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SAN JACINTO RIVER AUTHORITY

Sublethal Toxicity Evaluation
The Woodlands
Wastewater Treatment Plant No. 1

SUBMITTED TO:

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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ALAN PLUMMER ASSOCIATES, INC.
ENVIRONMENTAL ENGINEERS • DESIGNERS • SCIENTISTS

EA Engineering, Science, and Technology, Inc.

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WASTEWATER TREATMENT PLANT NUMBER 1

SUBLETHAL TOXICITY EVALUATION

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ATTACHMENTS

A PDF Files: Laboratory Reports, Industrial User Survey (2007-2008) and TCEQ Permit on Disc

B Laboratory Report, August 8, 10, and 12, 2005 – ENVIRON

C Laboratory Report, September 12, 14 and 16, 2005 – ENVIRON

D Laboratory Report, December 5, 7, and 9, 2005 – ENVIRON

E Laboratory Report, February 2006 – ENVIRON

F Laboratory Report, May 10, 2006 – ENVIRON

G Laboratory Report, May 8, 2006 – EA

H Laboratory Report, January 24, 2007 – EA

I Laboratory Report, October 8, 2007 – EA

J Laboratory Report, October 10, 2007 - EA

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LIST OF ABBREVIATIONS

APAI	Alan Plummer Associates, Inc.
HCO_3^-	bicarbonate ion
C_{18}-SPE	C_{18} column solid phase extraction
CO_2	carbon dioxide
$CO_3^=$	carbonate ion
C. dubia	Ceriodaphnia dubia
$CaCO_3$	calcium carbonate
$CBOD_5$	five-day carbonaceous biochemical oxygen demand
D. magna	Daphnia magna
dia	diameter
EA	EA Engineering, Science and Technology, Inc.
EDTA	ethylenediaminetetraacetic acid
ENVIRON	ENVIRON International Corporation
EPA	U.S. Environmental Protection Agency
ft	feet
GAC	granular activated carbon
in	inches
IC_{25}	25% inhibition concentration
mg/L	milligrams per liter
MGD	million gallons per day
MH	moderately hard
NaThio	sodium thiosulfate
N	nitrogen
NPDES	National Pollutant Discharge Elimination System
NOEC	No-observed-effect concentration
Number	No.
%	percent
pH*i*	initial pH

LIST OF ABBREVIATIONS
(Continued)

P. promelas	Pimephales promelas
SIU	Significant Industrial User
SJRA	San Jacinto River Authority
SPE	solid-phase extraction
STE	sublethal toxicity evaluation
SWD	side-water depth
TCEQ	Texas Commission on Environmental Quality
TDS	total dissolved solids
TIE	Toxicity Identification Evaluation
TRE	Toxicity Reduction Evaluation
µm	micrometer
µS/cm	microsiemens per centimeter
w/	with
WET	Whole Effluent Toxicity
WWTP No. 1	The Woodlands Wastewater Treatment Plant Number 1
WWTP No. 2	The Woodlands Wastewater Treatment Plant Number 2
WWTP No. 3	The Woodlands Wastewater Treatment Plant Number 3

CHAPTER I

INTRODUCTION

The San Jacinto River Authority (SJRA) owns and operates The Woodlands Wastewater Treatment Plant Number 1 (WWTP No. 1). SJRA is authorized under its Texas discharge permit WQ0011401-001 to treat and dispose of treated wastewater from WWTP No. 1 to Panther Branch (Outfall 001) or, alternatively, to Harrison Lake (Outfall 002), which are tributaries to Spring Creek in Segment No. 1008 of the San Jacinto River Basin. The permitted annual average flow at Outfall 001 is 7.8 million gallons per day (MGD) and the permitted annual average flow at Outfall 002 is 0.6 MGD. SJRA is also subject to the federal National Pollutant Discharge Elimination System (NPDES) Permit No. TX0054186. The NPDES permit limits for conventional pollutants for Outfall 001 are presented in Table I-1.

Table I-1

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

Wastewater Treatment Plant No. 1 Permit Effluent Limits

Parameter	Daily Average Effluent Limit*
Ammonia-Nitrogen, 30-day Average	3 mg/L maximum
$CBOD_5$, 30-day Average	10 mg/L maximum
Dissolved Oxygen, at all times	4.0 mg/L minimum
Total Suspended Solids, 30-day Average	15 mg/L maximum

*The above permit limits are the same for both the Texas discharge permit and the NPDES permit for Outfall 001.

Both permits require whole effluent toxicity (WET) testing with a water flea _Ceriodaphnia dubia_ (*C. dubia*) and a fish _Pimephales promelas_ (*P. promelas*) (common name fathead minnow). In August of 2005 SJRA initiated a sublethal toxicity evaluation (STE) in response to sporadic sublethal WET test failures in the *C. dubia* test. This STE effort was a proactive measure on the part of SJRA and not a permit requirement. SJRA has worked over the past three years to characterize and identify the cause of sporadic sublethal toxicity. The efforts have included the following:

- Toxicity characterizations
- Mock effluent testing
- WET testing with an alternative organism
- WET testing under carbon dioxide (CO_2) atmosphere
- Significant Industrial User Investigation
- Ion exchange treatment of effluent
- Source water WET and water chemistry testing
- Chemical analyses of effluent
- Chemical inventory of industrial and commercial businesses in the service area
- WWTP No. 1 and WWTP No. 2 WET comparisons

The following chapters provide a description of the WWTP No. 1 treatment processes and historical performance, an identification of the team conducting the STE, a summary of the routine WET test results since 2004, a summary of the STE studies, a comparison of WET test data for WWTP No. 1 to WET test data for Wastewater Treatment Plant Number 2 (WWTP No. 2), and conclusions based on currently available data.

CHAPTER II

WASTEWATER TREATMENT PLANT NO. 1 DESCRIPTION

WWTP No. 1 is one of three plants providing wastewater services to the community of The Woodlands, Texas, a master-planned community. The September 2008 estimated population of The Woodlands is 88,123.

WWTP No. 1 has been in operation since 1975. The service area for WWTP No. 1 is dominated by residential users. Additional customers include office buildings, commercial and retail businesses, and hotels. Industrial users contribute a minimal percentage of the effluent.

This chapter identifies the location of the plant, describes the treatment system, and summarizes historical effluent quality data. It also provides information about the water supply.

LOCATION

WWTP No. 1 is located in the southern part of The Woodlands. The Woodlands is located in Montgomery County, Texas, 25 miles north of downtown Houston. Figure II-1 is a general location map, and Figure II-2 is a map of The Woodlands.

TREATMENT PROCESS DESCRIPTION

Since 1975 WWTP No. 1 has undergone several expansions to comply with effluent permit limits and to accommodate increasing flows, up to the current capacity of an annual average discharge of 7.8 MGD. WWTP No. 1 includes an activated-sludge process that is operated in a complete-mix mode. A flow diagram of the existing liquid and solids treatment processes is presented in Figure II-3. Table II-1 is a list of the major treatment units and their respective sizes.

WWTP No. 1 uses chemical addition for effluent disinfection and in sludge treatment processes. Effluent disinfection is accomplished with chlorine gas followed by de-chlorination with sulfur dioxide. Polymer is added to the sludge to aid in de-watering. Prior to using the polymer for sludge de-watering, SJRA contracted with a laboratory to

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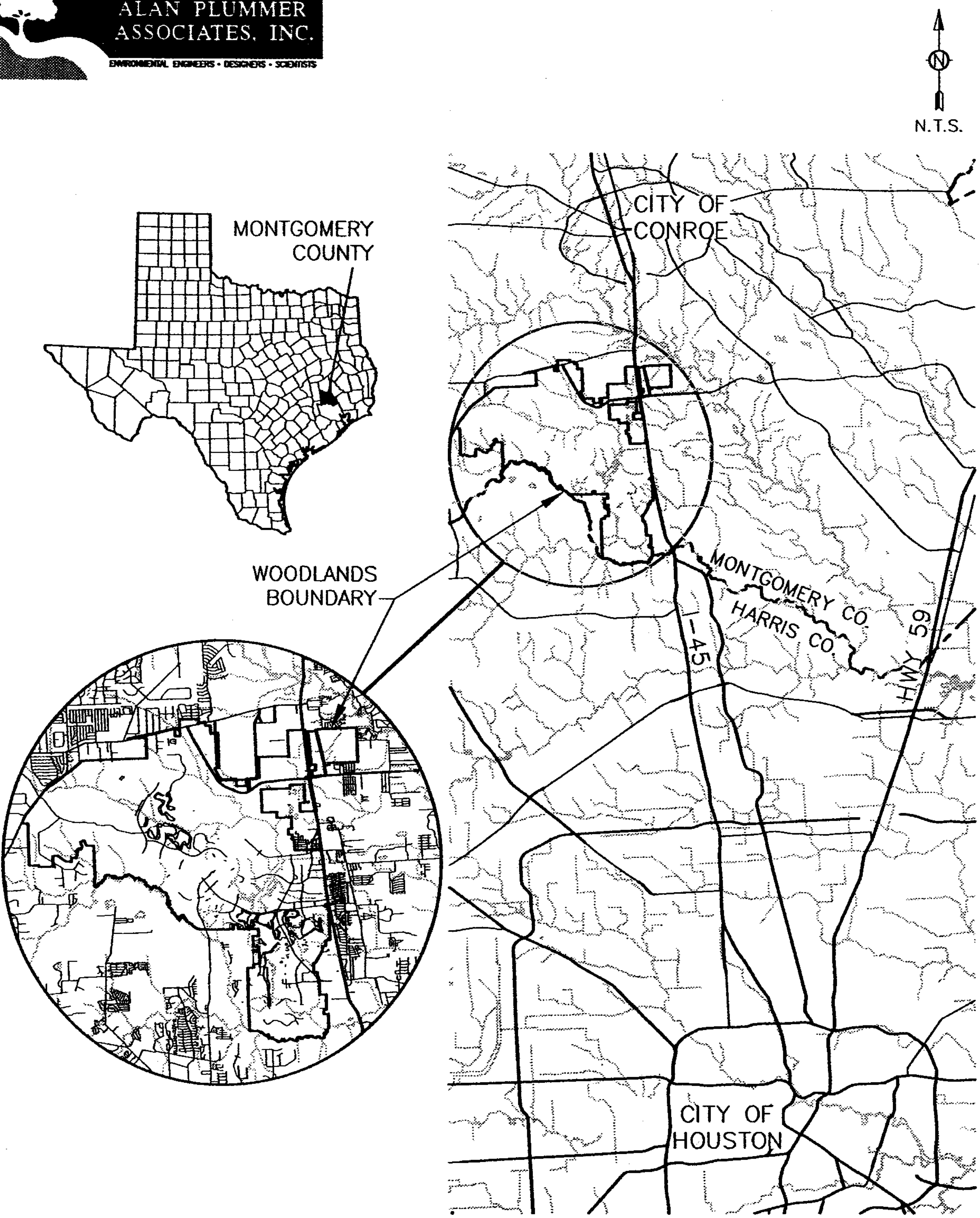

