

October 19, 2006

Tam Doduc, Chair
State Water Resources Control Board
P.O. Box 100
Sacramento, CA 95812-0100



Dear Chairwoman Doduc:

Thank you for the opportunity to comment on California's proposed final 2004-2006 Clean Water Act Section 303(d) list. We carefully reviewed the proposed final listing decisions and supporting documentation and have concluded that 99% of the State's assessment determinations are consistent with federal listing requirements. We identified 10 additional water body-pollutant combinations that appear to meet federal listing requirements and urge the State Board to include these combinations on the final list (see Table 1, below). We also identified 9 water body-pollutant combinations that do not need to be listed and should be removed from the final list (see Table 2). In several other cases, we could not determine from the decision documents the specific basis for the State's assessment determinations and will need to further review the State's decision rationales upon receipt of the final submittal. Finally, as the State is already working on the 2008 assessment, we include several recommendations to improve the assessment approach used to develop the 2004-2006 list. This letter summarizes our review of individual assessments and overall process concerns; Enclosure 1 describes our review of individual assessment decisions.

I. Overview

As the State's Section 303(d) list submission is long overdue, we urge the State Board to adopt its final 2004-2006 list without further delay. After we receive your final submittal package, we will review the assessment documentation and, if necessary, supporting data and information in the State's administrative record to determine whether the final list meets federal listing requirements. EPA believes the State's highest priority at this point should be to complete its work on the 2004-2006 list now and focus upon development of the 2008 Integrated Report containing Section 303(d) list and Section 305(b) report information.

EPA commends the State for its considerable effort to respond to public comments and evaluate recently submitted data and information. We support the State's decision to list several waters for invasive species in the Central Valley and North Coast Regions and two Central Valley waters for temperature. We also support the decisions concerning the following waters and pollutants as recommended in EPA's comments on the draft list:

- list Laguna de Santa Rosa for nitrogen and phosphorus,
- delist Lower Lost River for temperature,
- list several Dominguez Channel/Los Angeles Harbor segments for toxic pollutants,
- delist Santa Monica Bay for chlordane,
- delist San Gabriel River Reach 3 for toxicity, and
- list Anaheim Bay for dieldrin.

We continue to support the State's decision to delist waters for which no data or information could be found to support prior listings or the listings were determined to be invalid based on reassessment of available data and information.

Consistent with the Listing Policy, the State's Section 303(d) list includes a subcategory of impaired waters with completed TMDLs (Water Quality Limited Segments Being Addressed). We note that based on its assessments of some waters for which TMDLs have been completed, staff concluded that several of these waters are no longer impaired. Although EPA disagrees with several of these assessments, we would not disapprove the State's decision not to list them as federal listing guidance indicates States are not required to include on the Section 303(d) list impaired waters for which TMDLs have been completed. Our comments, therefore, do not focus on these water body assessments.

II. Additional Listing Recommendations

Our review of the listing record indicates several waters not proposed for listing may exceed the applicable water quality standards. We urge the State to consider listing the waters identified in Table 1. The following sections discuss the basis for these recommendations.

Table 1: Additional Listing Candidates

Reg. Bd.	Water Body	Pollutant
3	Chumash Creek	dissolved oxygen
4	Consolidated Slip	benzo[a]anthracene
	Los Angeles/Long Beach Inner Harbor	copper zinc
	Los Angeles/Long Beach-Fish Harbor	benzo[a]-pyrene
	Los Angeles/Long Beach Outer Harbor	sediment toxicity
	San Buenaventura Beach	coliform bacteria
5	Feather River- N. Fork below L. Almanor	copper
7	New River (Imperial)	copper
9	Loveland Reservoir	pH

A. Toxic Pollutant Assessments

The California Toxics Rule (CTR) contains numeric water quality standards for toxic pollutants that are applicable to most California waters. The CTR provides that toxic pollutant criteria are not to be exceeded more than once in three years on average. Assessment decisions for toxic pollutants must be consistent with this allowable exceedance frequency. We also recommend consideration of the magnitude of excursions and excursion frequency to assess water quality standards compliance. The State's application of a binomial statistical method to

assess toxic pollutant attainment of water quality standards appears inconsistent with this CTR provision and has resulted in omission of several waters that should be listed for toxic pollutants. For example, N. F. Feather River and New River appear to exceed CTR standards for copper.

B. Conventional Pollutant Assessments

Listing assessments of conventional pollutants such as dissolved oxygen (DO), pH, and bacterial indicators must be consistent with the provisions of applicable water quality objectives in each Basin Plan. For example, Regional Basin Plan standards for dissolved oxygen typically provide that minimum values are “not to be exceeded at any time” or should be evaluated based on the 85th or 90th percentile. Several Basin Plans provide that bacterial indicator objectives may not be exceeded in more than 10% of available samples. The Policy’s binomial statistical approach applies an allowable 25% exceedance rate for conventional pollutants that appears inconsistent with many applicable objectives. We recommend direct application of allowable exceedance rates specified in Basin Plans. In cases where the Basin Plans do not specify allowable exceedance frequencies for conventional pollutants, we recommend application of a 10% exceedance rate for conventional pollutants, as described in EPA guidance (EPA, 2002; EPA 1997). Several additional waters (e.g. Chumash Creek for DO, Loveland Reservoir for pH, and Mission Bay for coliform bacteria) should be evaluated for listing based on these considerations.

C. Sediment Chemistry and Toxicity Assessments

The Listing Policy states that sediment chemistry shall be used as a basis for listing if supported by evidence of related sediment toxicity or benthic community impacts. In some cases (e.g., Los Angeles/Long Beach Harbor and Consolidated Slip), waters were not listed although available data showed evidence of elevated sediment chemistry levels for individual pollutants and either sediment toxicity or benthic community impacts. These assessments may support listings of these waters and pollutants.

III. Additional Delisting Recommendations

Our review of the assessments of two waters in the Los Angeles Region found that some pollutants are proposed for listing that do not exceed water quality standards for the pollutants in question (see Table 2). In the case of the freshwater portion of Dominguez Channel, the proposed listings for several toxicants were based on samples collected at saltwater sites downstream from this segment that are unrepresentative of the freshwater segment. In the case of Walnut Creek toxicity, recent toxicity sampling results found no toxicity in Walnut Creek (a tributary to San Gabriel River). We have attached the draft sampling report to this letter (see Enclosure 2). As EPA is working with the Regional Board to complete TMDLs both for toxicants in the Dominguez Channel area and for toxicity in San Gabriel River watershed, we believe the Section 303(d) listings should accurately reflect the actual causes of impairment based on the most representative available data sets.

Table 2: Additional Delisting Candidates

Reg. Bd.	Water Body	Pollutant
4	Dominguez Channel (above Vermont Ave)	aldrin Chem A chlordane DDT dieldrin PCBs chromium PAHs
	Walnut Creek	toxicity

IV. Waters For Which the Basis For Decisions Was Unclear

We appreciate your staff's efforts to work with us to explain unclear assessment decisions. However, analysis provided in the listing record and by your staff was insufficiently clear for EPA to determine whether several waters and pollutants meet federal listing requirements. We urge the State to clarify the basis for its assessment that the waters in Table 3 should not be included on the final list.

Table 3. Waters For Which Decisions Are Unclear

Reg. Bd.	Water Body	Pollutant
1	Klamath River	sediment
	Dutch Bill Creek	dissolved oxygen phosphate
	Lancel Creek	dissolved oxygen
	Pocket Creek	dissolved oxygen phosphate
	Austin Creek	phosphate
	Big Sulfur Creek	phosphate
	Santa Rosa Creek	phosphate
	Russian River	phosphate
	Usal Creek	temperature
	Winchuck River	sediment
	Humboldt Bay	dioxin
2	Stege Marsh	toxicity
3	San Luis Obispo Creek	nutrients
4	Ormond Beach	coliform bacteria
	Malibu Creek	invasive species
9	Mission Bay	pathogens

A. Nutrient Effects Assessments

For many waters, the State declined to apply narrative biostimulation objectives to assess waters for nutrient-related impairments due to an apparent concern that available assessment criteria are not fully reliable (e.g., Russian River and several tributaries for phosphate). The State is required to evaluate potential violations of the narrative objectives (40 CFR 130.7(b)(3)).

The State conducted this assessment to support its listing of Laguna de Santa Rosa and other waters for nitrogen and/or phosphorous and should do so for other waters for which nutrient data are available.

B. Klamath River and Tributary Sediment Assessments

When the State previously proposed to list Klamath River for sediment, EPA commented that the State should clarify that its listing did not apply to waters in Indian Country as the State lacks jurisdictional authority over those waters. In response, the State proposed not to list any of Klamath River and/or its tributaries for sediment. The fact sheet prepared for Klamath River sediment indicates several lines of data and information were provided to support potential sediment listings of Klamath River and several tributary Creeks. The data and information in the State's records (potentially including data collected by the Yurok tribe near its tribal boundaries and comments submitted during the comment period) may support sediment listings of Klamath River or its tributaries upstream from Indian Country. The State must clarify how it considered the available data and information and, if warranted, list portions of Klamath River and/or its tributaries for sediment outside tribal boundaries.

C. Evaluation of Data and Information Submitted by Commenters

We appreciate your staff's efforts to consider data and information submitted by public commenters. In most cases, it appears staff did a good job of considering public comments. However, for some waters, we could not determine from the responsiveness summary or fact sheets how staff considered information submitted during the comment period (e.g., information regarding Klamath River sediment, Humboldt Bay dioxin, Malibu Creek invasive species, and several beaches addressed in Heal the Bay's comments. Please clarify how the State considered the data and information submitted for these waters in your final decision and submittal.

V. Assessment Process Concerns

To develop the Section 303(d) list, staff applied the Listing Policy adopted in September 2004. In our comments on the Listing Policy and draft 2004-2006 Section 303(d) list, EPA expressed concern the Policy would be applied in a manner inconsistent with federal listing requirements and applicable water quality standards. Briefly, these concerns involve:

- application of "weight of evidence" analysis procedures
- listing thresholds used for toxic, bacterial and some conventional pollutants that are inconsistent with applicable water quality standards,
- minimum sample size requirements,
- interpretation of narrative water quality standards, and
- documentation prepared to support decisions.

A. Weight of Evidence Assessment

The Listing Policy includes provisions that require the State to conduct a weight of evidence analysis of different lines of evidence that may collectively indicate water quality impairment even when single lines of evidence do not indicate impairment (see Section 3.11).

During the development of the Listing Policy, EPA and other commenters were assured that these provisions would be implemented in accordance with this principle and that the analysis of single lines of evidence is a “first step” in the analysis (Listing Policy Responsiveness Summary, p. B-20). We are concerned that staff now appear to take the position that the “weight of evidence” provisions can be applied to evaluate only those lines of evidence that were not evaluated through other assessment provisions in the Policy (303(d) List Response to Comments, p. 11). This is inconsistent with the plain language of the Listing Policy, which states “When all other Listing Factors do not result in the listing of a water segment but information indicates non-attainment of standards, a water segment shall be evaluated to determine whether the weight of evidence demonstrates that a water quality standard is not attained.” (Section 3.11).

Moreover, as the staff interpretation would enable the State to avoid considering lines of evidence that are existing and readily available, the interpretation is inconsistent with the federal requirement that States consider all existing and readily available data and information in the assessment process (40 CFR 130.7(b)). This interpretation would also enable staff to rely upon decision rules contained in the Listing Policy that are inconsistent with the provisions of applicable water quality standards concerning the allowable duration and frequency of excursions (e.g., for toxic pollutants covered under the California Toxics Rule). Finally, the staff interpretation would enable the State to avoid assessing attributes of data and information that the other listing factors do not consider (e.g., magnitude, duration, and timing of water quality objective excursions and synergistic effects of related pollutants that could indicate nonattainment of standards.) The State Board should direct staff to apply the Policy’s “weight of evidence” provisions not as a rare exception but as a regular practice to ensure all evidence is fully and carefully considered in the assessment process.

B. Review Thresholds Inconsistent with Water Quality Standards

As discussed in sections II.A. and II.B. above, staff relied improperly on the Listing Policy’s binomial decision rules to assess compliance with numeric water quality standards in Basin Plans and the CTR. The binomial decision rules set the allowable exceedance frequency at levels less stringent than provided in the applicable water quality standards. As a result, several waters that exceed the numeric standards are not included on the final list. While the binomial decision rules may be used reasonably as screening tools, the State Board should direct staff not to rely solely on these erroneous decision rules to make final listing determinations in the next listing cycle.

C. Improper Use of Minimum Sample Size Requirements

For several waters and pollutants, staff apparently did not consider listings because available data sets did not meet minimum sample size expectations set in the Listing Policy. Several of the minimum sample sizes are inconsistent with the provisions of applicable Basin Plan and California Toxics Rule water quality standards. While use of minimum sample sizes may be used as a screening tool, final assessments should not be limited in the next listing cycle by minimum sample sizes unless specified in the applicable water quality standards.

