the recommended fWERs.

The process outlined in the Guidelines for calculating criteria is driven, in most cases, by the toxicity results for the four lowest tested genera. For the acute criterion, invertebrates are not among the most sensitive genera tested and do not drive the calculation of the acute criterion. Although the 1999 Criteria Update discusses the fact that under soft water conditions, *Hyalella* may be more sensitive than other species (1999 Update pp. 31 and 40), this information was not used to adjust the criteria. Consequently, it is not possible to adjust the acute criteria using the WER without recalculating the criteria using the information that EPA chose not to include in the criteria. Additionally, because conditions in Southern California do not include low hardness waterbodies, this information is not applicable to the SSOs for these waterbodies. As a result, the acute criterion was not adjusted based on the results of this study. The chronic criterion is driven by invertebrate test results and chronic SSOs are being proposed based on the study results. The calculation process described here is for the chronic criterion calculations.

In the 1999 Ammonia Criteria, chronic tests on 10 genera and the associated genus mean chronic values (GMCVs) are used to calculate the chronic criteria. Of these species, four are invertebrates and five are fish genera. The first step in the SSO calculation process was to multiply the fWERs by the four invertebrate GMCVs in the criteria. The fish GMCVs were not multiplied by the fWER. The next step was to recalculate the Final Chronic Value (FCV)[2] using the steps presented in the Guidelines as follows.

1. Order the invertebrate data (multiplied by the fWER) and the fish data from lowest to highest.
2. Assign ranks (R) from 1 to 10 to the ordered data.
3. Calculate the cumulative probability (P) for each data point as R/(N+1).
4. Select the four data points that have cumulative probabilities closest to 0.05.
5. Using those values, calculate the FCV using the following equations:

$$S^2 = \frac{\sum((\ln GMCV)^2) - ((\sum(\ln GMCV)^2/4)}{\sum(P) - ((\sum(\sqrt{P}))^2/4)}$$

$$L = \frac{(\sum(\ln GMCV) - S(\sum(\sqrt{P}))}{4}$$

$$A = S(\sqrt{0.05}) + L$$

$$FCV = e^A$$

The design of the calculation process listed above is to determine a criteria value that will protect 95% of aquatic species. Because there were only 10 genera used to calculate the FCV for ammonia, the calculation process results in a FCV that is lower than any of the ammonia concentrations (GMCVs) demonstrated to cause toxicity to the tested organisms (i.e. the calculation process “assumes” that a hypothetical aquatic species exists that is more sensitive than those that have been tested because of the small data set used in the calculation).

To calculate the FCV, all of the toxicity results were normalized to a pH of 8.0 and temperature of 25°C. Therefore, the FCV calculated from this dataset is determined at this pH and temperature. In the 1999 criteria calculations, this dataset results in a FCV that is lower than the lowest GMCV (*Hyalella*) by about 15% (i.e., the FCV is 85.4% of the *Hyalella* GMCV). Calculating the FCV using datasets normalized to different pHs and temperatures results in different degrees of extrapolation below the lowest GMCV. For that reason, the 1999 ammonia criteria document assumed that it was appropriate to use the FCV at pH 8.0 and temperature 25 °C to calculate the criteria equation for all pH and temperature values. To accomplish this, the difference

[2] The FCV is the value used to determine the criteria that is estimated to be protective of 95% of all species that could be impacted by ammonia.

between the FCV and the lowest GMCV was calculated and incorporated into the criteria equation. The criteria were then determined to be the chronic pH relationship multiplied by 85.4% of the lower of (1) the appropriate fish GMCV (different depending on whether or not early life stages of fish are present) and (2) the temperature adjusted *Hyalella* GMCV.

For the purposes of recalculating the criteria, new FCVs were calculated using datasets in which the invertebrate GMCVs had been multiplied by the fWER. Then, the difference between the FCV and the lowest GMCV (determined after multiplying the invertebrate data by the fWER) was calculated. If the difference was lower than 85.4% (as assumed in the 1999 ammonia criteria), the criteria value of 85.4% was used because it is not appropriate to have a greater degree of extrapolation below than the lowest GMCV value than that assumed in the original criteria calculations. The 1999 Criteria Update discusses the appropriate degree of extrapolation for the criteria (p. 76). Because the degree of extrapolation varies depending on the conditions chosen to determine the criteria (i.e. if the data is adjusted to a different pH and temperature before calculating the criteria), using the same approach as provided in the criteria document was chosen as the appropriate degree of extrapolation for this study. If the difference was higher than 85.4%, the criteria were calculated as the chronic pH relationship multiplied by this new difference (percentage) multiplied by the lower of (1) the lowest appropriate fish GMCV (early life stage present or absent) and (2) the new temperature-adjusted *Hyalella* GMCV.