FIGURE II-1

SAN JACINTO RIVER AUTHORITY
WASTEWATER TREATMENT PLANT NO. 1
SUBLETHAL TOXICITY EVALUATION
GENERAL LOCATION MAP

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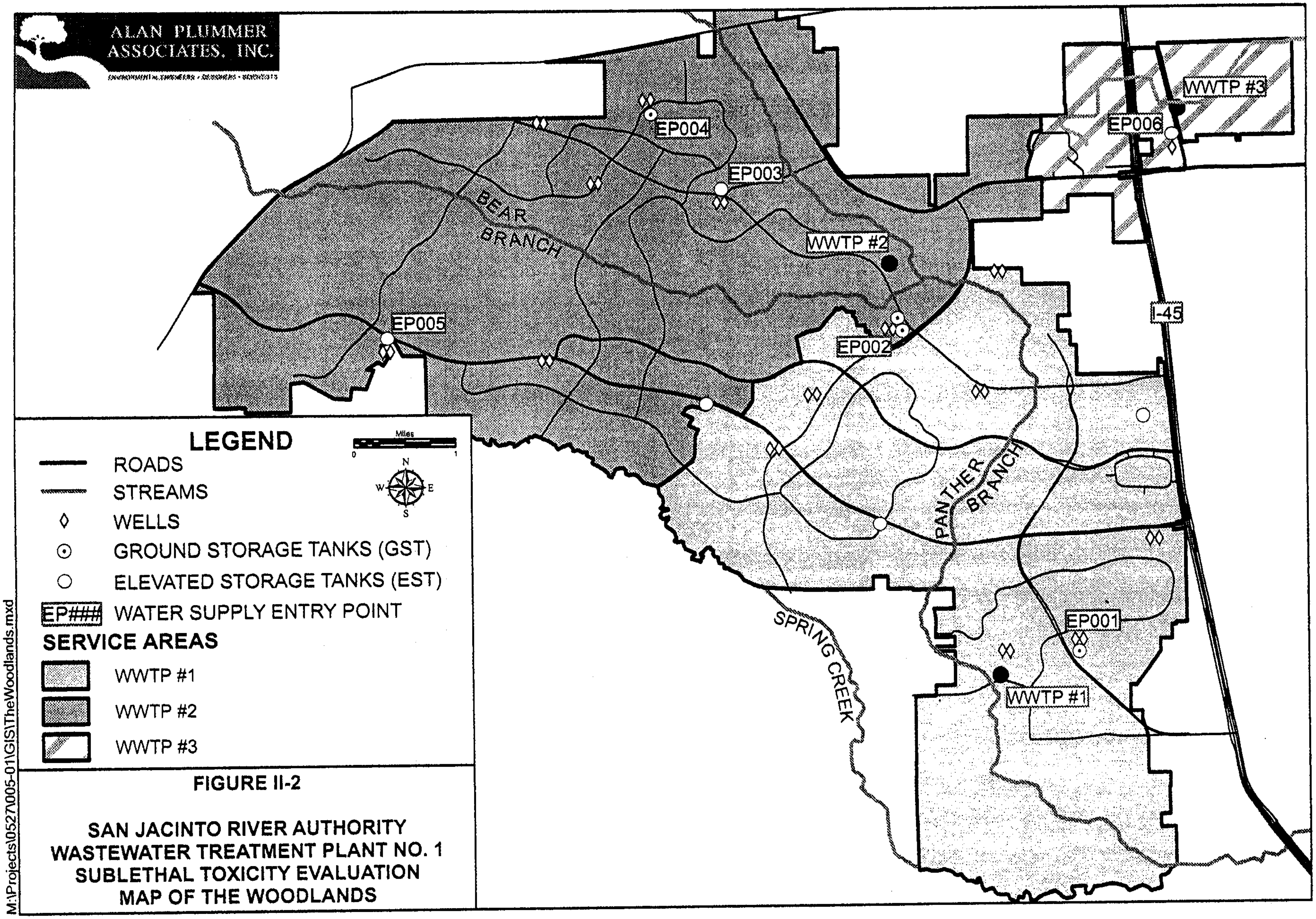

FIGURE II-2

**SAN JACINTO RIVER AUTHORITY
WASTEWATER TREATMENT PLANT NO. 1
SUBLETHAL TOXICITY EVALUATION
MAP OF THE WOODLANDS**

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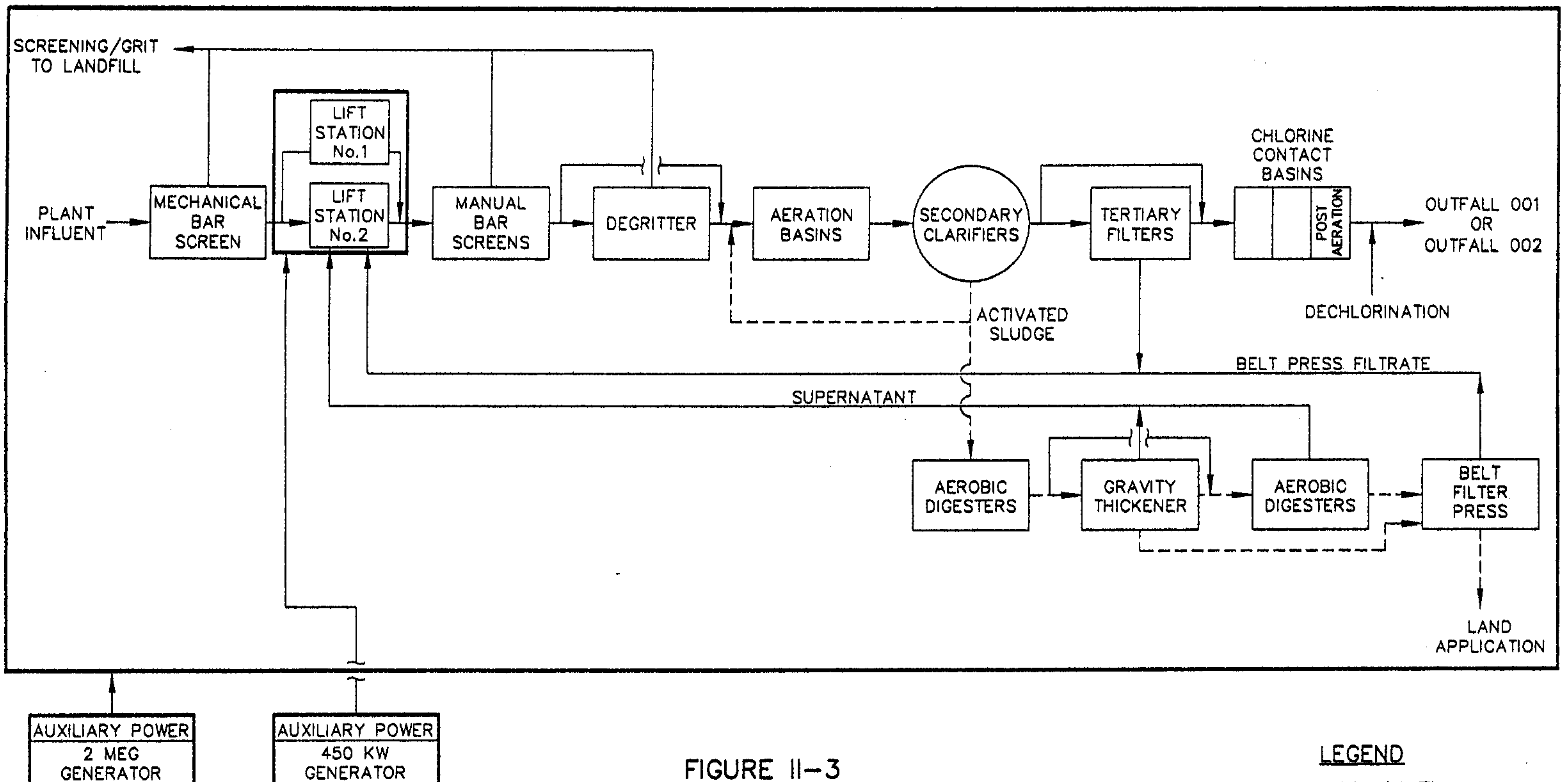

FIGURE II–3

SAN JACINTO RIVER AUTHORITY
WASTEWATER TREATMENT PLANT NO. 1
SUBLETHAL TOXICITY EVALUATION

WASTEWATER TREATMENT PLANT NO. 1 FLOW DIAGRAM

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Table II-1

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

Wastewater Treatment Plant No. 1 Treatment Units

Type of Unit	Number of Units	Size
Mechanical Bar Screen	1	3.5 foot wide
Entrance Structure Degritter	1	22 ft x 10 ft, 16-ft SWD Vortex Type Unit
Fine Bar Screens	3	3.16 ft x 7.8 ft long
Aeration Basins	1 1 2	90 ft x49 ft x14.5 ft SWD 90 ft x50 ft x14.5 ft SWD 75 ft x75 ft x16 ft SWD w/ Diffused Air System
Final Clarifiers	3	87 ft dia, 10 ft SWD
Low head Automatic Backwash Filters	2	60 ft x 21 ft x1.3 ft SWD
Chlorine Contact Basins	1 2	20 ft x 27.7 ft x 9 ft SWD 60 ft x 21 ft x10 ft SWD
Aerobic Digesters	1 2 1 1	47 ft x 23.5 ft x 23.5 ft SWD 50 ft x 40 ft x 18 ft SWD 55 ft dia, 12 ft SWD w/1.5 ft cone 45 ft dia,15 ft SWD w/1.5 ft cone
Gravity Thickener	1	35 ft dia,11.5 ft SWD w/3.0 ft cone
Belt Press	1	2-meter unit

conduct tests of the polymer to confirm that the polymer would not negatively impact WET tests of the effluent. The polymer test data are provided on the enclosed disc.

HISTORICAL EFFLUENT QUALITY

WWTP No. 1 routinely produces high quality effluent exceeding permit requirements. Figure II-4 presents historical effluent quality data for conventional pollutants for the period January 2004 through September 2008. Five-day carbonaceous biochemical oxygen demand ($CBOD_5$) removal efficiency is high. For example, the average for September 2008 was 98%.

In addition, to the extent that there may be potential toxicants in the raw wastewater, these, too, appear to be effectively removed by the treatment processes. Results of effluent analyses, including priority pollutant analyses, conducted for the August 2007 Texas discharge permit application and the 2006 NPDES permit application are provided on the enclosed disc. With a few exceptions, pollutant concentrations are below the detection limits. Concentrations of the pollutants that were detected are very low and are not at levels that would be expected to impact WET test results.