D. Application of Narrative Water Quality Objectives

As discussed in Section IV.A above, staff declined to apply narrative water quality objectives in assessing some waters and pollutants for which numeric water quality standards are not in place. For the next listing cycle, all narrative objectives should be applied in the assessment process.

E. Decision Record Is Convolved and Excessively Large

We recommend that the State reconsider how it documents its assessment determinations. We and many other commenters found it extremely difficult to determine the basis for staff's assessment determinations based on review of the voluminous record provided to support the proposed list. First, individual fact sheets often do not clearly explain the data and information considered and specific basis for the assessment determination. Second, the fact sheets and other material were organized in several documents in a convoluted manner. Third, the overall size of the record (4945 pages) made it difficult to carefully review the basis for individual decisions. The State should review how other states organize their Section 303(d) list and Integrated Report documentation and consider revising its approach to documenting its decisions. For example, the State of Arizona organized its most recent Integrated Report documentation in an easy-to-follow tabular form by watershed, which enabled the State to capture its entire Section 303(d) and 305(b) reporting decision in one 331 page document.

During our review of the final State list submittal, it is possible we may identify additional waters that meet federal listing requirements or that require additional explanation of the State's decision. We will discuss these waters with your management team if identified. We would be happy to discuss our comments at your convenience and look forward to receiving the 2004-2006 Section 303(d) listing decision in the near future. If you have any questions, please call me at (415) 972-3572 or David Smith at (415) 972-3416.

Sincerely yours,

/original signed by/

Alexis Strauss
Director, Water Division

Enclosures:

1. Specific comments on proposed final 2004-2006 California Section 303(d) list
2. "Wet and Dry Weather Toxicity in the San Gabriel River"

Enclosure 1: Specific comments on proposed final 2004-2006 California Section 303(d) list

Table 1: Candidates for Inclusion on the Section 303(d) List				
RB	Proposed assessment	Waterbody name	Pollutant	Comment & Recommendation
3	Delist	Chumash Ck	DO	Available data indicate Basin Plan numeric WQO for DO is violated in greater than 10% of samples (40/245). State should retain listing this waterbody for this pollutant.
4	Do Not List	Consolidated Slip	Benzo[a]-anthracene	Assessment record is incomplete. Fact sheet shows evidence of sediment toxicity and sediment chemistry exceedences for this compound (15/53 samples). This is sufficient evidence of impairment based on narrative WQOs. State should include this pollutant on list for this segment.
4	Do Not List	LA/LB Harbor—Inner Harbor	Cu Zn	Assessment record shows sediment quality exceedences for Cu (18/627) and Zn (35/716) samples (CSTF database). There is also evidence of sediment toxicity and benthic community effects for this waterbody. These are multiple lines of evidence of impairment based on narrative WQOs. State should include pollutants on list for this segment.
4	Do Not List	LA Harbor—Fish Harbor	Benzo[a]-pyrene	Assessment record is incomplete. Fact Sheet shows evidence of sediment toxicity and sediment chemistry exceedences for this compound (11/13 samples). This is sufficient evidence of impairment based on narrative WQOs. State should include this pollutant on list for this segment.
4	Do Not List	LA/LB Outer Harbor	Sediment toxicity	Available data show sediment toxicity exists in this waterbody. (9/37 samples are moderately or highly toxic). This is sufficient evidence of impairment based on narrative WQOs. State should include this pollutant on list for this segment.
4	Delist	San Buenaventura Beach	coliform bacteria	Fact sheet indicates WQOs violated in more than 10% of results (44/401 samples). State should retain on list based on exceedences of numeric WQOs.
5	Do Not List	Feather River – North Fork (below Lake Almanor)	Cu	Available data indicate numeric CTR standards are violated for Cu (10/124) samples. State should list pollutant for this segment.

Table 1: Candidates for Inclusion on the Section 303(d) List				
RB	Proposed assessment	Waterbody name	Pollutant	Comment & Recommendation
7	Do Not List	New River (Imperial)	Cu	Available data indicate numeric WQO violations for Cu (10/24) samples. State should list pollutant for this segment.
9	Do Not List	Loveland Reservoir	pH	Available data indicate greater than 10% exceedences of Basin Plan numeric WQO for pH (35/212) samples. State should list pollutant for this segment.

Table 2: Candidates for Exclusion from the Section 303(d) List				
RB	Proposed assessment	Waterbody name	Pollutant	Comment & Recommendation
4	List	Dominguez Channel freshwater (lined portion above Vermont Ave.)	Aldrin, Chem A, Chlordane, DDT, Dieldrin, PCBs	In the 2005 draft factsheets, State provided evidence that the fish tissue sample (TSMP, 1992) was collected at downstream site in estuary portion, not in freshwater area (above Vermont Ave). EPA has confirmed this using lat/long data for sample site (405.12.04). Also, the freshwater stream flow is solely downstream, so there is neither flow nor pollutant transfer from downstream estuarine waters up into the upstream, freshwater segment (lined portion). State should delist these six pollutants for this waterbody.
4	List	Dominguez Channel freshwater (lined portion above Vermont Ave.)	Cr PAHs	Fact sheet states there are sediment exceedences for these two pollutants however there are no sediment results in this freshwater segment. Existing water column data does not show exceedences for these two pollutants (LACDPW). State should delist these two pollutants for this waterbody.
4	List	Walnut Creek	toxicity	Thorough examination of available data including new toxicity results (SCCWRP 2006, see enclosure 2) show no impairment in this segment. This segment should be delisted.

RB	Proposed assessment	Waterbody name	Pollutant	Comment & Recommendation
1	Do not list	Klamath River/ Streams tributary to Lower Klamath River	sediment	EPA concurs with the decision not to list portions of the Klamath River located in Indian Country for sediment as the State lacks jurisdictional authority to list waters in Indian Country. However, the fact sheet prepared to support the Klamath River sediment is unclear as to staff's assessment of whether data and information in the record are sufficient to support listing portions of the Klamath River and/or its tributaries that are not located in Indian Country. The fact sheet(s) indicate some lines of evidence of sediment impairment in these waters. Please clarify the technical basis for the decision to not include on the list for sediment either Klamath River segments or tributary waterbody segments located outside tribal boundaries.
1	Do Not List	Winchuck River	sediment	Fact sheet indicates some lines of evidence of sediment impairment in this waterbody. Please clarify the technical basis for the decision to not include this segment on the list.
1	Do Not List	Tributaries to Russian River- Dutch Bill Creek, Lancel Creek, Pocket Creek	dissolved oxygen	Available data indicate greater than 10% of samples collected in these creeks exceeded the Basin Plan's minimum D.O. objective, which is not to be exceeded "at any time". State has not provided clear information establishing sufficient rationale for not listing these waters.
1	Do Not List	Dutch Bill Creek, Pocket Creek, Austin Creek, Big Sulfur Creek, Santa Rosa Creek, Russian River	phosphate	It is unclear from the fact sheets how the State considered available data for phosphate and/or ortho-phosphate results. State needs to identify numeric guideline for this pollutant and apply it for assessment decisions for these waters (and possibly others in California). State has not provided clear information establishing sufficient rationale for not listing these waters.
1	Do Not List	Usal Creek	Temperature	It is unclear from the fact sheet how the State considered available temperature results for this waterbody.
1	Do Not List	Humboldt Bay	dioxin	It was unclear from the fact sheets and responsiveness summary how the State considered data submitted by Humboldt Baykeeper comments which the commenter alleges is sufficient to support a

Table 3: Basis for assessment decision is unclear				
RB	Proposed assessment	Waterbody name	Pollutant	Comment & Recommendation
				dioxin listing of Humboldt Bay. Please clarify how the State evaluated the data submitted for Humboldt Bay.
2	Do Not List	Stege Marsh	toxicity	Available data appear to indicate substantial amphipod toxicity in this waterbody and the presence of several toxicants that may cause or contribute to the observed toxicity. Please provide a clearer rationale for the decision not to list for toxicity. In addition, fact sheet refers to planned remedial action. If the State is asserting that required controls on pollutant sources in Stege Marsh are expected to result in attainment of applicable toxicity standards, please provide more information to support a conclusion that these controls will be sufficient to ensure attainment of the applicable standards.
3	Delist	San Luis Obispo Creek	Nutrients	State has placed this waterbody in Being Addressed Category; however the nutrient TMDL has not been approved. EPA's review of existing draft TMDL indicates significant revision is required prior to approval. State should retain this waterbody-pollutant combination on impaired waters list until nutrient TMDL has been approved.
4	Delist	Consolidated Slip	Dieldrin	Fact sheet states the original listing was based on tissue MTRL values and sediment EDL values however staff's assessment did not make to OEHHA values or sediment guidelines identified with Listing Policy. State should retain this segment on list or provide good cause for delisting.
4	Delist	Ormond Beach	Coliform bacteria	Available data show numeric WQOs violated in greater than 10% of results (33/279 samples). Fact sheet indicates even higher exceedence rates at certain monitoring stations. It is unclear from the fact sheets if staff's analysis of 1999-2001 data record included examination of individual pathogen results (i.e, enterococci, fecal, or total coliform data). State must provide good cause for delisting or should retain on list for exceedences of bacterial indicators.

Table 3: Basis for assessment decision is unclear				
RB	Proposed assessment	Waterbody name	Pollutant	Comment & Recommendation
9	Delist	Mission Bay shoreline	Pathogens	Available data indicate greater than 10% exceedances of Basin Plan Bacteria WQOs (2016 of 17,847 samples for all pathogen results). It is unclear from the fact sheets and responsiveness summary if staff's analysis of 2001-2003 data record included examination of individual pathogen results (i.e, enterococci, fecal, and total coliform data). Also unclear if state performed an evaluation of data for geomean exceedences. State should retain on list for bacterial indicators based on exceedences of numeric WQOs.

WET AND DRY WEATHER TOXICITY IN THE SAN GABRIEL RIVER

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DRAFT October 5, 2006

INTRODUCTION

Urban watersheds receive a multitude of potential pollutants that can affect aquatic life (Bay et al. 1996, Ackerman et al. 2005, Tiefenthaler and Stein 2005). The San Gabriel River, located on the border between Los Angeles and Orange Counties in southern California, is an ideal example of the ways in which aquatic life may be impacted by potential pollutants. Sources of potential pollutants include treated sanitary wastewaters from five Water Reclamation Plants (WRPs) and untreated urban runoff from approximately 350 km² of developed land discharged into the river via a municipal separate storm sewer system, as well as once-through cooling waters from two power generating stations that is mixed with low volume industrial and sanitary wastes then discharged into the watershed's estuary.

To complicate the fate and transport of anthropogenic pollutants and their resultant effects on aquatic life, the hydrology of many urban watersheds is often highly modified. For example, three major dams were constructed in the upper undeveloped reaches of the San Gabriel River watershed in order to capture, retain, and utilize wet season runoff for potable water use during the dry season. While this provides much needed water for the citizens of Los Angeles, the upper watershed is now hydrologically disconnected from the urbanized lower watershed. The result is that natural waters are unavailable for mixing and dispersion when anthropogenic sources discharge to the river downstream. Even greater hydromodification exists in the urbanized lower San Gabriel River watershed. Many miles of the river in this portion of the watershed are lined with concrete in an effort to reduce flooding and property damage, but this modification also results in the maximum exposure of pollutants to aquatic life through the loss of natural stream and treatment processes. Where unlined channels exist in the lower watershed, temporary dams are inflated to enhance groundwater recharge.

In response to pollutant inputs and hydrologic modification, many urban watersheds have been the focus of water quality regulatory efforts. Urban Los Angeles once again provides a good example. More than 180 waterbodies in the Los Angeles region have been placed on the United States Environmental Protection Agency's (EPA's) list of impaired waters. This list, also referred to as the 303(d) list (referring to section 303d of the Clean Water Act), identifies locations impacted by specific pollutants that can result in toxicity to aquatic life and other impacts. The effect of the 303(d) list is the mandate for future regulation (termed a total maximum daily load or TMDL), which will require the mitigation of these pollutant inputs.

In the San Gabriel River watershed, managers have been implementing mitigation to negate the effects of these pollutant inputs. Over the past 10 years, WRPs in the San Gabriel River watershed have installed additional treatment processes, costing over \$40 million, that have improved the water quality of their discharges. By contrast, little mitigation in terms of structural controls has occurred within the San Gabriel River watershed. Approximately \$__ has been spent on structural best management practices to control pollutant inputs from urban runoff.