The net effect of this calculation procedure in most cases is that for waterbodies without early life stages of fish present, the site specific objective basically becomes the national criterion multiplied by the fWER. However, when early life stages of fish are present, the objective is dependent on the fish data and will not always be the national criterion multiplied by the fWER. The calculations for the various stations are included as Appendix 4-Site-Specific Objective Calculations. An example calculation is presented below for SGR1.

1. Multiply the invertebrate data by the fWER of 1.64 for SGR1 and rank the results from lowest to highest:

Genus/Species	GMCV	Rank	Cumulative Probability (P)	GMCV*fWER (1.64) for invertebrates	New Rank	New P
Hyalella	1.45	1	0.09	2.37	1	0.09
Musculium	2.26	2	0.18	3.70	4	0.36
Lepomis	2.85	3	0.27	2.85	2	0.18
Pimephales	3.09	4	0.36	3.09	3	0.27
Micropterus	4.56	5	0.45	4.56	5	0.45
Catostomus	4.79	6	0.55	4.79	6	0.55
Ictalurus	8.84	7	0.64	8.84	7	0.64
Daphnia	12.3	8	0.73	20.1	8	0.73
Ceriodaphnia	16.1	9	0.82	26.4	9	0.82

2. Calculate the FCV using the equations above:

$$S^2 = \frac{\sum((\ln(2.37)^2, \ln(2.85)^2, \ln(3.09)^2, \ln(3.70)^2) - ((\sum((\ln(2.37), \ln(2.85), \ln(3.09), \ln(3.70))^2 / 4)}{\sum(0.09, 0.18, 0.27, 0.36) - ((\sum(\sqrt{0.09}, \sqrt{0.18}, \sqrt{0.27}, \sqrt{0.36})^2 / 4)} = \mathbf{2.01}$$

$$L = \frac{(\sum(\ln(2.37), \ln(2.85), \ln(3.09), \ln(3.70)) - \sqrt{2.02}(\sum(\sqrt{0.09}, \sqrt{0.18}\sqrt{0.27}\sqrt{0.36})}{4} = \mathbf{0.43}$$

$$A = \sqrt{2.01}(\sqrt{0.05}) + 0.43 = \mathbf{0.75}$$

$$FCV = e^{0.75} = \mathbf{2.1}$$

3. Calculate the difference between the lowest ranked GMCV and the FCV:

$$\frac{FCV}{\mathit{Lowest}\ \mathrm{GMCV}} = \frac{2.1}{2.37} = 0.89$$

4. Replace 0.854 in the criteria equation with the difference calculated in Step 3. Multiply the Hyalella GMCV from the criteria document (1.45) by the fWER and replace 1.45 in the equation with the new GMCV.

Original criteria equation with Early Life Stages of Fish Present:

$$CCC = \left(\frac{0.0676}{1 + 10^{7.688-pH}} + \frac{2.912}{1 + 10^{pH-7.688}}\right) * \mathbf{0.854} * MIN(2.85, \mathbf{1.45} * 10^{0.028*(25-T)})$$

Site-Specific Objective Equation:

$$CCC = \left(\frac{0.0676}{1 + 10^{7.688-pH}} + \frac{2.912}{1 + 10^{pH-7.688}}\right) * \mathbf{0.89} * MIN(2.85, \mathbf{2.37} * 10^{0.028*(25-T)})$$

As shown in this example calculation, the component of the criteria equation designed to protect fish species (2.85 in the last part of the equation) is not adjusted by the site-specific objective calculation.

PROTECTION OF FISH SPECIES

Even though the WER used to determine site-specific objectives is only based on the *Hyalella* results, the calculation of site-specific objectives described above is inherently protective of fish species. The calculation process described above is based on national EPA guidance for calculating aquatic life criteria. Through the calculation process, the toxicity values for invertebrates were adjusted based on the WER, but the fish toxicity values were not changed. The calculation process uses these toxicity values to determine a site-specific objective that is protective. The resulting SSOs are the pH relationship multiplied by the lower of 1) the *Hyalella* value adjusted by the WER and 2) the most sensitive fish value. Because the toxicity values for fish species were not changed as a result of the WER, the site-specific objectives are protective of fish at the same level as the 1999 Ammonia Criteria.

As an additional step to ensure the protection of fish species in the local waterbodies, the calculated site-specific objectives were compared to the toxicity values (EC20ss) for fathead minnows found during the toxicity testing. The following table summarizes the toxicity result and the corresponding site-specific objective for the site at the same pH.