WATER SUPPLY FOR SERVICE AREA

WWTP No. 1 effluent has low hardness and high alkalinity, which is a result of the water quality of the source water. The water supply for The Woodlands is groundwater. Groundwater is pumped from the Evangeline and Jasper Aquifers using wells located throughout The Woodlands. The Evangeline and Jasper Aquifers are part of the Gulf Coast Aquifer, which spans the Gulf Coast of Texas from the border with Mexico to the border with Louisiana. The well locations are shown on Figure II-2.

Due to increasing demand, water levels in the aquifers have been decreasing. In response to the decreasing well water levels and to accommodate increasing water demand in The Woodlands and its surrounding areas, SJRA is in the early planning phases of a project to develop nearby Lake Conroe water as a supplement to The Woodlands water supply.

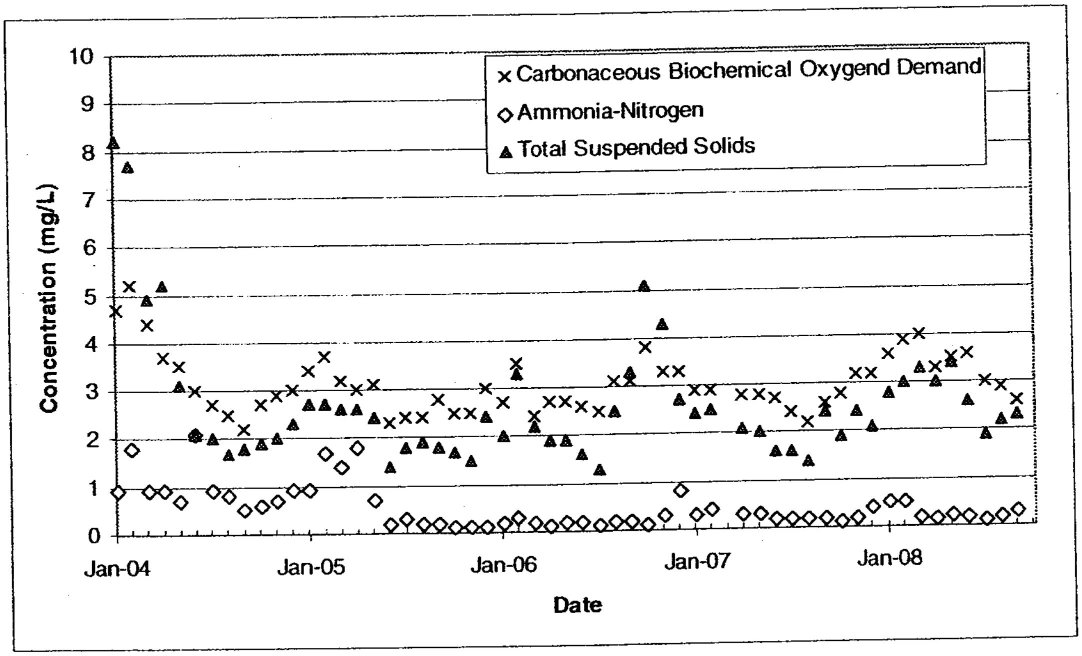

Figure II-4

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

Historical Effluent Quality
Monthly Average

CHAPTER III
SUBLETHAL TOXICITY EVALUATION TEAM

To conduct the STE, SJRA engaged the services of two nationally recognized WET testing laboratories, a consultant, and an analytical laboratory. The team members and their respective responsibilities are as follows:

- ENVIRON International Corporation (ENVIRON), formerly Advent Group, Inc., located in Nashville, Tennessee, performed all routine WET tests and some of the STE investigations discussed in this report.

- EA Engineering, Science and Technology, Inc., (EA) located in Sparks, Maryland, performed the remainder of the STE investigations.

- Eastex Environmental Laboratories, Inc., Coldspring, Texas, performed analytical testing. Eastex subcontracted some analyses to Anacon, Inc., in Houston, Texas; and to Test America, in Nashville, Tennessee, and Pittsburg, Pennsylvania. Eastex also evaluated Significant Industrial Users during 2007 and 2008.

- Alan Plummer Associates, Inc., (APAI) in Austin, Texas, assisted with the coordination and evaluation of the STE.

CHAPTER IV

COMPLIANCE WHOLE EFFLUENT TOXICITY TESTING

The Texas discharge permit and the NPDES permit for WWTP No. 1 specify that WET testing be conducted using short-term chronic toxicity tests with *C. dubia*, (3-brood survival and reproduction test) and *P. promelas* (7-day larval survival and growth test). Permit requirements for WET testing have changed over time. Table IV-1 lists the applicable dilution series and critical dilutions for both the NPDES and Texas discharge permits since 1989. Other changes to the WET permit requirements are not discussed in this report and are not relevant to the work presented.

WET test results are assessed by comparing the statistically determined no-observed-effect concentration (NOEC) to the critical dilution. An NOEC below the critical dilution constitutes a WET test failure. Lethal WET test failures are very infrequent. The last lethal *C. dubia* WET test failure was reported in January 2002, and the last lethal *P. promelas* WET test failure pre-dates that.

The results of routine and accelerated WET tests conducted for WWTP No.1 effluent from 2004 through September 2008 in compliance with permit requirements are summarized in Table IV-2. Sublethal WET test failures for the WWTP No. 1 effluent are sporadic and, typically, occur in the 3-brood *C. dubia* test. There are no WET test failures for lethality during this period.

Sublethal WET test failures in the *C. dubia* test have occurred in 35% of the WET tests conducted since January 2004. Since the critical dilution was increased to 85% in January 2006, the WET test failure rate has been 43%. There is no apparent pattern for sublethal WET test failures, seasonal or otherwise.

Sublethal WET test failures in the *P. promelas* test have occurred on only three occasions since March 2004. Two of these tests had a parallel test run on the same sample. The parallel tests did not exhibit test failures.

This report focuses on the causes of WET test failures in *C. dubia* tests. Henceforth, in the report, unless specified otherwise, a WET test refers to a 3-brood *C. dubia* survival and reproduction WET test.

All laboratory reports for the *C. dubia* WET tests (since January 2004) are available in pdf files on the enclosed disc. Attachment A provides a table of contents for the disc.

Table IV-1

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

Whole Effluent Toxicity Test
Dilution Series
(Percent Effluent)[1]

Date of Permit Issuance	National Pollutant Discharge Elimination System Permit	Texas Discharge Permit
9/1/1989	23, 32, **45**, 62, 86	
3/14/1990		25, 34, **46**, 63, 86
12/29/1995		23, 32, 45, **55**, 62
1/17/2006		27, 36, 48, 64, **85**
9/28/2007	29, 39, 52, **69**, 92	
10/16/2008		25, 33, 44, 59, **78**

1) The critical dilution is underlined and bolded.

Table IV-2

San Jacinto River Authority
Wastewater Treatment No. 1
Sublethal Toxicity Evaluation

Whole Effluent Toxicity Test Compliance Results for WWTP No. 1

First Sample Date	*Ceriodaphnia dubia*		*Pimephales promelas*	
	Survival(1)	Reproduction(1)	Survival(1)	Growth(1)
January 6, 2004	P	P	P	P
February 3, 2004	P	P	NT	NT
March 9, 2004	P	P	P	F
April 6, 2004	P	P	P	P
May 4, 2004	P	P	NT	NT
June 8, 2004	P	P	P	P
July 13, 2004	P	P	NT	NT
August 3, 2004	P	F	NT	NT
September 14, 2004	P	F	P	P
October 12, 2004	P	F	NT	NT
November 16, 2004	P	P	NT	NT
December 14, 2004	P	P	P	P
January 4, 2005	P	P	P	P
February 8, 2005	P	P	NT	NT
March 8, 2005	P	P	P	P
April 5, 2005	P	P	NT	NT
May 3, 2005	P	P	NT	NT
June 7, 2005	P	P	P	P
July 12, 2005	P	P	NT	NT
August 9, 2005	P	F	NT	NT
September 13, 2005	P	F	P	P
October 4, 2005	P	P	NT	NT
November 8, 2005	P	P	NT	NT
December 6, 2005	P	F	P	P
January 10, 2006	P	P	P	P
February 7, 2006	P	F	NT	NT
March 7, 2006	P	F	P	P
April 11, 2006	P	P	NT	NT

(1)P=Pass/F= Fail/NT=Not Tested
(2)Duplicate tests exhibited different WET test results.

Table IV-2

San Jacinto River Authority
Wastewater Treatment No. 1
Sublethal Toxicity Evaluation

Whole Effluent Toxicity Test Compliance Results for WWTP No. 1

First Sample Date	*Ceriodaphnia dubia*		*Pimephales promelas*	
	Survival[1]	Reproduction[1]	Survival[1]	Growth[1]
May 9, 2006	P	F	NT	NT
June 6, 2006	P	F	P	P/F [2]
July 11, 2006	P	F	NT	NT
August 8, 2006	P	P	NT	NT
September 12, 2006	P	P	P	P/F [2]
October 3, 2006	P	P	NT	NT
November 7, 2006	P	Invalid	NT	NT
November 28, 2006	P	P	NT	NT
December 5, 2006	P	P	P	P
January 23, 2007	P	F	NT	NT
February 6, 2007	P	P	NT	NT
March 6, 2007	P	P/F[2]	P	P
April 12, 2007	P	P	NT	NT
May 8, 2007	P	P	NT	NT
June 5, 2007	P	P	Control Failure	Control Failure
July 10, 2007	P	P	NT	NT
August 7, 2007	P	P	NT	NT
September 11, 2007	P	F	P	P
October 9, 2007	P	F	NT	NT
November 13, 2007	P	P	NT	NT
December 4, 2007	P	P	P	P
February 5, 2008	P	Invalid	P	P
March 11, 2008	P	P	NT	NT
May 6, 2008	P	F	P	P
June 25, 2008	P	P	P	P
July 22, 2008	P	F	P	P
August 12, 2008	P	F	NT	NT
September 23, 2008	P	F	NT	NT

[1]P= Pass / F= Fail / NT=Not Tested
[2]Duplicate tests exhibited different WET test results

CHAPTER V

SUBLETHAL TOXICITY EVALUATION OVERVIEW

SJRA's response to sporadic sublethal WET test failures has been to conduct a study similar to a Toxicity Reduction Evaluation (TRE). A TRE may be required for persistent significant lethality in WET tests of effluent. However, neither the Texas discharge permit nor the NPDES permit for WWTP No. 1 requires such an evaluation for sublethal effects. SJRA's effort to identify potential causes of the test failures has been voluntary.

DESCRIPTION OF SUBLETHAL RESPONSE

This report describes WET test failures in the WWTP No. 1 effluent in several ways, using terms such as, persistent, consistent, and sporadic. To be clear regarding the use of these terms, this report incorporates the following definitions:

Persistent: the tendency for WET test failures of a single effluent sample to be reproducible over time.