The objective of this study was to evaluate the impact of pollutants on aquatic life in the highly urban lower watershed of the San Gabriel River. Impact to aquatic life was assessed through the use of toxicity testing. Four specific goals were identified: 1) assess the magnitude of toxicity at selected locations throughout the San Gabriel River watershed; 2) determine whether or not this magnitude changes seasonally; 3) if toxicity exists, identify the responsible toxicants; and 4) compare the magnitude of toxicity in this study to studies conducted historically in the San Gabriel River watershed to evaluate the effectiveness of watershed management actions.

MATERIAL AND METHODS

Toxicity in the San Gabriel River watershed was evaluated by separating the study into wet weather and dry weather components (Figure 1). The wet weather component consisted of four sampling sites located at the downstream end of major reaches that receive urban runoff. Twenty-liter flow weighted composites were sampled during three storm events on December 29, 2004 (5.3 cm precipitation), April 22, 2005 (2.2 cm precipitation), and January 1, 2006 (3.7 cm precipitation). The dry weather component consisted of sampling a total of 10 sites that included the same four sites sampled during wet weather, plus an additional six sites strategically located in the immediate vicinity of WRP discharges or urban runoff inputs. Dry weather samples were collected at least three days after rain events. Twenty-liter samples were collected from each site during dry weather on a monthly basis from March 2005 to February 2006. Within seven months of this study's initiation, an additional six sites were added for dry weather sampling, all in a single tributary (North Coyote Creek), as a result of observed toxicity. All sites from the Coyote Creek subwatershed, including the additional sites in North Coyote Creek, were sampled until August 2006.

All samples were tested for toxicity using *Ceriodaphnia dubia* examining both acute (lethality) and chronic (reproductive success) endpoints. Testing was initiated within 36 hours of sample collection using undiluted sample and a negative control following standard EPA protocols (EPA 2003a; Table 1). Test organisms were obtained from in-house brood cultures and test duration/exposure lasted until 60% of the surviving females in the control had released three broods (typically between six and seven days). Test solutions were renewed daily.

Toxicity was defined as a 25%, or greater, organism response in the sample exposure relative to control organism response (i.e., <75% survival or reproduction in the 100% sample exposure). In addition, hypothesis testing was conducted following EPA guidelines (EPA 2003a). Hypothesis testing consisted of the nonparametric Fisher's Exact Test for the survival endpoint and an analysis of variance (ANOVA) followed by a multiple comparison procedure for the reproduction endpoint. The parametric Dunnett's Test was used to identify statistically significant differences from the control for reproduction data that were normally distributed with homogeneous variances. The nonparametric Steel's Many-One Test was employed when the data failed normal distribution or equality of variance assumptions.

If a sample was defined as toxic, a toxicity identification evaluation (TIE) was initiated (EPA 1991, 1993b). TIE testing used the remaining sample, stored at 4° C, within seven days of baseline test conclusion. For those samples in which only the reproductive endpoint elicited a toxic response, only 100% and control concentrations were evaluated in the TIE. In these cases, the TIE consisted of a full seven-day chronic test with each sample manipulation consisting of 10 replicates, with daily renewals. For those samples where the survival endpoint elicited a toxic response, three dilutions (25%, 50%, 100%) and a control were evaluated using four replicates containing five test organisms each. In the case of a TIE in response to survival, the exposure duration was 96 hours, with renewal after 48 hours.

The TIE manipulations focused on both characterization and identification phases (EPA 1991, 1993b). These manipulations included: 1) pH adjustment; 2) aeration; 3) Ethylenedinitrilo-

Tetraacetic Acid (EDTA); 4) Sodium thiosulfate (STS); 5) filtration; 6) piperonyl butoxide (PBO); 7) anion exchange column; 8) solid phase extraction (SPE); 9) SPE elution; and 10) no manipulation. By conducting each of these manipulations, the results, alone or in combination, can help to identify the responsible toxicant(s) (Table 2).

All quality assurance/quality control criteria were met for this study. These criteria included all of the test acceptability criteria (Table 1). In addition, positive control samples using reference toxicants (copper chloride) confirmed the relative sensitivity and stability of test organisms during the course of the study.

RESULTS

Wet Weather

None of the storms sampled during this study were acutely or chronically toxic to *Ceriodaphnia*. At all four sites, during all three storms, survival and reproduction were greater than 75% relative to controls.

Dry Weather

Eighteen of 194 (9%) total dry weather samples exhibited chronic toxicity during this study (Table 3). Twelve of 194 (6%) total dry weather samples exhibited acute toxicity during this study. All of the dry weather samples that exhibited chronic toxicity also exhibited acute toxicity. In only one case was statistically significant toxicity observed when the response was less than 25% relative to controls (Station 15, Jan 2006). Only once was toxicity greater than 25% relative to controls and not statistically significant (Station 15, Mar 2006).

All observed toxicity during this study was from Coyote Creek (Table 3). No toxicity was observed in Walnut Creek, San Jose Creek, or San Gabriel River Reaches 1 or 3. Widespread toxicity in Coyote Creek was observed in April 2005. As a result, an additional six stations upstream were added between July and October 2005. Widespread toxicity was observed again in August 2005. Widespread toxicity was not observed again for the remaining 12 months (September 2005 to August 2006).

In the two events for which widespread toxicity was observed in Coyote Creek (April and August 2005), the toxicity appeared to originate in the upper portions of the tributary (Figure 2). In April 2005, 100% reproductive impairment was observed at the site sampled furthest upstream (site 10) and reproductive success remained minimal moving downstream. *Ceriodaphnia* survival was also severely impacted at the furthest upstream station, then survival slowly increased downstream of the WRP discharge (Sites 7 and 6) indicating a potential dilution effect from the WRP effluent. In August 2005, severe reproductive impairment was again observed at the site sampled furthest upstream (site 14) and reproductive success remained minimal moving downstream. *Ceriodaphnia* survival was more sporadic moving downstream during this event. Seventy eight percent survival was measured at site 14 and decreased to 0% survival for downstream Sites 13 and 12. Survival increased to 100% at site 11, but fell back to 0% survival

for the remaining seven miles of Coyote Creek. The sudden increase in survival at Site 11 remains unexplained.

Dry Weather TIE Testing

Seven TIEs were initiated during the study on dry weather samples exhibiting a 25% or greater effect (Table 4). Toxicity was no longer present for three of the samples (sites 9 and 10 March 2005, site 15 March 2006); consequently, no toxicant was identified.

Organophosphorus pesticides, most likely diazinon, were identified as the causative agent in one sample (site 9 April 2005). This result was based on the exclusive removal of toxicity using SPE and the addition of PBO, which removes non-polar organic toxicants and inhibits toxicity due to diazinon, respectively (Figure 3). The SPE was sequentially eluted and these fractions were subsequently tested. Toxicity was recovered in the 80% methanol elution of the SPE column, a fraction associated with many organophosphorus pesticides including diazinon (Figure 4). Finally, 1,700 µg/L diazinon was quantified in the sample using Enzyme-Linked Immuno-Sorbant Assay (ELISA) techniques.

A non-polar organic toxicant(s), possibly a surfactant(s), was identified as the causative agent in the remaining three samples (site 10 April, June, and August 2005). This result was based on the removal of toxicity using SPE. Toxicity was recovered in the 75% methanol elution, a fraction commonly associated with organophosphorus pesticides with surfactant toxicity recovery also documented (Norberg-King et al. 2005). An anion exchange column was used on two samples, with complete removal of toxicity observed in one sample (June 2005) and partial removal in the other (August 2005). This may be indicative of anionic surfactants, but might also suggest the presence of some trace metals. Elution of the anion column would help to confirm anionic surfactant toxicity, but attempts to recover toxicity from the anion column were not successful. However, other treatments to identify trace metals did not reduce toxicity (i.e., EDTA), which helps to rule-out metals as a major source of toxicity. Aeration partially removed toxicity in the April 2005 sample. Some surfactants can be removed or partially removed through aeration. Finally, PBO did not reduce toxicity, and levels of diazinon in these three samples were low (<100 µg/L).

DISCUSSION

Toxicity was not widespread in the San Gabriel River watershed over the 18 months examined during this study. No toxicity was observed at any site during any of the storm events sampled. Similarly, no toxicity was observed in four of the five major reaches in the lower watershed during dry weather. In Coyote Creek where toxicity was observed, the toxicity was intermittent, and occurred only during six of the 18 sampling periods. This was despite an adaptive monitoring strategy, in which the number of sites sampled in Coyote Creek was doubled and the sampling period was extended by six months.

The lack of toxicity observed in this study was in direct contrast to historical studies in this watershed. While 9% of the samples were toxic in 2005/06, 55% of the samples collected for a

similar study in 1992/93 were toxic (UC Davis 1995). Moreover, toxicity was observed in only a single reach (Coyote Creek) in 2005/06, while UC Davis (1995) identified toxicity in all five major reaches in the lower San Gabriel River watershed.

The difference in toxicity from tests conducted 14 years ago is likely due to changes in water quality. UC Davis (1995) concluded that toxicity in the San Gabriel River watershed was likely due to non-polar organics and possibly ammonia. This is not unexpected as there are multiple WRPs discharging to the San Gabriel River; these treated effluent discharges comprise roughly 80% of flow during the dry season, contributing as much as 99% of the total ammonia input (Ackerman et al., 2005). In 1992/93, ammonia levels averaged over 10 mg/L. In 2003, however, the WRPs fully implemented nitrification and denitrification treatment (NDN) processes, which subsequently reduced discharged ammonia levels more than 80% to an average of less than 2 mg/L (Figure 5). Thus, a reduction in toxicity for reaches in the San Gabriel River watershed dominated by WRP effluents can be easily explained.

The lack of toxicity observed in the current study is consistent with other toxicity data collected in recent years. In 2005, a probability-based watershed survey was conducted in the entire San Gabriel River watershed, and 7% of the stream-miles were considered toxic to *Ceriodaphnia* (Stein and Bernstein, in prep). Even this toxicity, however, was eliminated after a TIE and subsequent follow-up investigations helped identify and eliminate the illicit discharge responsible (T. Fleming, personal communication).

A second example of reduced toxicity in recent years was observed in routine toxicity monitoring required in the vicinity of the WRPs as a part of their National Pollutant Discharge Elimination System (NPDES) permit requirements (Appendix B). Between June 2003 and June 2006, only 14% of the 269 total samples from 14 different sites exhibited toxicity (i.e., greater than 25% response relative to controls). For this period, toxicity was largely constrained to Coyote Creek (6% of total number of samples) and the uppermost portions of San Jose Creek (6% of total number of samples). Coyote Creek is the same tributary in which the current study found intermittent toxicity. The uppermost section of San Jose Creek was not monitored during the current study.

In contrast to the main stem of the San Gabriel River where significant resources have been expended to reduce pollutant inputs and minimize toxicity, much less effort has been spent on identifying and remediating sources of toxic pollutants in the Coyote Creek subwatershed. As a result, the toxicity in Coyote Creek has remained. The frequency of toxicity in Coyote Creek has remained similar between 1992/93 and 2004/05; roughly 12% to 22% of the samples were considered toxic. Pesticides available for application by homeowners continue to be one toxicant of concern. Diazinon was identified in 2004/05 (this study), as well as in the 1992/93 study (UC Davis 1995). The toxicity observed in urban runoff-dominated reaches during this study was intermittent, which is consistent with contributions by homeowner pesticide use (Schiff and Tiefenthaler 2003), illegal/illicit discharges, and observations in other dry weather runoff toxicity studies (Greenstein et al. 2004).

ACKNOWLEDGEMENTS

Toxicity testing was conducted by the Los Angeles County Sanitation Districts (Jay P. Bottomley and the staff of the San Jose Creek Water Quality Laboratory) and Nautilus Environmental, Inc. (Chris Stransky, Howard Bailey and staff). Wet weather sampling was conducted by Mactec Engineering, Inc. (Jay Shrake and staff). Dry weather sampling was conducted by the Los Angeles County Sanitation Districts (Misty Rogalski and Jeaneal Davis) and the Southern California Coastal Water Research Project (Dario Diehl, David Tsukada, Dawn Petschauer and Diana Young). Information management was provided by Southern California Coastal Water Research Project (Jeff Brown and Larry Cooper). This project was partially supported by the Los Angeles Regional Water Quality Control Board.

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Figure 1. Map of the lower San Gabriel River Watershed including dry and wet weather sampling locations.