Table 21. Comparison of Fathead Toxicity to Proposed SSOs

Site	Test Date	Endpoint	EC20 (Total Ammonia mg-N/L)	pH	Temperature	Recommended SSO
LA1	7/23/02	Biomass	18.2	7.7	20.1	4.9
LA2	7/23/02	Biomass	13.4	7.7	19.7	5.0
BW1	9/24/02	Biomass	12.4	7.8	19.0	3.6
SGR1	9/24/02	Biomass	13.0	7.8	19.0	4.1
SGR2	7/23/02	Biomass	20.4	7.8	19.5	5.3
CC1	7/9/02	Biomass	15.2	7.8	20.5	4.4
SJC1	7/9/02	Biomass	18.4	7.8	20.2	3.3
RH1	7/9/02	Biomass	15.5	7.7	19.8	5.3
SCR1	9/24/02	Biomass	13.5	7.8	20.0	5.0
SCR2	7/9/02	Biomass	14.8	7.8	20.7	4.7

As shown in Table 21, the levels observed to cause toxicity to fathead minnows at the various sites are three to four times higher than the proposed site-specific objectives. Consequently, the proposed SSOs are protective of fish species based on the tests conducted for this study and the method for calculating the SSOs.

PROTECTION OF RARE, ENDANGERED, THREATENED OR LOCALLY IMPORTANT SPECIES

The final step in the development of a SSO is to determine if it remains protective of any rare, endangered, threatened or locally important species (important species). For the watersheds in this study, three species were identified that fit into this category: unarmored three-spine stickleback (*Gasterosteus aculeatus williamsoni*), Santa Ana sucker (*Catostomus santaanae*), and steelhead trout (*Oncorhynchus mykiss*). To determine if the new objectives were protective of these species, available information on ammonia sensitivity was compiled and compared to the FCV calculated above for each site for which possible habitat exists for one or more of these important species. If the available ammonia toxicity data demonstrated that the ammonia sensitivity for one or more of the important species is lower than the FCV, the FCV for the site would be lowered to the lowest species sensitivity and the objectives recalculated.

In the criteria document, acute and chronic data are available for three species in the same genus as steelhead trout (*Oncorhynchus clarki, Oncorhynchus mykiss, and Oncorhynchus nerka*) and one species in the same genus as the Santa Ana sucker (*Catostomus commersoni*). Because the criteria are based on genus values and not species values, the use of data from species in the same genus as the important species is appropriate. For the unarmored three-spine stickleback, data are not available in the criteria document for this species or any species in the same genus. A review of other available data was conducted to identify other sources of information on the ammonia toxicity of the stickleback. Only one study was found on a species related to the unarmored threespine stickleback, the three-spine stickleback (*Gasterosteus aculeatus*). The data are presented in the 1989 Saltwater Ammonia Criteria for salinities of 11 g/kg and 34 g/kg. Additionally, the data are acute data and presented as unionized ammonia rather than total ammonia. Because the data are saltwater data, the conversion of unionized data to total data is difficult and the data are not directly comparable to the freshwater data. However, the acute saltwater data are the only data available for this genus for comparison. Data were also found in the Ecotox database on a species in the same family as the unarmored stickleback, the ninespine stickleback (*Pungitius pungitius*). The endpoint for this test was general histological changes and the temperature and pH data for the test were not available. If general histological changes are considered to be a chronic endpoint, the result can be used as an approximation of the freshwater chronic sensitivity level. However, it is not known whether an adjustment is needed to make a direct comparison at pH 8.0 and temperature 25°C. Although neither of these studies provides data that are directly comparable to the site-specific FCVs, the results provide an indication as to whether or not the unarmored three-spine stickleback is significantly more sensitive than the other species and might need additional protection. Table 22 summarizes the sensitive levels for the important species.

Table 22. Total Ammonia Sensitivities of Important Species

Species	Chronic toxicity level at pH 8, temp 25 (mg-N/L)	Acute toxicity level at pH 8 (mg-N/L)
Santa Ana sucker	>4.79	38.1
Steelhead trout	<4.16 (8.5) [1]	11.23 [2]
Unarmored three-spine stickleback	14[4]	12.2 [3]

1. The data for the genus *Oncorhynchus* were not included in the calculation of the chronic criteria because of the variability in the testing results and the different lengths and endpoints of the studies conducted. Only one species had a result that was used to calculate a species mean chronic value (*Oncorhynchus nerka*). This value is the value used for comparison in this analysis. The value in parentheses is the genus mean chronic value that would be calculated if all of the results were used from all of the species.
2. Value for *Oncorhynchus mykiss* (rainbow trout) to which the final acute value was lowered in the calculation of the 1999 Ammonia Criteria to ensure protection of this species.
3. Estimated total ammonia concentration for lower salinity value (11 g/kg). Unionized value is 2.09 mg-N/L. This value is significantly higher than the most sensitive saltwater species (0.434 mg-N/L unionized ammonia).
4. Value for *Pungitius pungitius* at an unknown pH and temperature. Converted from 1.0 meq/L result in Ecotox.