Consistent: the tendency for WET test failures of an effluent to be reproducible at similar levels across samples and over time.

Sporadic: the tendency for WET test failures of an effluent to occur intermittently across samples and over time.

SCOPE OF SUBLETHAL TOXICITY EVALUATION

During most of the study period (August 2005 through September 2008), SJRA conducted monthly compliance tests pursuant to the requirements of the NPDES permit. When monthly testing was no longer required, beginning in November 2007, quarterly compliance tests were conducted. However, SJRA continued to test monthly for toxic effects using screening tests during the period from February 2008 through September 2008, in months when routine compliance WET tests were not conducted.

The screening tests were conducted on a single sample. The same sample was used for each daily renewal. Screening tests were conducted using a control and a 100% effluent sample.

Compliance or screening samples that exhibited a WET test failure were used for subsequent studies. During the course of the STE, it was determined that, when trying to observe the effects of treatments for the toxicity characterization study, or other studies, a marginal sublethal signal could not be differentiated from test variability and artifactual toxicity induced by the treatments. Therefore, during the latter period of the STE, only samples that exhibited a 50% reduction in reproduction, compared to the control, were generally used for toxicity characterizations and other studies. Moderately hard (MH) laboratory water was used as the control for all studies except where the study description specifically states otherwise.

The initial phase of the STE consisted of toxicity characterization studies comparable to those conducted during a Phase I, Tier 1 study for a TRE. These initial studies are described in Chapter VI. The initial phase was followed by the following additional studies:

- WET tests of mock effluent
- WET tests of the water supply
- Ion exchange studies
- Evaluations of the effect of colloidal solids
- Evaluations of the effect of conducting WET tests under a CO_2 atmosphere
- WET testing using the water flea, <u>*Daphnia*</u> <u>*magna*</u> *(D. magna)*

These additional studies were developed based on observations in the initial phase and are presented in Chapter VII and Chapter VIII.

INDUSTRIAL SURVEY

SJRA regulates the quality of industrial discharges to its WWTPs and has done so since 1976. In addition, the Texas discharge permit for The Woodlands WWTP No. 2 (issued February 18, 2003), required SJRA to develop and submit an industrial pretreatment program for all of The Woodlands. SJRA submitted the pretreatment program in December 2004 for TCEQ's review and comment. To date, SJRA has not received comments or notifications from TCEQ concerning the proposed pretreatment program. However, SJRA continues to monitor and evaluate industrial users based on its industrial regulation that was adopted in 1976.

SJRA recently conducted a survey of Significant Industrial Users (SIUs). For each SIU, a list of chemicals present, including raw materials, products, and cleaning materials, was compiled. The survey results are included on the enclosed disc. The survey of SIUs did not identify any chemical that may be related to the sporadic and marginal WET test failures of the WWTP No.1 effluent.

In particular, SJRA focused on industrial detergents, because foaming was observed during one of the laboratory studies. The survey and site visits determined that none of the businesses or industries discharge detergents in amounts that would be expected to impact WET test results. In addition, none of the toxicity characterization studies suggested that a surfactant was the cause of test failures.

TEST INTERPRETATIONS

When interpreting STE studies, a method was needed to account for test variability to minimize the possibility that test variability would be misinterpreted as a study effect; for example, the effect of a characterization treatment. This section discusses the variability inherent in sublethal test results and describes how that variability was addressed when interpreting STE data.

Sublethal test results vary substantially when performing retests of a sample of consistent quality. This is demonstrated by the reference toxicant charts developed by WET laboratories. WET laboratories run reference toxicant tests monthly using a consistent range of concentrations of a known toxicant to verify that their test organisms are performing within an acceptable range. The results are tracked for 20 consecutive tests.

Figure V-1 presents reference toxicant charts for EA and ENVIRON laboratories. The variability shown on these charts is within the acceptable bounds (plus or minus 2 standard deviations of the mean) established in the test methods manuals. These charts are representative of the results obtained by experienced, qualified WET laboratories.

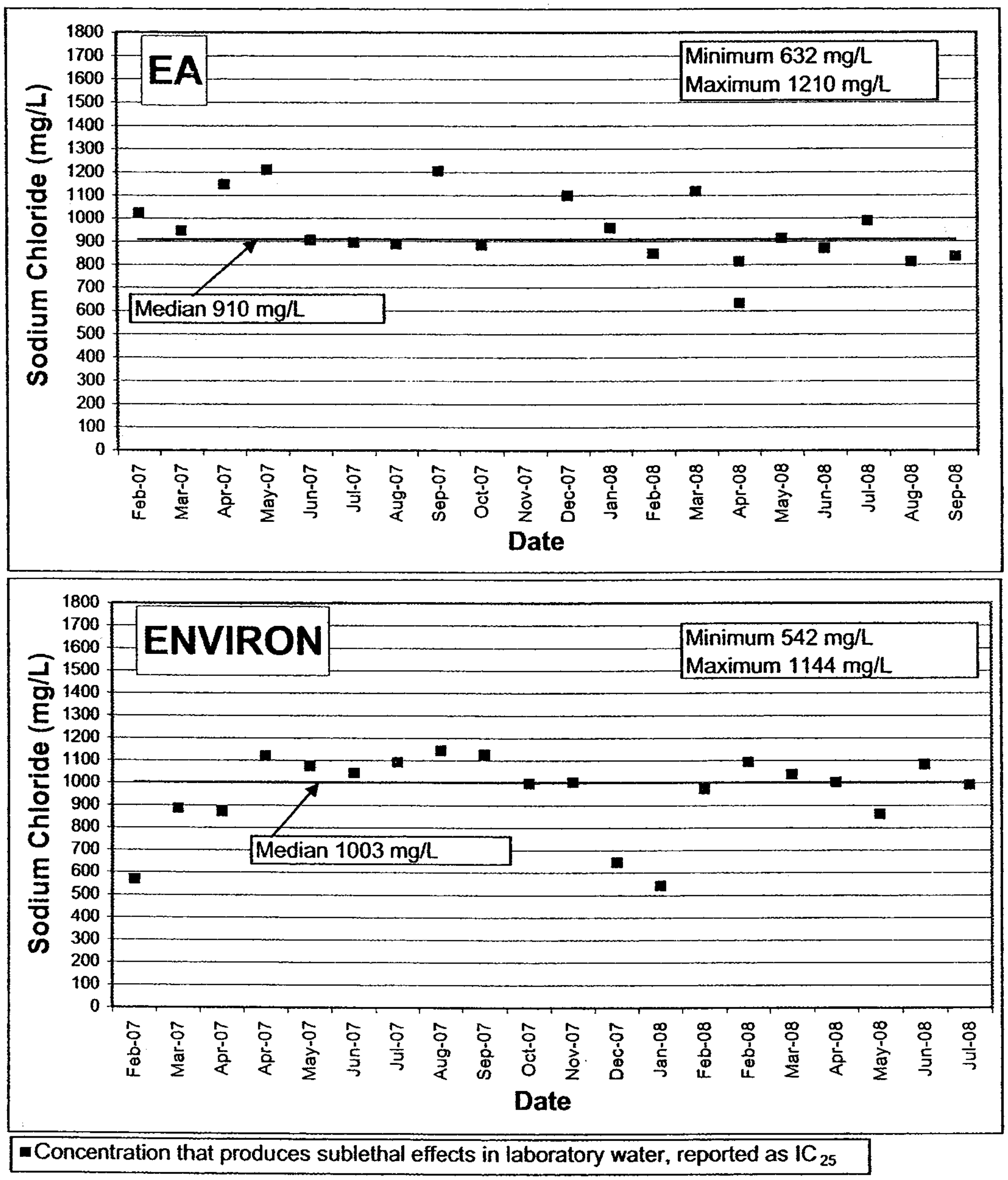

Figure V-1

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

Comparison of Reference Toxicity Data for Environ and EA

Both laboratories use sodium chloride (NaCl) as the reference toxicant and report sublethal effects as IC_{25} (the concentration of NaCl that produces a 25% reduction in reproduction). Both laboratories report the median concentration of NaCl that produced a 25% reduction in reproduction as being approximately 900-1000 mg/L. The median concentrations for the two laboratories are within 10% of each other. However, in any single test, the concentration that produces a 25% reduction in reproduction can vary by as much as 34% or more. A concentration of 600 mg/L or less produced a 25% reduction in some tests. In other tests, the concentration producing a 25% reduction was greater than 1,100 mg/L.

For the purpose of this study, in order to account for this variability, it was assumed that, if the number of neonates in a treated sample did not differ by 25% or more from the number of neonates in the baseline sample or control sample, depending on the comparison being made, the treatment effect was insignificant. The use of a 25% difference in interpreting study results is analogous to the use of IC_{25} as the definition of a sublethal effect in a WET test.

For clarification, this study uses percent difference in two ways as follows:

- A minimum of 50% difference between a sample and a control was established as the basis for deciding whether to initiate STE studies on a sample. This is further explained in Chapter VI.
- A minimum of 25% difference between a baseline sample and a treated sample is used in interpreting STE study results as having an effect.

CHAPTER VI
TOXICITY CHARACTERIZATION STUDIES

SJRA initiated characterization studies in August 2005 with the objective of first characterizing and then identifying the cause of sublethal WET test failures. Fourteen samples have been characterized. This chapter describes the results of the toxicity characterization studies conducted on WWTP No.1 effluent.

BACKGROUND

Permit compliance test or screening test samples exhibiting WET test failures were used for the toxicity characterization studies. Baseline tests were conducted in parallel with the characterization studies to provide a basis for comparing the treated and untreated effluent samples. Baseline testing consisted of conducting a WET test on an undiluted effluent sample.

For the first three characterization studies (August, September, and December 2005) all three sub-samples used in the compliance WET test were characterized. For the fourth characterization study (February 2006), the three sub-samples used in the compliance WET test were combined and then tested. The remaining three characterization studies (May 2006, January 2007, and October 2007) were preceded by initial chronic toxicity tests of each sub-sample, in addition to the baseline tests run concurrently with the characterization study. The sub-sample that exhibited the strongest sublethal effect in the initial chronic toxicity testing was selected for the subsequent characterization study. In May 2006 all three sub-samples exhibited approximately the same sublethal effect, and characterizations were performed on two of the three sub-samples.

Fourteen effluent samples have been characterized since August 2005 using Phase I, Tier 1 EPA protocols for toxicity identification evaluation (TIE) characterization treatments[1] or a similar approach. The initial nine samples were subjected to a limited

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[1]U.S. Environmental Protection Agency. Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I, EPA/600/6-91/005F. Office of Research and Development, Environmental Research Laboratory, Duluth, MN, (1992).

number of treatments. Complete Phase I Tier 1 TIE-type characterizations, as described in the EPA manuals, were conducted on the remaining five samples.