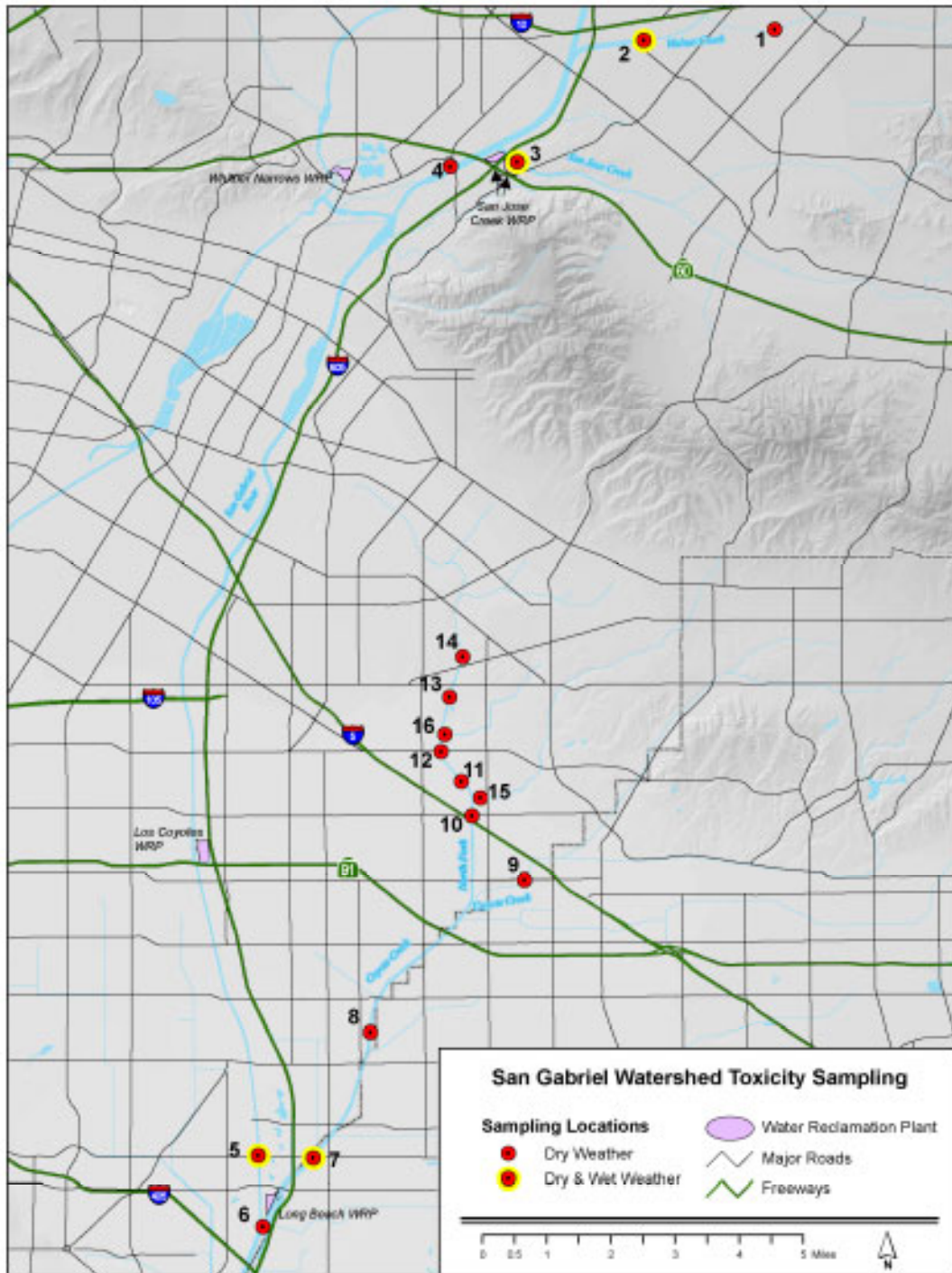


Figure 2. Survival in Coyote Creek on (A) April 2005 and (B) August 2005.

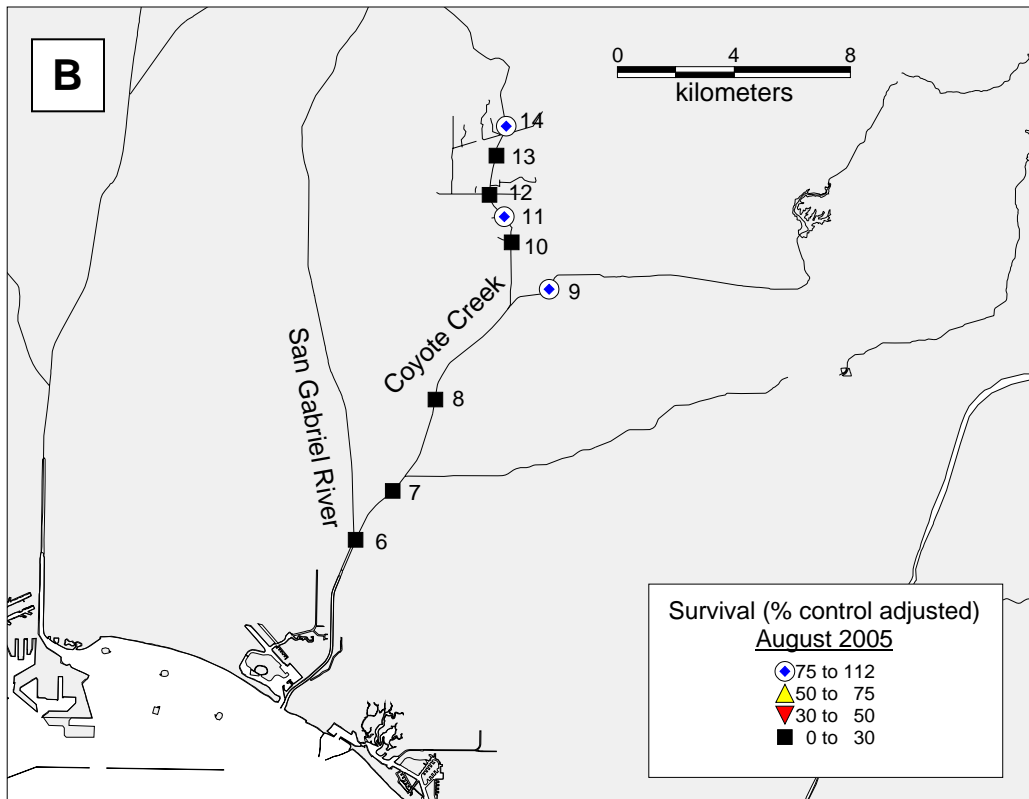
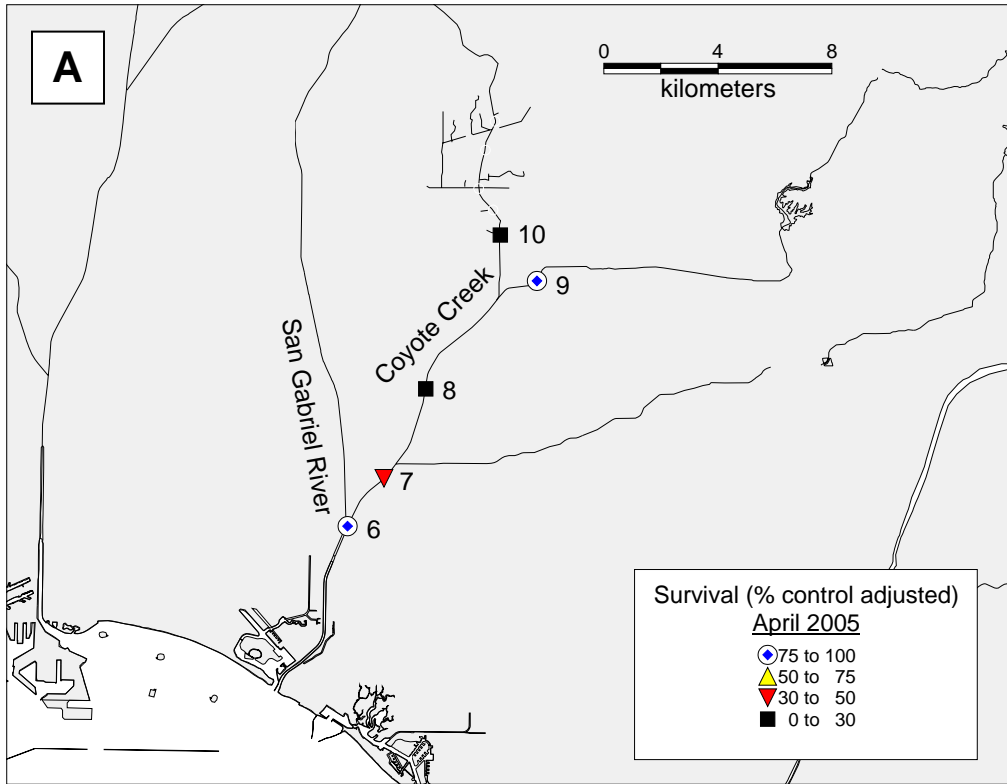


Figure 3. Acute Phase I TIE - site 9 sample collected on April 21, 2005.

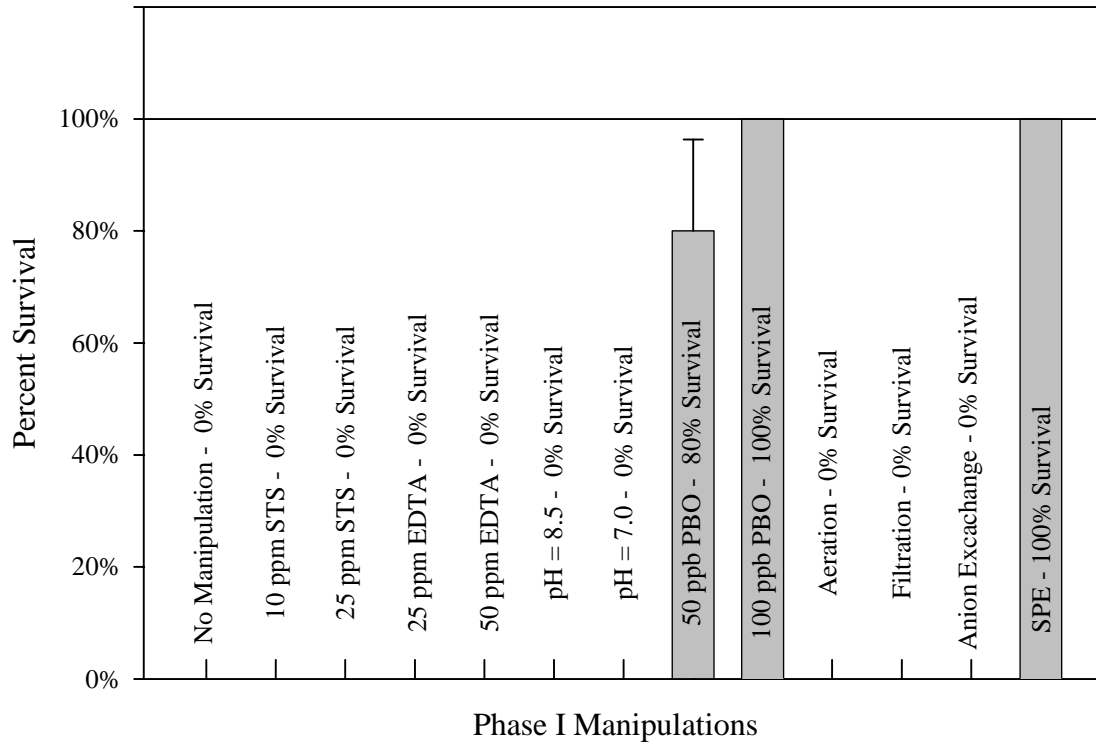


Figure 4. Acute Phase I TIE Solid Phase Extraction Elution Testing - Site 9 sample collected on April 21, 2005.