The FCVs calculated for the site-specific objectives range from 1.88 to 2.38 mg-N/L. None of the FCVs are higher than the chronic toxicity levels presented in Table 22 and the acute criteria were not adjusted based on this study. The data used to assess the unarmored three-spine stickleback do not indicate that this species is significantly more sensitive than either of the other important species or than the calculated FCVs. Based on the above discussions, no adjustments are necessary to make the SSO calculations protective of important species.

RECOMMENDED SITE-SPECIFIC OBJECTIVES FOR AMMONIA

Based on the procedure discussed in this section, the proposed SSO criteria equations for each site based on the recommend fWERs in Table 20 are shown in Table 23.

Table 23. Proposed Site-Specific Objective Equations for Ammonia by Site

LA1	**ELS Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.854 * MIN(2.85, 2.85 * 10^{0.028*(25-T)})$ **ELS Not Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.854 * 2.85 * 10^{0.028*(25-Max(T,7))}$
LA2	**ELS Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.854 * MIN(2.85, 2.85 * 10^{0.028*(25-T)})$ **ELS Not Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.854 * 2.85 * 10^{0.028*(25-Max(T,7))}$
BW1	**ELS Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.92 * MIN(2.85, 2.03 * 10^{0.028*(25-T)})$ **ELS Not Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.92 * 2.03 * 10^{0.028*(25-Max(T,7))}$
SGR1	**ELS Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.89 * MIN(2.85, 2.37 * 10^{0.028*(25-T)})$ **ELS Not Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.89 * 2.37 * 10^{0.028*(25-Max(T,7))}$
SGR2	**ELS Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.854 * MIN(2.85, 3.34 * 10^{0.028*(25-T)})$ **ELS Not Present**

$$CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.854 * 3.34 * 10^{0.028*(25-Max(T,7))}$$

Table 23 cont'd. Proposed Site-Specific Objective Equations for Ammonia by Site

SCR1	**ELS Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.854 * MIN(2.85, 3.24 * 10^{0.028*(25-T)})$ **ELS Not Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.854 * 3.24 * 10^{0.028*(25-Max(T,7))}$
SCR2	**ELS Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.854 * MIN(2.85, 3.20 * 10^{0.028*(25-T)})$ **ELS Not Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.854 * 3.20 * 10^{0.028*(25-Max(T,7))}$
SJC1	**ELS Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.92 * MIN(2.85, 2.02 * 10^{0.028*(25-T)})$ **ELS Not Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.92 * 2.02 * 10^{0.028*(25-Max(T,7))}$
RH1	**ELS Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.854 * MIN(2.85, 3.04 * 10^{0.028*(25-T)})$ **ELS Not Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.854 * 3.04 * 10^{0.028*(25-Max(T,7))}$
CC1	**ELS Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.854 * MIN(2.85, 2.96 * 10^{0.028*(25-T)})$ **ELS Not Present** $CCC = \left(\frac{0.0676}{1+10^{7.688-pH}} + \frac{2.912}{1+10^{pH-7.688}}\right) * 0.854 * 2.96 * 10^{0.028*(25-Max(T,7))}$

Table 24 provides example objectives based on the site-specific equations listed above for different pHs. The table allows comparison of the site-specific objectives determined in this study at each of the sampling locations.

Table 24. Example Site Specific Objectives (Total Ammonia-N in mg/L) at Different pHs

		pH							
	Temperature	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**
LA1	20	9.6	9.2	8.2	6.0	3.4	1.5	0.67	0.37
LA2	20	9.6	9.2	8.2	6.0	3.4	1.5	0.67	0.37
BW1	20	7.4	7.1	6.3	4.6	2.6	1.2	0.52	0.29
SGR1	20	8.3	8.0	7.1	5.2	2.9	1.3	0.58	0.32
SGR2	20	11.2	10.8	9.6	7.1	3.9	1.8	0.79	0.44
CC1	20	9.9	9.5	8.5	6.2	3.5	1.6	0.70	0.39
SJC1	20	7.4	7.1	6.3	4.6	2.6	1.2	0.52	0.29
RH1	20	10.2	9.8	8.7	6.4	3.6	1.6	0.72	0.40
SCR1	20	10.9	10.5	9.3	6.8	3.8	1.7	0.76	0.42
SCR2	20	10.8	10.3	9.2	6.8	3.8	1.7	0.75	0.42

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