Complete Phase I Tier 1 TIE-type characterization treatments provide a set of data that, when interpreted as a whole, may result in classifying the group to which the substance(s) causing WET test failures belongs: for example, non-polar organic compounds, certain groups of heavy metals, or volatile compounds. The following treatments were employed as part of the complete characterization studies:

- Ethylenediaminetetraacetic acid (EDTA) addition
- Sodium thiosulfate (NaThio) addition
- Aeration
- Filtration
- Solid Phase Extraction with a C_{18} column (C_{18}-SPE)
- Graduated pH 6.5 adjustment
- Graduated pH 7.5 adjustment
- Graduated pH 8.5 adjustment

Effluent was subjected to these treatments and retested for residual toxicity. The data were interpreted by comparing the results of the tests of the treated sample to the results of a parallel baseline test conducted with unaltered effluent. Details about the procedures of the characterization treatments and their respective purposes can be found in the referenced EPA manual.

The following sections summarize the results of the characterization studies. The summaries do not discuss the results of each individual treatment but focus on the overall interpretation of the efforts. Additional discussion of the results can be found in the laboratory reports.

Complete laboratory reports of the characterization studies are provided in the following attachments:

Attachment	Sample Date – Laboratory
B	August 8, 10, and 12, 2005 – ENVIRON
C	September 12, 14 and 16, 2005 – ENVIRON
D	December 5, 7, and 9, 2005 – ENVIRON
E	February 2006 – ENVIRON
F	May 10, 2006 – ENVIRON
G	May 8, 2006 – EA
H	January 24, 2007 – EA
I	October 8, 2007 – EA
J	October 10, 2007 - EA

INITIAL PARTIAL CHARACTERIZATIONS

Upon observation of a sublethal toxic response in the August 2005 compliance WET test (sublethal NOEC of 55%), ENVIRON conducted a limited set of toxicity characterization treatments on each of the three sub-samples. The treatments consisted of granular activated carbon (GAC), EDTA, and NaThio. Similarly, limited characterization treatments were conducted on each of the three sub-samples of the September 2005 and December 2005 WET test samples after these samples exhibited sublethal responses. The September sample was only treated with GAC. The December sample was treated with GAC and C_{18}-SPE. The only treatment consistently used on all samples was GAC. The results of these initial, partial characterizations are summarized in Table VI-1.

Meaningful interpretation of the August and December samples is not possible because of the very marginal reduction in reproduction in the baseline samples compared to the controls. None of the sub-samples differ from the respective controls by more than 21%.

Therefore, the results for all of the August and September treated samples may be representative of responses in the test variability range of $\pm$25%, except for the August 10 sample that was treated with GAC. After treatment, the August 10, sample exhibited 28% less reproduction than the baseline sample, which suggests that GAC treatment increased the toxicity of the sample. This is believed to be an anomalous result because GAC typically does not increase the toxic effect. The test result does, however, show that random anomalies do occur in characterization studies.

Table VI-1
San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

Partial Toxicity Characterization Studies
August, September, and December 2005

Date	Control	Baseline		GAC			C_{18}-SPE			EDTA			NaThio		
	Average Number of Neonates	Average Number of Neonates	Difference from Control (%)	Average Number of Neonates	Difference from Control (%)	Difference from Baseline (%)	Average Number of Neonates	Difference from Control (%)	Difference from Baseline (%)	Average Number of Neonates	Difference from Control (%)	Difference from Baseline (%)	Average Number of Neonates	Difference from Control (%)	Difference from Baseline (%)
8/8/2005	29	26	-12	27	-9	+4	Not tested			24	-17	-6	22	-25	-15
8/10/2005	29	25	-14	18	-39	-28	Not tested			22	-22	-9	19	-34	-23
8/12/2005	28	26	-9	31	+8	+19	Not tested			24	-16	-8	20	-29	-22
9/12/2005	26	17	-35	29	+15	+78	Not tested			Not tested					
9/14/2005	26	13	-49	29	+15	+124	Not tested			Not tested					
9/16/2005	26	15	-43	24	-7	+63	Not tested			Not tested					
12/5/2005	28	24	-15	30	+9	+28	21	-25	-12	Not tested					
12/7/2005	28	22	-21	26	-6	+19	23	-19	+3	Not tested					
12/9/2005	28	22	-20	29	+4	+29	18	-37	-21	Not tested					

In September, GAC was the only treatment used. All three treated sub-samples exhibited reproduction that was more than 25% greater than reproduction in the baseline sample and within $\pm$25% of that exhibited by the control. GAC appears to have removed the toxic effects of this sample.

The initial partial characterizations did not successfully identify the character of the substance causing WET test failures, and there was concern that the limited number of treatments used was not providing sufficient information. Therefore, SJRA directed the laboratory to conduct complete Phase I Tier 1 TIE-type characterizations. The complete Phase Tier 1 TIE-type characterization studies that were conducted are discussed in the following section.

COMPLETE TOXICITY CHARACTERIZATION STUDIES

Five complete toxicity characterization studies were conducted with samples collected in February 2006, May 2006, January 2007, and October 2007. Each of the five complete characterization studies was preceded by initial chronic toxicity tests to determine if the sublethal response was consistent and, in the case of all but the February 2006 sample, to identify which of the sub-samples had the strongest effect and was, therefore, best suited for the characterization study. For the study using the February 2006 sample, the three sub-samples were combined prior to conducting the characterization study in order to ensure sufficient sample volume would be available for all treatments.

The remaining characterization studies were performed using individual sub-samples collected as part of a compliance WET test. After the February 2006 sampling event, SJRA increased the sample volume collected for all compliance tests so that sufficient sample volume would be available to perform a characterization study on any one of the three sub-samples.

In May 2006, characterization studies were conducted on the first and second sub-samples. The second sub-sample was characterized by ENVIRON in May 2006, and the first sub-sample was characterized by EA in June 2006.

The second sub-sample from the January 2007 test was used for a characterization study conducted by EA in February 2007. The first sub-sample from the October 2007 test was used for the characterization study conducted by EA in October 2007.

The following sections provide a summary of each of the complete toxicity characterization studies. The results are summarized in Table VI-2.

February 2006 Characterization Studies

After a compliance WET test failure in February 2006 (sublethal NOEC of 62%), a toxicity characterization study was initiated. This study was unsuccessful because the sublethal response in the parallel baseline test was minimal (7% reduction in reproduction compared to the control). A baseline test of the sample conducted prior to the characterization showed a marginal sublethal response, 26% reduction in reproduction in the baseline sample compared to the control.

However, this study does provide further evidence that toxicity characterization treatments can produce artifactual toxicity when sublethal effects are being evaluated. The samples treated with filtration and aeration exhibited substantial sublethal effects compared to both the control and the baseline tests, as did the C_{18}-SPE treatment.

May 2006 Characterization Studies

In May 2006, the compliance WET test exhibited a sublethal WET test failure (sublethal NOEC of 62%). Subsequent baseline tests of the three sub-samples exhibited a similar reduction in reproduction in each of the three samples (an average of 29 neonates in the control; and an average of 17.2, 16.5, and 18.5 neonates in the first, second, and third sub-samples, respectively). Characterization studies were subsequently conducted by both ENVIRON and EA. ENVIRON used the May 10 sub-sample for their study, and EA used the May 8 sub-sample for their study.

Table VI-2

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

Complete Toxicity Characterization Studies
February and May 2006; January and October 2007

Treatment	Laboratory														
	ENVIRON			EA			ENVIRON			EA			EA		
	Sample Date														
	Feb 2006[1]			May 8, 2006			May 10, 2006			Jan 24, 2007			Oct 8, 2007		
	Average Number of Neonates	Difference from Control (%)	Difference from Baseline (%)	Average Number of Neonates	Difference from Control (%)	Difference from Baseline (%)	Average Number of Neonates	Difference from Control (%)	Difference from Baseline (%)	Average Number of Neonates	Difference from Control (%)	Difference from Baseline (%)	Average Number of Neonates	Difference from Control (%)	Difference from Baseline (%)
Control MH	29		+8	29		+68	26		+95	24		+32	24		+137
Baseline	27[2]	-7		17	-40		13	-49		18	-24		10	-58	
EDTA	25	-14	-7	16	-45	-8	23	-13	+69	11	-51	-36	9	-65	-17
NaThio	27	-9	-2	17	-43	-3	23	-11	+72	16	-32	-10	8	-68	-24
Activated Carbon	32 [6]	+8	+16	21 [7]	-26	+24	28	+7	+109	21 [6]	-10	+19	8 [7]	-67	-22
Aeration	21	-30	-24	19	-34	+10	27	+2	+99	17	-26	-2	10	-59	-4
Filtration	19	-35	-30	20	-30	+17	18	-31	+34	15	-36	-16	9	-63	-13
C_{18}-SPE	16	-45	-40	20	-32	+14	23	-12	+72	20	-14	+13	8.1	-67	-21
pH 6.5	29	-3	+5	15	-49	-15	24	-10	+76	18	-22	+3	NT[5]		
pH 7.5	NT[5]			11		-37	22	-15	+65	16	-30	-8	NT[5]		
pH 8.5	26	-13	-6	4.4[4]	-85		23	-12	+71	6	-74	-66	NT[5]		

1) Initial baseline test showed reduced reproduction (21.7 neonates, 26% of control
2) Lethality, 2 of 5 adults died
3) Solid phase extraction was performed with an Oasis HLB column. The WET test had 60% lethality. The laboratory attributed the lethality to a bacterial bloon
4) Laboratory attributed effect to total dissolved solids
5) NT - Not tested
6) GAC
7) PAC

The similar results from each of three baseline tests suggest that the sublethal response was probably due to the same compound or compounds being present in all sub-samples. However, the characterization results were inconsistent between the two laboratories. For the EA studies with the May 8, 2006, sub-sample, none of the treatments produced a 25% improvement in reproduction. However, in the ENVIRON studies with the May 10, 2006, sub-sample, all treatments increased the number of neonates by more than 25%. Neither of the characterization studies provides any information regarding what substances may have caused the sublethal response.

The pH adjustment studies at pH 7.5 and pH 8.5 substantially reduced reproduction. This is likely due to the increase in total dissolved solids (TDS) that occurred due to the addition of the acids and bases needed to adjust the pH.

Studies completed on samples collected through May 2006 demonstrated that meaningful characterization studies could not be conducted with a sample exhibiting a marginal sublethal response. The observed response to many of the characterization treatments appears to be representative of artifactual toxicity resulting from sample manipulation or the variability of the WET test itself. Consequently, in the summer of 2006, it was decided that characterization tests would not be conducted unless a sub-sample exhibited a reduction in reproduction of 50% or more in compliance tests.

January 2007 Characterization Studies

The January 2007 compliance WET test exhibited a sublethal WET test failure (sublethal NOEC of 32%). Subsequent baseline tests on the three sub-samples exhibited a 48%, 51%, and 42% reduction in reproduction, respectively, compared to the control. Therefore, a characterization study was conducted on the most toxic (second) sub-sample of the January 2007 test.