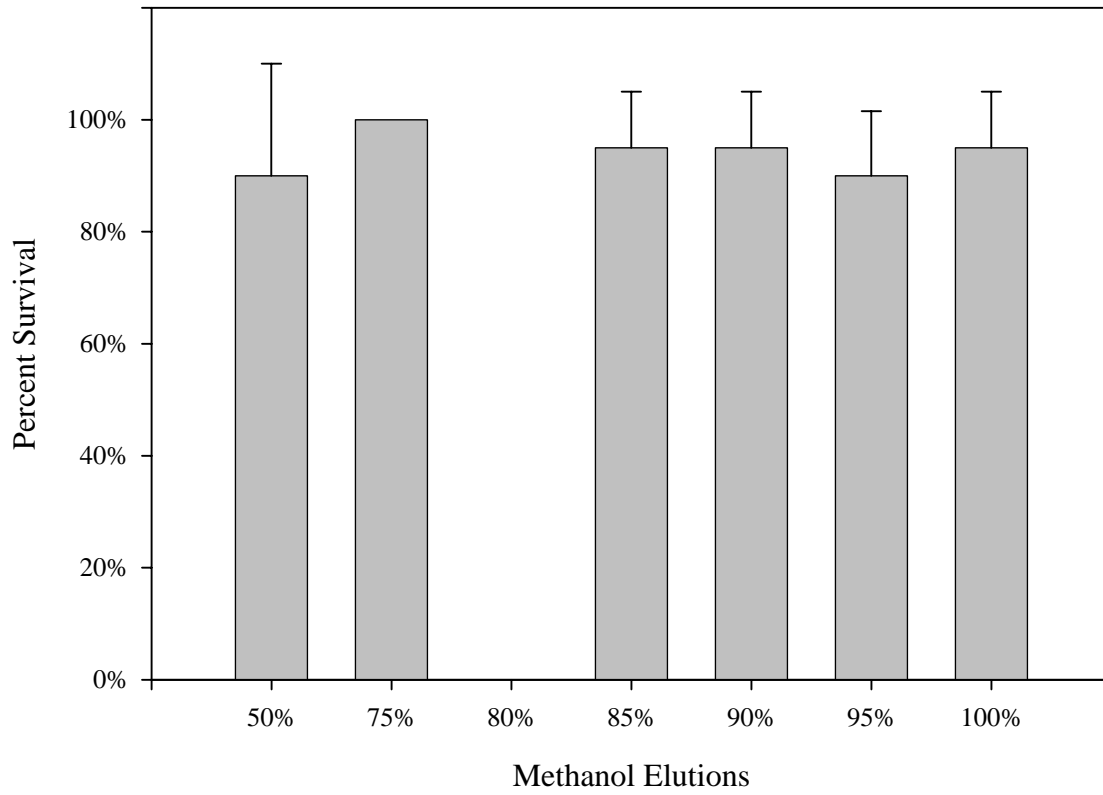


Figure 5. Time series plot of ammonia concentrations in final effluent and receiving water immediately downstream of the Los Coyotes WRP in the lower San Gabriel River Watershed. NDN plant upgrades were completed in June 2003.

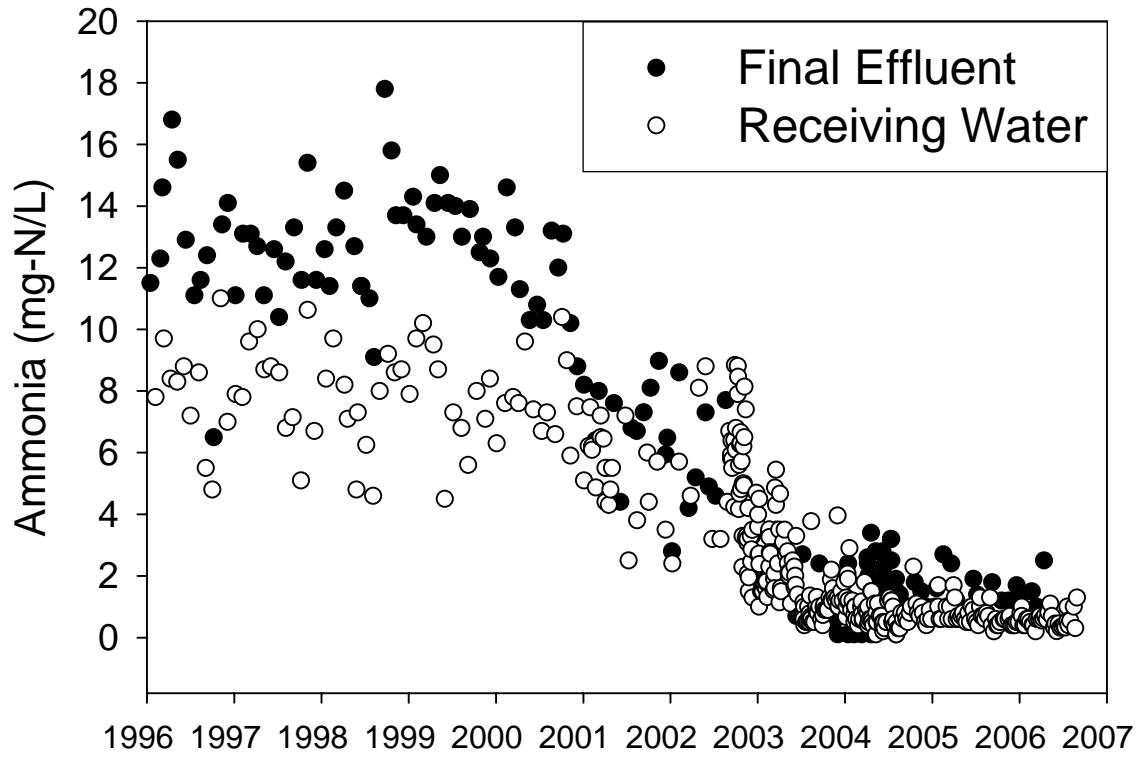


Table 1. Test conditions and requirements.

Test Organism:	<i>Ceriodaphnia dubia</i>
Organism Source:	In-house Cultures
Organism Age at Initiation:	<24 hours old and released within an eight hour period
Test Duration:	Until 60% or ore of the surviving females have three broods
Concentrations Tested:	0% and 100%
Solution Renewal:	Daily
Feeding:	0.1 ml YCT and 0.1 <i>Selenastrum</i> algal suspension daily
Test Chamber:	50 ml Disposable
Solution Volume:	15 ml
Control Water:	Either diluted mineral water (8 parts deionized water: 2 parts Perrier® water) or Reconstituted deionized water (hard)
Number of Replicates:	10
Organisms per Replicate:	1 assigned by blocking by known parentage
Photoperiod:	16 hours light (50-100 ft-c), 8 hours dark
Test Temperature:	25 ± 1° C.
Endpoints Measured:	Survival and Reproduction
Test Acceptability Criteria:	80% or greater survival with an average of 15 or more young per surviving female in the control organisms. 60% of surviving females in the controls must produce three broods within 8 days.

Table 2. Toxicity Identification Evaluation sample manipulations and their respective interpretations.

TIE Sample Manipulation	Expected response
pH Adjustment (pH 7 and 8.5)	Alters toxicity in pH sensitive compounds (i.e., ammonia and some trace metals)
Aeration	Reduces toxicity attributable to volatile, sublutable, and/or easily oxidizable compounds
Ethylenedinitrilo-Tetraacetic Acid (EDTA) Addition	Chelates trace metals, particularly divalent, cationic metals
Sodium thiosulfate (STS) Addition	Reduces toxicants attributable to oxidants (i.e., chlorine) and some trace metals
Filtration	Removes toxicity related to and/or associated with particulates
Solid Phase Extraction (SPE) with C ₁₈	Removes toxicity associated with non-polar organics (i.e., pesticides, surfactants)
Sequential Solvent Extraction of with C ₁₈ Column	SPE extraction can be used to confirm toxicity due to nonpolar organic compounds. Sequential extraction using solvents of gradually decreasing polarity can separate these compounds into fractions providing further toxicant resolution and isolation for chemical analysis
Piperonyl Butoxide (PBO)	Removes toxicity caused by metabolically activated pesticides (i.e., organophosphorous pesticides). Increases toxicity attributable to pyrethroid pesticides
Anion Exchange	Removes toxicity associated with anionic compounds, including some trace metals and surfactants
No Manipulation	For comparing the relative effectiveness of other manipulations and quantifies the persistence of toxicity in the stored sample

Table 3. Summary of dry weather *Ceriodaphnia dubia* toxicity from San Gabriel River from March 2005 through August 2006.

Location	Month of Sample Collection																	
	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
	2005	2005	2005	2005	2005	2005	2005	2005	2005	2005	2006	2006	2006	2006	2006	2006	2006	2006
Walnut Creek																		
1	-	-	-	-	-	-	-	-	-	-	-	-						
2	-	-	-	-	-	-	-	-	-	-	-	-						
San Jose Creek Reach 1																		
3	-	-	-	-	-	-	-	-	-	-	-	-						
San Gabriel River Reach 3																		
4	-	-	-	-	-	-	-	-	-	-	-	-						
San Gabriel River Reach 1																		
5	-	-	-	-	-	-	-	-	-	-	-	-						
Coyote Creek																		
6	-	S ¹	-	-	-	L ¹ S ¹	-	-	-	-	-	-						
7	-	L ¹ S ¹	-	-	-	L ¹ S ¹	-	-	-	-	-	-						
8	-	L ¹ S ¹	-	S ¹	-	L ¹ S ¹	-	-	-	-	-	-						
9	S ¹	-	-	-	-	-	L ¹ S ¹	-	-	-	-	-	-	-	-	-	-	-
10	S ¹	L ¹ S ¹	-	L ¹ S ¹	-	L ¹ S ¹	-	-	-	-	-	-	-	-	-	-	-	-
11					-	S ¹	-	-	-	-	-	-	-	-	-	-	-	-
12					-	L ¹ S ¹	-	-	-	-	-	-	-	-	-	-	-	-
13					-	L ¹ S ¹	-	-	-	-	-	-	-	-	-	-	-	-
14					-	L ¹ S ¹	-	-	-	-	-	-	-	-	-	-	-	-
15								-	-	-	- ¹	-	S	-	-	-	-	-
16								-	-	-	-	-	-	-	-	-	-	-

Shaded = samples not collected

- Not Toxic – effects less than 25% relative to control.

L = Lethal effect; toxicity less than 75% relative to control

S = Sub-lethal effect; reproduction less than 75% relative to control

¹ – Statistically significant from control

Table 4. Summary of dry weather TIE results.

Site	Sample Date	TIE RESULTS (Survival in 100%)										
		No Manipulation	STS ^a	EDTA ^b	pH 7.0	pH 8.5	PBO ^c	Aeration	Filtration	Centrifuge	SPE	Anion
#9	Mar 2005	Sample No longer Toxic										
#10	Mar 2005	Sample No longer Toxic										
#10	Apr 2005	0%	0%	0%	0%	0%	0% ^d	35%	0%	NT	87.5% ^e	NT
#10	Jun 2005	0%	0%	0%	0%	0%	0%	10%	10%	30%	100% ^e	100%
#10	Aug 2005	0%	0%	0%	0%	0%	0%	0%	0%	NT	100% ^e	0% ^f
#9	Sep 2005	0%	0%	0%	0%	0%	100% ^g	0%	0%	NT	100% ^e	0%
#15	Mar 2006	Sample No longer Toxic										

NT = Not tested

a – Sodium thiosulfate addition, two treatments of 10 and 25 ppm

b – Ethylenedinitrilo-tetraacetic acid addition, two treatments of 25 and 50 ppm

c – Piperonyl butoxide addition, two treatments of 50 and 100 ppb

d – 5% survival observed in the 50 ppb treatment with 0% survival in the 100 ppb treatment

e – Toxicity recovered in only the 75% methanol elution

f – Survival observed in lower concentrations of the sample indicating partial toxicity removal

g – 80% survival observed in 50 ppb treatment and 100% survival in 100 ppb treatment

Appendix A
Study Monitoring Results

Table A 1. Dry weather baseline chronic toxicity testing results for Sites #3 through #10 using grab samples collected on March 31, 2005.

Sample	Mean Survival (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)	Mean Reproduction (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)
Control	100% (0)	100% (0)	30.5 (3.2)	100% (10.4)
Site #3	100% (0)	100% (0)	29.2 (2.0)	95.7% (6.5)
Site #4	100% (0)	100% (0)	34.4 (6.1)	113% (20.0)
Control	100% (0)	100% (0)	31.9 (2.9)	100% (9.1)
Site #5	100% (0)	100% (0)	31.0 (2.4)	97.2% (7.5)
Site #6	100% (0)	100% (0)	30.2 (5.9)	94.7% (18.4)
Control	100% (0)	100% (0)	34.5 (1.9)	100% (5.5)
Site #7	100% (0)	100% (0)	34.7 (2.5)	101% (7.4)
Site #8	100% (0)	100% (0)	32.8 (8.1)	95.1% (23.4)
Control	100% (0)	100% (0)	33.6 (4.1)	100% (12.3)
Site #9	100% (0)	100% (0)	24.6 ^a (3.6)	73.2% ^a (10.6)
Site #10	100% (0)	100% (0)	20.8 ^a (3.5)	61.9% ^a (10.5)

a: response statistically significant from control.

Table A 2. Dry weather baseline chronic toxicity testing results for Sites #3 through #10 using grab samples collected on April 21, 2005.

Sample	Mean Survival (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)	Mean Reproduction (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)
Control	90% (30.8)	100% (34.2)	31.6 (10.1)	100% (32.0)
Site #3	80% (42.2)	88.9% (46.8)	26.2 (14.5)	82.9% (46.0)
Site #4	90% (31.6)	100% (35.1)	29.4 (11.1)	93.0% (35.0)
Site #5	90% (31.6)	100% (35.1)	26.2 (10.4)	82.9% (32.9)
Site #6	90% (31.6)	100% (35.1)	1.1 ^a (3.5)	3.5% ^a (11.0)
Site #7	40% ^a (51.6)	44.4% ^a (57.4)	0 ^a (0)	0% ^a (0)
Site #8	0% ^a (0)	0% ^a (0)	0 ^a (0)	0% ^a (0)
Site #9	90% (31.6)	100% (35.1)	28.7 (12.7)	90.8% (40.3)
Site #10	0% ^a (0)	0% ^a (0)	0 ^a (0)	0% ^a (0)

a: response statistically significant from control.