The baseline test conducted as part of the characterization study exhibited only a 24% reduction in reproduction when compared to the control. Nevertheless, the characterization study was performed. None of the treatments increased reproduction by 25% compared to the baseline.

Three of the treated samples exhibited reproduction within ±25% of the control (as did the baseline sample). These treatments were GAC, C_{18}-SPE, and pH 6.5. However, because of the overlap in the potential reproduction ranges of the control and the baseline samples, it is not possible to determine if these results are representative of an unchanged baseline result or if they are representative of a reduction in toxicity to the extent that the sample results are now in the range of the control sample. This is an example as to why a 50% reduction in reproduction in the baseline sample is needed in order to be able to distinguish normal variability of the WET test from the effects of the characterization treatments.

The reduction in reproduction in the pH 8.5 treatment is likely to be due to artifactual toxicity due to increased TDS resulting from the chemicals added to adjust the pH. The increase in TDS is reflected in the conductivity data collected during the test.

In addition, it should be noted that EDTA treatment increased the toxic response. Avoiding artifactual toxicity due to EDTA addition can be a challenge in lethal TREs and may be more so in sublethal TREs.

None of the treatments resulted in increased reproduction. Therefore, no data were obtained to aid in determining the cause of sublethal WET failures.

October 2007 Characterization Studies

The October 2007 compliance WET test exhibited a sublethal WET test failure. Subsequent baseline tests on the three sub-samples exhibited an 81%, 63%, and 63% reduction in reproduction, respectively, in the three sub-samples. This was the largest reduction in reproduction (compared to the control) of any sample tested during the STE. The subsequent baseline test of the first sub-sample during the characterization study had a reduction in reproduction of 58%. Even though this sub-sample exhibited the strongest sublethal response observed during the study, none of the treatments substantially reduced or increased the sublethal response.

SUMMARY OF TOXICITY CHARACTERIZATIONS

Toxicity characterization studies are frequently successful when investigating lethal WET test failures. However, performing successful characterizations of the causes of sublethal WET test failures is much more difficult, and may be impossible, especially if the WET test failures are expressed in a marginal response and occur sporadically, as is the case with the SJRA tests. As shown above, characterization studies of the WWTP No. 1 effluent did not successfully identify a chemical group producing the WET test failures.

This problem has previously been identified. Goodfellow and McCulloch reviewed the success rate of 89 TIEs. They found that when toxicity strength was minimal and the toxic events occurred infrequently, the TIE was successful only 40% of the time[2].

The toxicity characterization treatments produced inconsistent results. At times (May 10, 2006, ENVIRON study) every treatment reduced the sublethal response. At other times, (October 8, 2007, EA study), even with a sample that demonstrated a strong sublethal response, no treatment reduced the sublethal response.

The September 2005 ENVIRON study exhibited increased reproduction as a result of GAC treatment. However, no other treatments were applied to these samples. Therefore, this result could be in the same category as the May 10, 2006, ENVIRON study where all treatments improved reproduction. In other assessments, GAC generally decreased effluent toxicity, but these decreases were not beyond the range of improvements of 25% and could have been within the range of test variability. GAC also occasionally increased WET.

It was not possible to identify a class of substances that might be responsible for the sublethal WET test failures using Phase 1 Tier 1 TIE-type protocols. However, the results of the characterization studies, combined with knowledge about the unusual ionic makeup of The Woodlands water supply, led to the hypothesis that the random test

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[2]McCulloch, W.M. "Minimal Toxicity Necessary for an Effective TIE" from Toxicity Identification Evaluations and Reduction Evaluations SETAC Short-Course. 2006 SETAC North America Annual Meeting. Milwaukee, WI

failures are a result of the ion balance in the water supply. This hypothesis is consistent with the results of the characterization studies, since none of the characterization treatments is designed to alter the ion balance of the sample.

Further, it was hypothesized that the ion balance present is borderline with respect to the potential to produce a sublethal test response; i.e., depending on the sensitivity of the particular organisms used in the test, a sublethal response might or might not be exhibited. Therefore, additional studies were proposed in order to determine if this hypothesis could be confirmed. These studies are summarized in a subsequent chapter.

CHAPTER VII
ION IMBALANCE CONFIRMATION STUDIES

The results of the toxicity characterization studies were inconsistent and did not identify any group(s) of substances as the likely cause of the sublethal test failures. However, inconclusive results in toxicity characterization studies can indicate that an ion imbalance is the cause of WET failures. WWTP No.1 effluent was observed to have an unusual ion balance. Alkalinity is very high (approximately 200 mg/L as $CaCO_3$); and hardness is low (approximately 50 mg/L as $CaCO_3$) most of the time. Therefore, it was hypothesized that variability in the sensitivity of the test organisms to the ionic makeup of the effluent is the likely cause of the sporadic test failures.

To confirm ion imbalance as a cause of WET test failures, a weight-of-evidence approach is often required. Several types of studies were conducted to evaluate the role of the ionic composition in the WET test results. The studies included analytical testing, WET tests of laboratory water adjusted to reflect the ionic composition of the effluent (mock effluent), WET tests of source water, and ion exchange treatment of the effluent. The results of each of these studies are summarized below.

MOCK EFFLUENT

The effluent ionic composition of the effluent was analyzed repeatedly, and a mock effluent was prepared that approximately mimicked the average concentrations of the major ions in the effluent. The results of the effluent analyses are presented in Table VII-1, and a representative analysis of the ionic composition of the mock effluent is shown in Table VII-2. The ionic formulation of the mock effluent was the same for all tests.

WET tests of the mock effluent were conducted ten times in parallel with effluent WET tests. The laboratory reports for the mock effluent WET tests are included on the enclosed disc. The data are summarized in Table VII-3. The results can be summarized as follows:

- In the December 2006 test, the number of neonates in the 100% mock effluent sample exceeded the number of neonates in the control.

Table VII-1

San Jacinto River Authority
Wastewater Treatment Plant No.1
Sublethal Toxicity Evaluation

Effluent Ion Analyses

Parameter (mg/L)	5/7/2006	8/7/2006	8/9/2006	8/11/2006	9/11/2006	9/13/2006	9/14/2006	9/15/2006	9/18/2006	9/19/2006	9/20/2006	9/21/2006	9/22/2006
Alkalinity (as $CaCO_3$)	196	207.8	199.2	198.8	207.9	199	202.8	200.6	202.4	194	196.2	199.8	201.4
Ammonia-N	--	0.8	0.4	0.2	0.1	0.1	0.2	0.5	0.2	0.3	< 0.1	0.3	< 0.1
Bromide	--	< 0.5	0.50	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Calcium	15.8	12.9	14.6	13.9	16.8	16.1	15.5	14.2	12.9	12.4	13	13.4	13.3
Chloride	120	110	110	115	105	110	115	120	110	110	110	120	110
Fluoride	--	0.8	1.2	1.1	1	1.3	1.4	1.4	1	0.9	0.9	1	1.1
Hardness (as $CaCO_3$)	52	64	54	50	56	67	56	53	48	45	64	51	58
Magnesium	2.88	2.77	3.09	2.88	2.8	3.24	3	3.13	2.73	2.64	2.77	2.71	2.9
Nitrate-N	19.1	12.5	19.18	22.63	11.68	17.78	14.55	13.1	10.9	16.43	12.43	12.83	15.3
Potassium	13.4	10.3	11.3	11	10.4	11.2	11.8	11.9	9.9	9.08	9.49	10.5	10.4
Sodium	182	217	189.88	194	177	212	201	212	187	197	188	178	197
Sulfate	45	47.5	61	61	76.8	63.6	54	61	53.2	60	53.2	47	48.6

Parameter (mg/L)	Maximum	Minimum	Average
Alkalinity (as $CaCO_3$)	208	194	200
Ammonia-N	0.8	<0.1	0.3
Bromide	0.5	< 0.5	< 0.5
Calcium	17	12	14
Chloride	120	105	113
Fluoride	1.4	0.8	1.1
Hardness (as $CaCO_3$)	67	45	55
Magnesium	3.2	2.6	2.9
Nitrate-N	22.6	10.9	15.3
Potassium	13	9.1	11
Sodium	217	177	195
Sulfate	77	45	56

Table VII-2

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

Mock Effluent Ion Analyses - December 2006

Analyte	Results (mg/L)
Alkalinity (as $CaCO_3$)	190
Calcium	15.3
Chloride	119
Fluoride	0.93
Hardness (as $CaCO_3$)	46
Magnesium	2.8
Nitrate-N	9.0
Potassium	18.8
Sodium	180
Sulfate	39

Table VII-3
San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

Mock Effluent Toxicity Test Results

Laboratory	Date	Control	Percent Mock Effluent			
			86		100	
		Average Number of Neonates	Average Number of Neonates	% Difference from Control	Average Number of Neonates	% Difference from Control
Environ	11/7/2006	17.9	13.5	-25		
Environ	12/5/2006	24.8			26.9	+8
Environ	1/23/2007	25.4			13.8	-46
Environ	2/6/2007	23.6			19.0	-19
EA	2/8/2007	23.5			19.6	-17
Environ	3/6/2007	26.0	15.2	-42	10.0	-62
Environ	4/12/2007	22.9	21.7	-5	22.9	0
Environ	5/8/2007	26.2	15.7	-42	13.5	-52
Environ	6/5/2007	28.6	27.2	-5	28.2	-1
Environ	7/10/2007	21.7	22.6	+4	17.7	-18

- In April and June of 2007, the number of neonates in the 100% mock effluent was approximately the same as in the control.
- In contrast, during the months of January, March, and May 2007, the 100% mock effluent exhibited reduced reproduction of 46%, 62%, and 52%, respectively, when compared to the control; and in November 2006 the 86% dilution exhibited 25% reduced reproduction.
- In the remaining three samples, the 100% mock effluent exhibited reduced reproduction of less than 20%.

The results of WET tests of the mock effluent are inconsistent. What is significant is that samples containing 86% to 100% mock effluent exhibited a sporadic sublethal response. The reduction in reproduction, compared to the control, was equal to or greater than 25% in four tests 40% of the time. This is approximately the same rate of test failure as that exhibited by the WWTP No. 1 effluent for samples containing 85% effluent. The mock effluent has an ionic composition similar to the effluent of WWTP No. 1 and should contain no pollutants. The results of this study serve to confirm the hypothesis that variability of test organisms to the ion composition of the wastewater effluent is the cause of the sporadic sublethal WET test failures exhibited by the WWTP No. 1 effluent.