Table A 3. Dry weather baseline chronic toxicity testing results for Sites #3 through #10 using grab samples collected on May 20, 2005.

Sample	Mean Survival (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)	Mean Reproduction (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)
Control	95% (22.9)	100% (24.1)	34.35 (5.8)	100% (17.0)
Site #3	100% (0)	105% (0)	38.3 (2.5)	112% (7.3)
Site #4	100% (0)	105% (0)	39.4 (2.2)	115% (7.0)
Site #5	100% (0)	105% (0)	39.3 (2.9)	114% (8.6)
Site #6	100% (0)	105% (0)	38 (2.5)	111% (7.4)
Site #7	100% (0)	105% (0)	37.4 (3.0)	109% (8.6)
Site #8	100% (0)	105% (0)	36.9 (3.6)	107% (10.6)
Site #9	100% (0)	105% (0)	36.6 (2.9)	107% (8.5)
Site #10	100% (0)	105% (0)	34.2 (2.4)	99.6% (7.1)

Table A 4. Dry weather baseline chronic toxicity testing results for Sites #3 through #10 using grab samples collected on June 23, 2005.

Sample	Mean Survival (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)	Mean Reproduction (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)
Control	100% (0)	100% (0)	20.6 (6.6)	100% (32.2)
Site #3	100% (0)	100% (0)	20.4 (7.3)	99.3% (35.4)
Site #4	100% (0)	100% (0)	23.7 (5.7)	115% (27.8)
Site #5	100% (0)	100% (0)	21.2 (5.8)	103% (28.1)
Site #6	100% (0)	100% (0)	19 (7.3)	92.5% (35.4)
Site #7	100% (0)	100% (0)	16.8 (4.2)	81.8% (20.6)
Site #8	100% (0)	100% (0)	0.7 ^a (1.1)	3.4% ^a (5.2)
Site #9	100% (0)	100% (0)	27.5 (6.5)	134% (31.8)
Site #10	0% ^a (0)	0% ^a (0)	0.5 ^a (1.6)	2.4% ^a (7.7)

a: response statistically significant from control..

Table A 5. Dry weather baseline chronic toxicity testing results for Sites #3 through #14 using grab samples collected on July 28, 2005.

Sample	Mean Survival (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)	Mean Reproduction (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)
Control	100% (0)	100% (0)	25.4 (6.1)	100% (24.2%)
Site #3	100% (0)	100% (0)	33.5 (1.6)	132% (6.5%)
Site #4	90% (31.6%)	90% (31.6%)	26.2 (10.3)	103% (40.5%)
Site #5	80% (42.2%)	80% (42.2%)	21.2 (12.1)	83.5% (47.7%)
Site #6	100% (0)	100% (0)	31.2 (4.1)	123% (16.2%)
Site #7	100% (0)	100% (0)	35.4 (3.4)	139% (13.4%)
Site #8	90% (31.6%)	90% (31.6%)	35.5 (7.2)	140% (28.3%)
Site #9	100% (0)	100% (0)	34.3 (2.8)	135% (10.8%)
Site #10	100% (0)	100% (0)	35.6 (5.6)	140% (22.2%)
Site #11	90% (31.6%)	90% (31.6%)	29.5 (13.6)	116% (53.6%)
Site #12	100% (0)	100% (0)	32.2 (2.4)	127% (9.4%)
Site #13	100% (0)	100% (0)	32.6 (5.6)	128% (22.1%)
Site #14	100% (0)	100% (0)	31.9 (11.6)	126% (45.9%)

Table A 6. Dry weather baseline chronic toxicity testing results for Sites #3 through #14 using grab samples collected on August 18, 2005.

Sample	Mean Survival (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)	Mean Reproduction (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)
Control	90% (31.5)	100% (35.0)	18.2 (10.3)	100% (56.6)
Site #3	100% (0)	111.1% (0)	20.7 (7.5)	114% (41.0)
Site #4	90% (31.6)	100% (35.1)	22.6 (11.6)	124% (64.0)
Site #5	80% (42.2)	88.9% (46.8)	17.1 (9.6)	93.6% (52.7)
Site #6	0% ^a (0)	0% ^a (0)	0 ^a (0)	0% ^a (0)
Site #7	0% ^a (0)	0% ^a (0)	0 ^a (0)	0% ^a (0)
Site #8	0% ^a (0)	0% ^a (0)	0 ^a (0)	0% ^a (0)
Site #9	100% (0)	111.1% (0)	24.1 (6.0)	132% (32.8)
Site #10	0% ^a (0)	0% ^a (0)	0 ^a (0)	0% ^a (0)
Site #11	90% (31.6)	100% (35.1)	0.9 ^a (1.7)	5.0% ^a (9.1)
Site #12	0% ^a (0)	0% ^a (0)	0 ^a (0)	0% ^a (0)
Site #13	0% ^a (0)	0% ^a (0)	0 ^a (0)	0% ^a (0)
Site #14	70% ^a (48.3)	77.8% ^a (53.7)	1.1 ^a (2.6)	6.0% ^a (14.3)

a: response statistically significant from control.

Table A 7. Dry weather baseline chronic toxicity testing results for Sites #3 through #16 using grab samples collected on September 29, 2005. Sites #3 through #9 were initiated with the Control #1 set of neonates, and sites #10 through #16 were initiated with the Control #2 set of neonates, and statistics for each site were run relative to the control set.

Sample	Mean Survival (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)	Mean Reproduction (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)
Control	100% (0)	100% (0)	29.8 (2.7)	100% (8.9)
Site #3	100% (0)	100% (0)	38.4 (6.0)	129% (20.2)
Site #4	100% (0)	100% (0)	34.4 (3.9)	115% (13.2)
Site #5	100% (0)	100% (0)	31.0 (4.5)	104% (15.3)
Site #6	100% (0)	100% (0)	30.0 (4.4)	101% (14.8)
Site #7	90% (31.6)	90% (31.6)	33.0 (5.7)	111% (19.2)
Site #8	100% (0)	100% (0)	32.5 (7.7)	109% (26.0)
Site #9	0% ^a (0)	0% ^a (0)	0 ^a (0)	0% ^a (0)
Control	90% (31.6)	100% (35.1)	22.3 (11.3)	100% (50.8)
Site #10	90% (31.6)	100% (35.1)	25.4 (13.5)	114% (60.7)
Site #11	100% (0)	111% (0)	31.7 (5.2)	142% (23.4)
Site #12	100% (0)	111% (0)	23.7 (7.3)	106% (32.8)
Site #13	100% (0)	111% (0)	22.2 (7.0)	99.6% (31.3)
Site #14	90% (31.6)	100% (35.1)	24.3 (9.1)	109% (41.0)
Site #15	90% (31.6)	100% (35.1)	26.8 (10.0)	120% (11.3)
Site #16	90% (31.6)	100% (35.1)	28.5 (11.3)	128% (50.8)

a: response statistically significant from control.

Table A 8. Dry weather baseline chronic toxicity testing results for Sites #3 through #16 (including the site designated “Site 11.5”) using grab samples collected on October 27, 2005.

Sample	Mean Survival (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)	Mean Reproduction (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)
Control	100% (0)	100% (0)	20.8 (9.0)	100% (43.4)
Site #3	100% (0)	100% (0)	34.3 (4.9)	165% (23.8)
Site #4	100% (0)	100% (0)	42.5 (3.7)	205% (17.9)
Site #5	90% (31.6)	90% (31.6)	34.1 (12.9)	164% (62.1)
Site #6	90% (31.6)	90% (31.6)	28.2 (5.6)	136% (26.8)
Site #7	100% (0)	100% (0)	36.6 (5.2)	176% (24.9)
Site #8	100% (0)	100% (0)	36.1 (5.3)	174% (25.3)
Site #9	100% (0)	100% (0)	31.8 (5.7)	153% (27.4)
Site #10	100% (0)	100% (0)	35.5 (2.5)	171% (12.1)
Site #11	100% (0)	100% (0)	32.4 (5.3)	156% (25.4)
Site #11.5	100% (0)	100% (0)	10.6 ^a (1.2)	51.1% ^a (5.7)
Site #12	100% (0)	100% (0)	34.0 (9.0)	164% (43.4)
Site #13	90% (31.6)	90% (31.6)	31.1 (11.4)	150% (54.9)
Site #14	100% (0)	100% (0)	35.6 (4.5)	172% (21.5)
Site #15	100% (0)	100% (0)	30.6 (4.7)	148% (22.5)
Site # 16	100% (0)	100% (0)	33.4 (5.6)	161% (26.8)

a: response statistically significant from control.

Table A 9. Dry weather baseline chronic toxicity testing results for Sites #3 through #16 using grab samples collected on November 15, 2005. Sites #3 through #9 were initiated with the Control #1 set of neonates, and sites #10 through #16 were initiated with the Control #2 set of neonates, and statistics for each site were run relative to the control set.

Sample	Mean Survival (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)	Mean Reproduction (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)
Control	100% (0)	100% (0)	32.5 (3.0)	100% (9.1)
Site #3	100% (0)	100% (0)	29.0 (5.6)	89.2% (17.2)
Site #4	100% (0)	100% (0)	37.4 (2.9)	115% (8.8)
Site #5	90% (31.6)	90% (31.6)	34.9 (5.9)	107% (18.2)
Site #6	90% (31.6)	90% (31.6)	26.4 (9.2)	81.2% (28.2)
Site #7	100% (0)	100% (0)	37.2 (4.0)	114% (12.5)
Site #8	90% (31.6)	90% (31.6)	31.5 (11.4)	97.0% (35.0)
Site #9	100% (0)	100% (0)	34.2 (5.9)	105% (18.1)
Control	100% (0)	100% (0)	27.5 (4.5)	100% (16.5)
Site #10	100% (0)	100% (0)	35.9 (6.6)	130% (24.0)
Site #11	100% (0)	100% (0)	39.5 (3.3)	144% (12.0)
Site #12	100% (0)	100% (0)	36.0 (4.2)	131% (15.3)
Site #13	100% (0)	100% (0)	34.8 (3.7)	126% (13.5)
Site #14	100% (0)	100% (0)	33.5 (4.7)	122% (17.1)
Site #15	100% (0)	100% (0)	32.2 (4.4)	117% (16.1)
Site #16	100% (0)	100% (0)	38.9 (2.9)	142% (10.5)

Table A 10. Dry weather baseline chronic toxicity testing results for Sites #3 through #16 using grab samples collected on December 8, 2005. Sites #3 through #9 were initiated with the Control #1 set of neonates, and sites #10 through #16 were initiated with the Control #2 set of neonates, and statistics for each site were run relative to the control set.

Sample	Mean Survival (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)	Mean Reproduction (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)
Control	100% (0)	100% (0)	32.4 (2.6)	100% (8.2)
Site #3	100% (0)	100% (0)	31.6 (4.0)	97.5% (12.3)
Site #4	100% (0)	100% (0)	30.1 (3.1)	92.9% (9.6)
Site #5	100% (0)	100% (0)	28.6 (2.8)	88.3% (8.6)
Site #6	100% (0)	100% (0)	29.9 (3.3)	92.3% (10.3)
Site #7	100% (0)	100% (0)	32.9 (2.9)	102% (9.0)
Site #8	100% (0)	100% (0)	32.0 (4.8)	98.8% (14.7)
Site #9	100% (0)	100% (0)	31.0 (3.6)	95.7% (11.1)
Control	100% (0)	100% (0)	32.1 (2.7)	100% (8.5)
Site #10	100% (0)	100% (0)	33.9 (3.3)	106% (10.3)
Site #11	90% (31.6)	90% (31.6)	31.2 (4.6)	87.5% (33.6)
Site #12	100% (0)	100% (0)	31.5 (2.4)	98.1% (7.4)
Site #13	100% (0)	100% (0)	27.6 (5.8)	85.8% (18.2)
Site #14	100% (0)	100% (0)	29.8 (6.6)	92.8% (20.5)
Site #15	90% (31.6)	90% (31.6)	26.2 (3.1)	81.7% (9.6)
Site #16	90% (31.6)	90% (31.6)	31.0 (3.9)	96.6% (12.3)

Table A 11. Dry weather baseline chronic toxicity testing results for Sites #3 through #16 using grab samples collected on January 20, 2006. Sites #3 through #9 were initiated with the Control #1 set of neonates, and sites #10 through #16 were initiated with the Control #2 set of neonates, and statistics for each site were run relative to the control set.