SOURCE WATER EVALUATION

The source of drinking water for The Woodlands is the Evangeline and Jasper Aquifers. The aquifers are part of the Gulf Coast Aquifer, which straddles the Texas coast from its southern border with Mexico north to, and beyond, the border with Louisiana. Water is pumped from wells within The Woodlands boundaries to storage tanks prior to being released to the distribution system. (Figure II-2 shows the locations of the wells, ground storage tanks, and elevated storage tanks.) The only treatment of the raw water is chlorination. The water system is a looped system. Therefore, the source of water supply for the wastewater received at WWTP No. 1 can be from any of five entry points. These entry points are shown on Figure II-2 as EP001 through EP005.

Quality of Source Water

Analytical data describing the quality of the source water are presented in Table VII-4. The source water for the WWTP No. 1 service area has low hardness and high alkalinity.

The hardness is lower than found in the WWTP No. 1 effluent, and the alkalinity is higher than that found in the effluent. This is expected, because minerals including calcium, are added to the water as it is being consumed and then released to the collection system, and alkalinity is lowered in the wastewater treatment process.

Table VII-4

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

Water Supply pH, Alkalinity, and Hardness

Parameter	Entry Point #1	Entry Point #2	Entry Point #3	Entry Point #4	Entry Point #5
Alkalinity (mg/L as CaCO3)	278	267	259	259	283
Hardness (mg/L as $CaCO_3$)	23.8	21.1	33.5	33.1	27.9
pH (std units)	7.4	7.8	7.7	7.6	7.9

<u>WET Tests of Source Water</u>

WET tests using source water for the WWTP No. 1 service area were initiated on August 12, 2008; August 13, 2008; and September 30, 2008. The first two WET tests were conducted with a water sample collected from the laboratory in the operations building of WWTP No. 1 on August 11, 2008. Prior to collecting the sample, the faucet was flushed for several minutes. For the September 30, 2008, test, a sample was collected from a storage tank, prior to entry into the water distribution system, but after chlorination. All samples were dechlorinated with NaThio prior to the WET test initiation.

The results of the tests are summarized in Table VII-5. Laboratory reports for the source water WET tests can be found in Attachments J-1 through J-3.

As shown, the source water exhibited a range of responses from no effect, to sublethal effects, to lethal effects. The different results of the WET tests appear to be due to differences in test organism sensitivity to the ion composition of the source water. Similar inconsistent test organism response to the ion matrix is observed in tests of the WWTP No.1 effluent and the mock effluent.

Table VII-5

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

Source Water Whole Effluent Toxicity Tests

Parameter	Laboratory		
	EA	ENVIRON	EA
Date of Sample Collection	8/11/2008	8/11/2008	9/29/2008
Date of WET Test Initiation	8/12/2008	8/13/2008	9/30/2008
Dilution Series %	Control /29/39/52/69/92/100		
NOEC Survival	100%	52%	100
NOEC Reproduction	29%	<29%	100
IC_{25} Reproduction	35%	11%	>100

In two tests, the source water produced a stronger WET test response than that typically observed in the WET test failures of the effluent. The stronger response is probably due to the greater imbalance between hardness and alkalinity, which causes more stress on the organisms.

ION EXCHANGE

The low hardness and high alkalinity of the WWTP No. 1 effluent are atypical for WWTP effluent. An ion exchange study was implemented to investigate the role of hardness and alkalinity in the sporadic sublethal WET test failures. The objective of the study was to determine if adjusting the ionic makeup of an effluent sample to more typical values would reduce the magnitude of the sublethal response.

In parallel tests, cation and anion exchange resins were used to alter the hardness and alkalinity of the effluent. Ion exchange treatments were employed three times on the October 8, 2007, effluent sample. The results of these three studies (which were conducted in December 2007, January 2008, and February 2008) are summarized below. The laboratory report for the ion exchange treatments is presented in Attachment I.

December 2007 Study

In the first study on December 11, 2007, split samples of effluent were treated with either a cation exchange resin or an anion exchange resin for approximately one hour. After treatment, the pH of the two samples was adjusted back to the initial pH. The water quality of the treated samples and the results of WET tests of the treated samples are shown in Table VII-6.

Both resins lowered hardness and alkalinity. The effluent treated with cation exchange resin exhibited acute lethal toxicity, probably caused by the very low hardness of only 4 mg/L as $CaCO_3$. The sample treated with anion exchange resin exhibited no lethal toxicity, and reproduction was 50% higher than in the baseline test but still 53% less than the control. However, the blank, MH laboratory water treated with anion exchange resin, demonstrated a 31% reduction in reproduction compared to the control. The reduction in reproduction in the treated blank sample suggests that either the ion exchange resin introduced a toxicant, or the ionic composition of the blank was unbalanced as a result of the anion exchange treatment.

Table VII-6

San Jacinto River Authority
Wastewater Treatment Plant No.1
Sublethal Toxicity Evaluation

December 2007 Ion Exchange Study
(October 8, 2007, Effluent)

Sample	7-Day Survival (%)	Average Number of Neonates	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	Conductivity (uS/cm)
Control	100	19.0			316
Baseline	60	6.0	194	44	933
Cation Exchange					
Blank	100	18.0			
Effluent	0	0.4	20	4	713
Anion Exchange					
Blank	100	13.2			
Effluent	100	9.0	40	24	1,026

January 2008 Study

A second, two-part study using ion exchange treatments of the October 8, 2007, effluent sample was initiated January 3, 2008. The procedures for this study were as follows:

- In the first part of the study, effluent was treated with a cation exchange resin. Then, the treated effluent sample was split into three aliquots; and the hardness, alkalinity and pH of the split samples were adjusted to match the ionic composition of typical soft water, moderately hard water, and WWTP No. 1 effluent, respectively.

- In the second part of the study, samples of effluent were treated with either anion or cation exchange resins, but the exposure times were shortened compared to the exposure times used in the December 2007 study. The alkalinity and hardness of the partial ion exchange samples were not adjusted. It was anticipated that a shorter exposure time would prevent complete exchange of the ions. Therefore, the hardness and alkalinity of the treated effluent would not be as low as that observed in the December study. The hypothesis was that a partial ion exchange would positively impact reproduction in the treated effluent.

- Two controls were used for this testing regimen. In addition to the standard MH control, a soft water control was tested. The soft water control allows assessment of organism sensitivity to water with a hardness similar to the hardness of the effluent. The hardness of the soft water control was 40 mg/L as $CaCO_3$, which is very similar to the hardness of the effluent (approximately 50 mg/L as $CaCO_3$).

- The study did not include treated blanks.

- Tests were performed with minimal replication.

The results of the January 2008 study, including the resultant hardness, alkalinity, and conductivity of the treated samples, as well as the WET test results, are presented in Table VII-7.

The following points are noted with respect to study results:

- All treated samples had reduced reproduction compared to the controls.

- The MH control had an average number of neonates of only 15.4, barely within the range acceptable for a routine WET test. This suggests that the *C. dubia* culture may have been stressed.

- The soft water control had only 13.2 neonates per adult.

Table VII-7

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

January 2008 Ion Exchange Study
(October 8, 2007, Effluent)

Sample Identification	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	Conductivity (µS/cm)	7-Day Survival (%)	Average Number of Neonates
Laboratory Water					
MH Control	58	92	318	80	15.4
Soft Water Control	30	40	164	60	13.2
Effluent Treatments					
Baseline	194	44	954	80	6.4
Cation Exchange Adjustment to Soft Water	44	36	775	80	4.6
Cation Exchange Adjustment to MH Water	56	92	898	80	5.4
Cation Exchange Adjustment to Original Effluent Quality	222	44	1,110	100	4.4
Partial Anion Exchange	84	44	256	100	9.8
Partial Cation Exchange	100	20	865	80	4.0

- The effluent with partial anion exchange treatment exhibited a 53% improvement in reproduction compared to the untreated effluent; but, reproduction was still 26% less than that exhibited by the soft water control and 36% less than the MH control.

- None of the effluent samples treated with cation exchange resin had better reproduction than the baseline sample, and some had up to 38% less reproduction. Even adjustment of the alkalinity and hardness to concentrations comparable to the MH control did not change this result.

February 2008 Study

The third ion exchange study was conducted on February 6, 2008. This study also used the October 8, 2007, effluent.

When reviewing the results of previous ion exchange studies, the following questions had arisen:

- Is there a toxic substance associated with the anion exchange resin that causes effluent samples treated with anion exchange resin to not achieve reproduction at the same rate as the control?

- Although treatment of the effluent with the anion exchange resin improves the relative balance of alkalinity and hardness, does it result in other ionic imbalances?

To address these questions, during the third ion exchange study a new supply of anion exchange resin was used, and a sample of MH laboratory water was treated to produce a treatment blank. The results of this study are presented in Table VII-8.

As shown in Table VII-8, the rate of reproduction in the baseline sample was slightly less than 25% of the rate of reproduction in the control. This is not a strong enough signal for this study to produce truly meaningful results. However, the following results are worth noting:

- Survival and reproduction in the effluent treated with anion exchange resin was virtually identical to the survival and reproduction in the untreated effluent.

- The laboratory water that was treated with anion exchange resin exhibited significant lethal and sublethal effects.

Table VII-8

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

February 2008 Ion Exchange Study
(October 8, 2007, Effluent)

Treatment	7-Day Survival (%)	Average Number of Neonates
Control	100	25.4
Baseline	100	19.2
Anion Exchange of Effluent	100	19.4
Anion Exchange of Blank	60	4.6

The cause of the strong lethal and sublethal effects in the blank is unknown. Either of the two previous hypotheses could be applicable: the resin has an associated toxicant, or the ion balance produced by treatment with the resin is stressful to the organisms. The latter seems more probable since a similar toxic response was not observed in the treated effluent sample.

SUMMARY OF ION EXCHANGE STUDY RESULTS

The ion exchange studies provided only limited information regarding whether or not the ionic composition of the effluent is the cause of sublethal WET test failures. The following observations can be based on the test results:

- The test of a soft water control, which has a hardness comparable to the WWTP No. 1 effluent, exhibited a 40% reduction in survival and a 14% reduction in reproduction compared to the test results for the MH control. This suggests that the sublethal effect may be, at least in part, attributable to the low hardness of the effluent.

- Ion adjustments of the effluent with a cation resin resulted in either no change in survival and reproduction rates or a decrease in survival and reproduction rates, even when the hardness and alkalinity of the treated effluent were adjusted to match the hardness and alkalinity present in the MH control.

- Ion adjustment of the effluent with an anion exchange resin resulted in increased reproduction compared to the rate of reproduction in the untreated effluent. However, the reproduction was not as high as reproduction in the control. This improvement may be attributable to the fact that the alkalinity was decreased by the ion exchange treatment. The fact that the treated effluent did not perform as well as either of the controls may be attributable to (1) the low hardness still present, (2) the fact that the alkalinity was still higher than that present in the controls, or (3) a combination of (1) and (2). As discussed in Chapter X, studies by Winger and Lasier found that increases in alkalinity tend to increase toxic effects; and decreases in hardness tend to increase toxic effects. The results of the anion exchange studies are consistent with those findings.