Sample	Mean Survival (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)	Mean Reproduction (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)
Control #1	100% (0)	100% (0)	33.7 (3.3)	100% (9.8)
Site #3	100% (0)	100% (0)	31.4 (5.9)	93.2% (17.4)
Site #4	100% (0)	100% (0)	34.4 (2.7)	102% (8.1)
Site #5	100% (0)	100% (0)	37.4 (2.7)	111% (7.9)
Site #6	90% (31.6)	90% (31.6)	30.4 (11.2)	90.2% (33.1)
Site #7	100% (0)	100% (0)	35.6 (3.5)	106% (10.4)
Site #8	100% (0)	100% (0)	35.4 (3.5)	105% (10.5)
Site #9	100% (0)	100% (0)	30.7 (5.1)	91.1% (15.0)
Control #2	100% (0)	100% (0)	33.5 (2.0)	100% (6.0)
Site #10	100% (0)	100% (0)	34.5 (3.2)	103% (9.5)
Site #11	100% (0)	100% (0)	30.3 (10.9)	90.4% (32.6)
Site #12	80% (42.2)	80% (42.2)	30.6 (12.1)	91.3% (36.2)
Site #13	100% (0)	100% (0)	30.2 (4.5)	90.1% (13.3)
Site #14	100% (0)	100% (0)	29.4 (4.1)	87.8% (12.4)
Site #15	100% (0)	100% (0)	26.5 ^a (2.0)	79.1% ^a (5.8)
Site #16	100% (0)	100% (0)	29.4 (3.6)	87.8% (10.8)

a: response statistically significant from control.

Table A 12. Dry weather baseline chronic toxicity testing results for Sites #3 through #16 using grab samples collected on February 23, 2006. Sites #3 through #9 were initiated with the Control #1 set of neonates, and sites #10 through #16 were initiated with the Control #2 set of neonates, and statistics for each site were run relative to the control set.

Sample	Mean Survival (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)	Mean Reproduction (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)
Control #1	90% (31.6)	100% (35.1)	23.8 (10.4)	100% (43.9)
Site #3	100% (0)	111.1% (0)	31.4 (3.2)	132% (13.6)
Site #4	100% (0)	111.1% (0)	35.7 (3.1)	150% (12.8)
Site #5	90% (31.6)	100% (35.1)	30.6 (11.4)	129% (47.7)
Site #6	70% (48.3)	77.8% (31.6)	23.6 (16.4)	99.2% (68.8)
Site #7	100% (0)	111.1% (0)	28.5 (5.5)	120% (23.0)
Site #8	90% (31.6)	100% (35.1)	25.6 (11.1)	108% (46.7)
Site #9	100% (0)	111.1% (0)	30.1 (4.8)	126% (20.3)
Control #2	100% (0)	100% (0)	26.2 (6.4)	100% (24.4)
Site #10	90% (31.6)	90% (31.6)	31.0 (12.6)	118% (48.0)
Site #11	90% (31.6)	90% (31.6)	24.9 (11.3)	95.0% (43.1)
Site #12	90% (31.6)	90% (31.6)	27.6 (11.3)	105% (43.1)
Site #13	100% (0)	100% (0)	30.6 (7.8)	117% (29.8)
Site #14	100% (0)	100% (0)	29.6 (7.4)	113% (28.3)
Site #15	90% (31.6)	90% (31.6)	28.8 (11.9)	110% (45.6)
Site #16	80% (42.2)	80% (42.2)	26.3 (14.3)	100% (54.5)

Table A 13. Dry weather baseline chronic toxicity testing results for Sites #9 through #16 using grab samples collected on March 24, 2006.

Sample	Mean Survival (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)	Mean Reproduction (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)
Control	100% (0)	100% (0)	24.9 (8.0)	100% (43.9)
Site #9	100% (0)	100% (0)	20.4 (9.9)	81.9% (39.7)
Site #10	100% (0)	100% (0)	24.7 (13.8)	99.2% (55.3)
Site #11	100% (0)	100% (0)	21.6 (15.9)	86.7% (63.8)
Site #12	100% (0)	100% (0)	26.0 (12.1)	104% (48.5)
Site #13	100% (0)	100% (0)	23.5 (10.9)	95.1% (44.1)
Site #14	100% (0)	100% (0)	22.7 (14.1)	91.2% (56.6)
Site #15	100% (0)	100% (0)	17.2 (15.5)	72.3% (65.1)
Site #16	90% (31.6)	90% (31.6)	24.6 (12.0)	98.8% (48.3)

Table A 14. Dry weather baseline chronic toxicity testing results for Sites #9 through #16 using grab samples collected on April 27, 2006.

Sample	Mean Survival (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)	Mean Reproduction (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)
Control	100% (0)	100% (0)	28.4 (2.5)	100% (8.9)
Site #9	100% (0)	100% (0)	30.2 (6.1)	106% (21.6)
Site #10	100% (0)	100% (0)	36.4 (4.1)	128% (14.5)
Site #11	100% (0)	100% (0)	38.8 (4.5)	137% (15.9)
Site #12	90% (31.6)	90% (31.6)	38.4 (3.7)	135% (13.1)
Site #13	100% (0)	100% (0)	36.1 (8.5)	127% (29.9)
Site #14	100% (0)	100% (0)	30.2 (8.4)	106% (29.4)
Site #15	100% (0)	100% (0)	34.0 (4.1)	120% (14.6)
Site #16	100% (0)	100% (0)	30.7 (2.9)	108% (10.2)

Table A 15. Dry weather baseline chronic toxicity testing results for Sites #9 through #16 using grab samples collected on May 19, 2006.

Sample	Mean Survival (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)	Mean Reproduction (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)
Control	100% (0)	100% (0)	28.4 (4.4)	100% (15.6)
Site #9	90% (31.6)	90% (31.6)	23.6 (8.2)	83.1% (29.0)
Site #10	100% (0)	100% (0)	28.8 (5.4)	101% (19.0)
Site #11	100% (0)	100% (0)	29.2 (5.3)	103% (18.5)
Site #12	100% (0)	100% (0)	29.2 (5.3)	103% (18.5)
Site #13	100% (0)	100% (0)	34.2 (3.3)	120% (11.7)
Site #14	100% (0)	100% (0)	33.8 (3.2)	119% (11.1)
Site #15	100% (0)	100% (0)	34.1 (2.8)	120% (9.7)
Site #16	100% (0)	100% (0)	32.0 (3.9)	113% (13.7)

Table A 16. Dry weather baseline chronic toxicity testing results for Sites #9 through #16 using grab samples collected on June 20, 2006.

Sample	Mean Survival (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)	Mean Reproduction (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)
Control	90% (31.6)	100% (31.6)	27.5 (8.3)	100% (30.3)
Site #9	100% (0)	111% (0)	25.6 (3.9)	93.1% (14.3)
Site #10	100% (0)	111% (0)	31.8 (6.4)	116% (23.3)
Site #11	100% (0)	111% (0)	31.5 (10.6)	114% (38.7)
Site #12	100% (0)	111% (0)	34.7 (2.9)	126% (10.6)
Site #13	100% (0)	111% (0)	31.9 (4.6)	116% (16.7)
Site #14	100% (0)	111% (0)	36.2 (3.6)	132% (13.3)
Site #15	100% (0)	111% (0)	35.2 (3.6)	128% (13.0)
Site #16	90% (31.6)	100% (31.6)	347 (3.3)	126% (12.1)

Table A 17. Dry weather baseline chronic toxicity testing results for Sites #9 through #16 using grab samples collected on July 27, 2006.

Sample	Mean Survival (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)	Mean Reproduction (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)
Control	100% (0)	100% (0)	27.2 (4.3)	100% (15.8)
Site #9	90% (31.6)	90% (31.6)	33.7 (2.3)	124% (8.3)
Site #10	100% (0)	100% (0)	33.9 (3.3)	125% (12.3)
Site #11	100% (0)	100% (0)	36.1 (3.0)	133% (10.9)
Site #12	90% (31.6)	90% (31.6)	29.4 (10.2)	108% (37.5)
Site #13	100% (0)	100% (0)	31.7 (4.8)	117% (17.8)
Site #14	100% (0)	100% (0)	32.5 (4.1)	119% (15.2)
Site #15	100% (0)	100% (0)	32.4 (3.9)	119% (14.4)
Site #16	100% (0)	100% (0)	29.6 (7.7)	108% (28.4)

Table A 18. Dry weather baseline chronic toxicity testing results for Sites #9 through #16 using grab samples collected on August , 2006.

Sample	Mean Survival (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)	Mean Reproduction (Std. Dev.)	Survival Response Relative to Control (Std. Dev.)
Control				
Site #9				
Site #10				
Site #11				
Site #12				
Site #13				
Site #14				
Site #15				
Site #16				

Appendix B
NPDES Monitoring Results

Appendix A: NPDES Chronic Toxicity Monitoring Conducted from June 2003 through June 2006 in the San Gabriel River Watershed

INTRODUCTION

The Sanitation Districts of Los Angeles County own and operate five Water Reclamation Plants (WRPs) that discharge in the San Gabriel River Watershed. Each WRP operates under an individual NPDES permit. These permits require toxicity tests be conducted at a number of receiving water stations within the watershed at defined frequencies. Results of recent chronic toxicity monitoring conducted as part of the NPDES monitoring program are presented in this appendix.

Toxicity results for receiving waters before June 2003 are not presented; the WRPs in the watershed added nitrification and denitrification (NDN) to their respective facilities in the first half of 2003. Before NDN, the effluent from the plants often had ammonia concentrations above 10 mg/L. After the addition of NDN, the ammonia effluent concentration from each plant averages less than 2 mg/L. Since ammonia concentrations at pre-NDN levels can cause toxicity and that potential source of toxicity has been greatly lessened (ammonia concentrations of 2 mg/L or less are not expected to cause chronic toxicity), only results from after the initiation of NDN is presented herein. In that way, it is known the ammonia contribution to toxicity has been minimized and other potential causes of toxicity can be investigated.

MATERIALS AND METHODS

Chronic toxicity test results are presented for four reaches within the San Gabriel River Watershed. Three different species were used at various times for the chronic toxicity tests during the June 2003 to June 2006 testing period. The test conditions and requirements followed for all these tests are contained in Table B.1, B.2, and B.3.

The locations of receiving water stations in the San Gabriel River Watershed are shown on Figure A.1. The tests conducted as part of NPDES chronic toxicity monitoring are listed in Table B.4. This data set consisted of 269 chronic toxicity tests using receiving water samples collected from San Gabriel River Reach 3 (27 tests), San Jose Creek Reach 1 (55 tests), San Gabriel River Reach 1 (121 tests), and Coyote Creek (66 tests).

All receiving water was monitored in dry weather conditions with no samples collected within 48 hours of any significant rain event. Testing was conducted by a California Department of Health Services-certified laboratory using USEPA Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving waters to Freshwater Organisms, 3rd or 4th Edition. Concurrent positive control reference toxicant testing meeting all required test acceptability criteria were conducted with each test. Acute (lethality) and chronic (reproduction or growth) endpoints were evaluated in the *Ceriodaphnia dubia* and *Pimephales promelas* tests. The green algae test (*Pseudokirchneriella subcapitata*) only consisted of a single chronic (cell density) endpoint.

One to three receiving water grab samples of 4 to 12 liters were collected depending on the species being tested. For the green algae test, a single grab consisting of 4 liters were collected and used for the entire test. For *Ceriodaphnia dubia* and *Pimephales promelas* testing, a minimum of three grab samples (with volumes ranging from 4 to 12 liters) were collected and used during the seven-day test. In all instances, each sample was first used within 36 hours of collection and used for subsequent renewals for no longer than 72 hours after collection. The number of receiving water locations and minimum frequency of testing was specified in the NPDES permits and ranged from monthly to quarterly depending on the permit.

The NPDES permits define chronic toxicity as a TUc (toxicity unit) of >1.0 with the TUc calculated as 100/NOEC (the no observable effect concentration (or NOEC) is the highest concentration not

statistically significant from the control). NPDES permits require the initiation of weekly accelerated testing for six weeks if the monthly median exceeds 1.0 TUC at the location immediately downstream from a WRP discharge. If two of six weekly accelerated tests exceed 1.0 TUC, the appropriate plant-specific Toxicity Reduction Evaluation workplan is initiated which most often includes the initiation of phase I toxicity identification evaluation (TIE) testing. This protocol was followed in all cases when the monthly median TUC exceeded 1.0.

To be consistent with other results included in this study, an effect of greater than 25% in 100% receiving water was identified as “toxicity”. However, statistically significant differences using EPA protocol outlined hypothesis testing methods are also noted.

Table B.1. Test conditions and requirements followed for all *Ceriodaphnia dubia* tests.

Test Organism:	<i>Ceriodaphnia dubia</i>
Organism Source:	In-house Cultures
Organism Age at Initiation:	<24 hours old and released within an eight hour period
Test Duration:	Until 60% or ore of the surviving females have three broods
Concentrations Tested:	At least 0% and 100%, in some instances intermediate concentrations were also tested
Solution Renewal:	Daily
Feeding:	0.1 ml YCT and 0.1 <i>Selenastrum</i> algal suspension daily
Test Chamber:	50 ml Disposable
Solution Volume:	15 ml
Control Water:	Reconstituted deionized water (hard or moderately hard)
Number of Replicates:	10
Organisms per Replicate:	1 assigned by blocking by known parentage
Photoperiod:	16 hours light (50-100 ft-c), 8 hours dark
Test Temperature:	25 ± 1° C.
Endpoints Measured:	Survival and Reproduction
Reference Toxicant Testing	Concurrent reference toxicant test conducted meeting all test acceptability requirements
Test Acceptability Criteria:	80% or greater survival with an average of 15 or more young per surviving female in the control organisms. 60% of surviving females in the controls must produce three broods within 8 days.

Table B.2. Test conditions and requirements followed for all *Pimephales promelas* tests.

Test Organism:	<i>Pimephales promelas</i>
Organism Source:	Commercial supplier
Organism Age at Initiation:	<24 hours old
Test Duration:	Seven days
Concentrations Tested:	At least 0% and 100%, in some instances intermediate concentrations were also tested
Solution Renewal:	Daily
Feeding:	1500 newly hatched <i>artemia</i> per beaker, twice daily
Test Chamber:	400 ml glass beakers
Solution Volume:	250 ml
Control Water:	Reconstituted deionized water (hard and moderately hard)
Number of Replicates:	4
Organisms per Replicate:	10
Photoperiod:	16 hours light (50-100 ft-c), 8 hours dark
Test Temperature:	25 ± 1° C.
Endpoints Measured:	Survival and Growth (biomass)
Reference Toxicant Testing	Concurrent reference toxicant test conducted meeting all test acceptability requirements
Test Acceptability Criteria:	80% or greater survival with an average of 0.25 mg/surviving larvae in the control organisms.

Table B.3. Test conditions and requirements followed for all *Pseudokirchneriella subcapitata* tests.

Test Organism:	<i>Pseudokirchneriella subcapitata</i>
Organism Source:	In-house Cultures and commercial suppliers
Organism Age at Initiation:	4 to 7 day-old cultures
Test Duration:	96 hours
Concentrations Tested:	At least 0% and 100%, in some instances intermediate concentrations were also tested
Solution Renewal:	None
Feeding:	None
Test Chamber:	50 ml Disposable
Solution Volume:	15 ml
Control Water:	Algal stock medium with EDTA prepared using deionized water
Number of Replicates:	4
Organisms per Replicate:	10,000 cells per ml
Photoperiod:	Continuous (360 - 440-c)
Test Temperature:	25 ± 1° C.
Endpoints Measured:	Growth (chlorophyll fluorescence)
Reference Toxicant Testing	Concurrent reference toxicant test conducted meeting all test acceptability requirements
Test Acceptability Criteria:	Mean cell density of 1×10^6 cells per ml in the controls and control CV equal to or less than 20%

Table B.4. NPDES receiving water chronic toxicity testing from June 2003 through June 2006

REACH	SITE	VALID NPDES CHRONIC TESTS CONDUCTED			
		<i>Pimephales promelas</i>	<i>Ceriodaphnia dubia</i>	<i>Pseudokirchneriella subcapitata</i>	TOTAL
San Gabriel River Reach 3	R11	12	3	1	16
	WN-RA	11	0	0	11
San Jose Creek Reach 1	POM-RA	1	16	0	17
	POM-RC	1	7	0	8
	POM-RD	1	7	0	8
	C1	8	0	1	9
	C2	8	4	1	13
San Gabriel River Reach 1	R2	7	0	1	8
	R3-1	21	16	0	37
	R4	21	18	0	39
	R9W	21	16	0	37
Coyote Creek	RA1	6	20	0	26
	RA	6	22	0	28
	R9E	6	6	0	12

RESULTS

Toxicity monitoring results are presented below for the four reaches in the San Gabriel River Watershed for which there are monitoring results. To be consistent with the toxicity results reported in the main report, results in this appendix are reported as not toxic if less than a 25% effect is observed in the site sample relative to the control. However, statistically significant differences are also noted.

San Gabriel River Reach 3

A total of 27 valid chronic toxicity tests have been conducted since June 2003 with samples collected at two receiving water stations in Reach 3 of the San Gabriel River (see Figure A.1). Both receiving water stations are located downstream of two discharge points of the San Jose Creek WRP. Effects greater than 25% were observed in only two tests. Statistically significant effects were observed in three of the 29 tests. A summary of these results is contained in Table B.5. No consistent toxicity was observed in this reach.

San Jose Creek Reach 1

A total of 55 valid chronic toxicity tests were conducted with samples collected from five receiving water stations in San Jose Creek Reach 1. Effects greater than 25% were observed in 17 of the 55 tests with statistically significant effects observed in 22 of the 55 tests. Most of the observed effects were in the samples from stations POM-RA, POM-RC, and POM-RD and not from stations C1 or C2 (14 tests and 3 tests, respectively). The Pomona WRP discharges upstream of station POM-RA and the San Jose Creek WRP has a discharge downstream of station C1 (and upstream of C2). A summary of the toxicity results is contained in A.6. As shown in the table, the majority of the observed toxicity in this reach has been confined to the upstream area of San Jose Creek.

The toxicity testing for stations POM-RA, POM-RC and POM-RD are governed by the NPDES requirements for the Pomona WRP. Accelerated receiving water monitoring and Toxicity Reduction Evaluation workplan initiation has been triggered on a few occasions at POM-RA as the result of observed toxicity but specific causes of the sporadic toxicity has not been identified. However, diazinon quantification conducted (using both enzyme-linked immuno-sorbent assays (ELISA) and EPA method 8141) revealed elevated levels of diazinon in the receiving water but not in the Pomona WRP effluent on at least one occasion.

San Gabriel River Reach 1

A total of 121 valid chronic toxicity tests were conducted with samples collected from four receiving water stations in Reach 1 of the San Gabriel River. Receiving water stations R2 and R3-1 are located downstream of a discharge point for the San Jose Creek WRP and upstream of the discharge point for the Los Coyotes WRP. Stations R4 and R9W are located downstream of the discharge point for the Los Coyotes WRP. Effects greater than 25% were observed in only three tests with statistically significant effects observed in six of the 121 tests. A summary of these results is contained in Table B.7. No consistent toxicity was observed in this reach.

Coyote Creek

A total of 66 valid chronic toxicity tests were conducted with samples collected from three receiving water stations in the lower portion of Coyote Creek. Receiving water station RA1 is located upstream of the discharge from Long Beach WRP and stations RA and R9E are located downstream of the discharge. Effects greater than 25% were observed in 15 of the 66 tests with statistically significant effects observed in 19 of the tests. Toxicity in the lower portion of Coyote Creek was observed much more frequently prior to January 2005 with only two of the 27 tests conducted in or after January 2005 exhibiting effects greater than 25%. Most of the toxicity observed at these stations has been attributed to sources upstream of the Long Beach WRP. A summary of these results is contained in Table B.8.

Monthly median and weekly accelerated testing was conducted at station RA on several occasions. Since the source of toxicity appeared to be coming from above the Long Beach WRP discharge (as evidenced

by the observed toxicity at the upstream station RA1), concurrent testing was also conducted at RA1. In most cases, the concurrent upstream testing confirmed that the source of toxicity was originating above the WRP discharge. The nature of the toxicity has not been determined.

Table B.5. Summary of NPDES chronic tests for Reach 3 of San Gabriel River.²

Month/Year Tested	Location	
	R11	WN-RA
August 2003	-	ns
October 2003	S ¹	ns
November 2003	-	-
February 2004	L ¹	ns
March 2004	ns	-
May 2004	-	ns
August 2004	-	-
November 2004	-	ns
March 2005	-	-
May 2005	- ¹	-
August 2005	-	-
November 2005	-	-
February 2006	-	-
March 2006	ns	-
May 2006	-	-

- not toxic; effect less than 25%.

S = sub-lethal (reproduction or growth) effect greater than 25%.

L = lethal/survival effect of greater than 25%.

ns = not sampled.

¹ Statistically significant from control.

² More than one test was conducted in certain months; all resulted are reflected.

Table B.6. Summary of NPDES chronic tests for San Jose Creek Reach 1. ²

Month/Year Tested	Location					
	POM-RA		POM-RC	POM-RD	C1	C2
August 2003	ns		ns	ns	ns	-
October 2003	ns		ns	ns	ns	-
February 2004	ns		ns	ns	ns	-
May 2004	ns		ns	ns	ns	-
August 2004	-		-	-	-	-
November 2004	L ¹ S ¹	-	-	-	- ¹	-
February 2005	S ¹	- ¹	S ¹	S ¹	-	ns
March 2005	-	S ¹		ns	ns	-
May 2005	ns		- ¹	S ¹	L ¹ S ¹	-
August 2005	ns		- ¹	-	-	-
November 2005	S ¹	S ¹	L ¹ S ¹	L ¹ S ¹	-	S ¹
December 2005	L ¹ S ¹		S ¹	ns	ns	ns
February 2006	-		-	L ¹ S ¹	- ¹	-
May 2006	-		-	-	L ¹ S ¹	S ¹ -

- not toxic; effect less than 25%.

S = sub-lethal (reproduction or growth) effect greater than 25%.

L = lethal/survival effect of greater than 25%.

ns = not sampled.

¹ Statistically significant from control.

² More than one test was conducted in certain months; all results are reflected.

Table B.7. Summary of NPDES chronic tests for Reach 1 of San Gabriel River.²

Month/Year Tested					
	R2	R3-1	R4		R9W
June 2003	ns	-	-		-
July 2003	ns	-	-		-
August 2003	ns	-	-		-
September 2003	ns	-	-		-
October 2003	ns	-	-		-
November 2003	ns	-	-		-
December 2003	ns	-	-		-
January 2004	ns	-	-		-
February 2004	ns	-	-		-
March 2004	ns	-	-		-
April 2004	ns	-	-		-
May 2004	ns	-	-		-
June 2004	ns	-	-		-
July 2004	ns	S ¹	-		-
August 2004	-	-	-		-
September 2004	ns	-	-		-
October 2004	ns	-	-		-
November 2004	-	-	-		-
December 2004	ns	-	-		-
January 2005	ns	-	-		-
February 2005	ns	-	ns		-
March 2005	-	-	-	-	-
April 2005	ns	-	-		-
May 2005	-	-	-		-
June 2005	ns	-	-		-
July 2005	ns	-	-		-
August 2005	-	-	-		-
September 2005	ns	-	-		-
October 2005	ns	- ¹	-		L ¹ S ¹
November 2005	-	- ¹	-		-
December 2005	ns	-	-		-
January 2006	ns	-	-		-
February 2006	-	-	-		-
March 2006	ns	-	-		-
April 2006	ns	-	- ¹	-	-
May 2006	-	-	-		-
June 2006	ns	-	-		S ¹

- not toxic; effect less than 25%.

S = sub-lethal (reproduction or growth) effect greater than 25%.

L = lethal/survival effect of greater than 25%.

ns = not sampled.

¹ Statistically significant from control.

² More than one test was conducted in certain months; all resulted are reflected.

Table B.8. Summary of NPDES chronic tests for Coyote Creek.²

Month/Year Tested	Location									
	RA1			RA				R9E		
July 2003	L ¹ S ¹			L ¹ S ¹				L ¹ S ¹		
October 2003	-			-				-		
January 2004	-			-				-		
April 2004	-			-				-		
July 2004	L ¹ S ¹			- ¹	-	L ¹ S ¹		S ¹		
August 2004	L ¹ S ¹	L ¹ S ¹	L ¹ S ¹	S ¹	-	S ¹		ns		
October 2004	-			- ¹				-		
November 2004	L ¹ S ¹	-	-	-	-	-		ns		
December 2004	-	-	-	- ¹	-	-	L ¹ S ¹	-	ns	
January 2005	S ¹		-	-			-		-	
February 2005	-	- ¹	-	-	-	-		ns		
April 2005	-			-				-		
July 2005	ns			Ns				-		
August 2005	L ¹			-				ns		
October 2005	-			-				ns		
November 2005	ns			Ns				-		
January 2006	-			-				-		
April 2006	-			-				-		

- not toxic; effect less than 25%.

S = sub-lethal (reproduction or growth) effect greater than 25%.

L = lethal/survival effect of greater than 25%.

ns = not sampled.

¹ Statistically significant from control.

² More than one test was conducted in certain months; all resulted are reflected.