The sporadic sublethal effects observed in WET testing of a mock effluent and WET testing of the water supply for The Woodlands support the hypothesis that the variability in the response of the test organisms to the ionic makeup of the effluent is the cause of sporadic WET test failures exhibited by WWTP No. 1 effluent. The third study, the ion exchange study was inconclusive because of the side effects of the ion exchange resin, as evidenced by toxicity being exhibited in blank controls.

CHAPTER VIII
OTHER STUDIES

In addition to the studies previously described, four other studies were conducted:

- A study of whether WET test results are affected by the presence or absence of colloidal solids.
- A study of whether WET test results are affected if the pH shift observed during WET tests is eliminated.
- A study of whether WET test results are affected by the use of an alternate organism, *D. magna.*
- A study of metals in the effluent.

Each of these studies is summarized below.

COLLOIDAL SOLIDS INVESTIGATION

Activated carbon treatments in the toxicity characterization studies produced inconsistent results; but, at times, the treatments appeared to reduce sublethal effects substantially. It was hypothesized that differences in the filtration step of the activated carbon treatment might have affected the outcome of the WET tests by removing more or less of the colloidal solids. To test the effect of filtration, two levels of filtration were employed: 1.0 micrometer (µm) and 0.45 µm. Samples were also treated with C_{18}-SPE and GAC prior to filtration because these treatments can have a filtering effect. The colloidal solids study was run on February 6, 2008. The October 8, 2007, effluent sample was used.

The study results are presented in Table VIII-1. The laboratory report can be found in Attachment I.

Very little was learned from this study. Reproduction in the baseline sample differed from the control by only 24%, which is not a sufficient difference to produce meaningful results. In addition, results were highly variable; and, contrary to expectations, filtration appeared to increase the toxic response significantly at times, even in the blank

Table VIII-1

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

Colloidal Solids Investigation
(October 8, 2007, Effluent Sample)

Sample	7-day Survival (%)	Average Number of Neonates
Control	100	25.4
Baseline	100	19.2
1 µm Filtration		
Effluent	80	10.2
Blank	60	15.8
0.45 µm Filtration		
Effluent	60	8.0
Blank	100	26.2
C_{18}-SPE + 0.45 µm Filtration		
Effluent	60	5.4
Blank	80	18.0
GAC + 0.45 µm Filtration		
Effluent	100	10.6
Blank	100	28.4

samples. (The blanks were treated samples of MH laboratory water.) All of the filtered effluent samples exhibited a significant sublethal effect, even though the unfiltered effluent sample did not.

These results suggest that something atypical was occurring in the laboratory during these tests. However, nothing in the results of this study supported the hypothesis that colloidal solids contribute to sample toxicity. Therefore, no further studies of this type were conducted.

CONTROL OF pH SHIFT USING A CARBON DIOXIDE BLANKET

Laboratories conducting WET tests routinely record the pH of the sample being tested. Each day the sample in the culture cups used for the test is replaced with a new aliquot of the sample. The pH of the sample added to the culture cups is measured, as well as the pH of the sample removed from the culture cups. Review of the pH data for the WWTP No. 1 WET tests shows that the pH drifts upwards from the time the effluent is added until it is removed approximately 24 hours later. In the highest dilutions, the upward shift is approximately 1 pH unit.

A study was initiated to determine whether reproduction is adversely impacted by the upward shift in pH in the WWTP No. 1 effluent samples. The pH in test chambers can be maintained relatively constant by conducting the WET test in an atmosphere that has been enriched with carbon dioxide (CO_2).

Two tests were conducted under pH-controlled conditions. The first test was initiated February 6, 2008; and the second test was initiated February 26, 2008. The October 8, 2007, effluent sample was used for the February 6 study. The February 26 study used effluent samples collected in 2007 on October 10, October 12, November 12, November 14, and November 16. The objective of this study was to compare test results for a sample that had exhibited sublethal effects in a compliance WET test (the October sample) and a sample that had not exhibited sublethal effects in a compliance WET test (the November sample).

Each sample was treated with CO_2 at the start of the test to achieve a pH of approximately 7.5. This is the pH observed when the sample was received at the laboratory.

The results of these tests are summarized in Table VIII-2 and Table VIII-3. The laboratory report for these tests is provided in Attachment I.

The character of the November sample is somewhat unclear. The November compliance test did not exhibit sublethal effects, but a baseline test of the November 12 sub-sample on December 4 exhibited a reduction in reproduction of 65%. However, in the test of the November sub-samples in ambient air during the February 26 study (which is equivalent to a baseline test), the November 12 sample had a higher rate of reproduction than the control. This result tends to support the premise that sublethal test results are highly variable.

Table VIII-2
San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

First pH Drift Study – February 6, 2008
(October 8, 2007, Effluent Sample)

Sample	7-Day Survival (%)	Average Number of Neonates	pH			
			Initial	Day[1] 2	Day[1] 5	Day[1] 6/7
Control	100	26.6	8.3	7.3	7.5	7.2
Baseline in Ambient Air	100	19.2	8.2	8.4	8.6	8.5
Baseline under 2.5% CO_2 Atmosphere	100	30	7.5[2]	7.4	7.7	7.4

[1]pH at the end of 24 hours in test cup.
[2]pH adjusted with CO_2 to pH observed when the sample was received at the laboratory.

Table VIII-3

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

Second pH Drift Study - February 26, 2008

Sample	7-Day Survival (%)	Average Number of Neonates	pH						
			Initial	Day 1[2]	Day 2[2]	Day 3[2]	Day 4[2]	Day 5[2]	Day 6[2]
Controlled Headspace 2.5% CO_2[1]									
Control	100	22.1	7.8	7.3	7.4	7.7	7.7	7.3	7.5
Effluent Sub-sample 10/10/2007	90	23.9	7.5[1]	7.6	7.4	7.6	7.7	7.4	7.6
Effluent Sub-sample 10/12/2007	100	19.4	7.5[1]	7.6	7.4	7.7	7.7	7.4	7.7
Effluent Sub-sample 11/12/2007	100	26.2	7.5[1]	7.6	7.4	7.8	7.7	7.5	7.7
Effluent Sub-sample 11/14/2007	90	24.6	7.5[1]	7.6	7.4	7.8	7.7	7.5	7.8
Effluent Sub-sample 11/16/2007	100	19.6	7.5[1]	7.6	7.4	7.8	7.7	7.5	7.8
Ambient Air									
Control	80	18.1	8.0	8.0	8.2	8.1	8.3	8.6	8.4
Effluent Sub-sample 10/10/2007	100	20.7	8.1	8.6	8.5	8.6	8.6	8.7	8.6
Effluent Sub-sample 10/12/2007	100	13.5	8.2	8.6	8.5	8.5	8.6	8.7	8.7
Effluent Sub-sample 11/12/2007	100	22.4	8.2	8.7	8.5	8.6	8.6	8.7	8.7
Effluent Sub-sample 11/14/2007	100	22.2	8.1	8.7	8.6	8.6	8.6	8.7	8.7
Effluent Sub-sample 11/16/2007	100	18.4	8.2	8.6	8.5	8.6	8.6	8.7	8.7

[1]pH adjusted with CO_2 to pH observed when the sample was received at the laboratory.
[2]pH at the end of 24 hours in test cup.

During the tests to determine the effect of controlling pH drift, only two baseline samples exhibited a reduction in reproduction of 25% or more when pH was not controlled, the October 8 and October 12 samples. In both cases, when these samples were tested under a CO_2-enriched atmosphere to control pH, the rate of reproduction increased so that the decrease in reproduction in the effluent sample, compared to the control, was less than 25%. In fact, the October 8 effluent sample had better reproduction than the control when tested in a CO_2-enriched atmosphere.

Two possibilities have been suggested with respect to why pH control may reduce sublethal effects. The first is that it affects the alkalinity of the sample. The second is that it changes the ionic form of the alkalinity that is present. Below pH 7.6, alkalinity is present as the bicarbonate ion (HCO_3^-). At pH 8.5 approximately 5% of the alkalinity is present as the carbonate ion ($CO_3^=$). The carbonate ion may be more stressful to *C. dubia* than the bicarbonate ion and thereby, produce the sublethal effects that have been observed.

The Phase 1 EPA manual suggests pH control studies to determine whether reduced toxic responses are due to ammonia or cationic metals. Neither ammonia nor cationic metals are believed to be the cause of WWTP No. 1 sublethal WET test failures. As shown in Figure II-4, the concentration of ammonia in the effluent is very low. With respect to metals, neither analytical tests for metals (summarized later in this chapter) nor the EDTA treatments indicated that a metal(s) is the cause of the sublethal test failures.

DAPHNIA MAGNA TESTS

If the sporadic sublethal WET test failures are concluded to be a result of the quality of the water supply, the question arises as to how the permit should address this. The alkalinity and hardness present in the effluent are not expected to impact aquatic life in the receiving stream. Therefore, it would be desirable to use a WET test that is sensitive to other substances that might produce WET test failures but not sensitive to the concentrations of alkalinity and hardness that are present.

It was decided to run a 7-day survival and reproduction WET test using *D. magna*, which is less sensitive to high TDS concentrations than *C. dubia*. The hypothesis was that *D. magna* may also be less sensitive to the concentrations of alkalinity and hardness present in WWTP No. 1 effluent.

The test with *D. magna* was initiated in November 8, 2007, using the October 10, 2007, sub-sample. The test followed ASTM Method STP-871. The results are presented in Table VIII-4, and the laboratory report is provided in Attachment J.

Table VIII-4

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

***Daphnia magna* 7-day Survival and Reproduction Test – November 8, 2007**
(October 10, 2007, Effluent Sample)

Sample	7-Day Percent Survival (%)	Average Number of Neonates
Control	100	64.3
100% Effluent	100	48.2

As shown in Table VIII-4, the *D. magna* had 25% reduced reproduction in the effluent compared to the control. This test suggests that *D. magna* is also sensitive to the ion composition of the effluent and is not a practical alternative to the *C. dubia* test.

CHEMICAL ANALYSES

In order to determine whether effluent metal concentrations could be responsible for the sporadic sublethal test failures, effluent samples were analyzed twenty-three times. The data are summarized in Table VIII-5. Most metal concentrations were reported as below the detection limit. Metals are not considered a cause of the sublethal WET test failures,

Table VIII - 5

San Jacinto River Authority
Wastewater Treatment No. 1
Sublethal Toxicity Evaluation

Effluent Metal Analyses

Metal	Sample Date											
	5/12/2006	6/5/2006	6/7/2006	6/9/2006	7/3/2006	7/10/2006	7/12/2006	7/14/2006	8/7/2006	8/9/2006	9/11/2006	9/13/2006
Arsenic, total (ppb)	<5.00	<5.00	<5.00	<5.00	-	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00
Cadmium, total (ppb)	<1.00	<1.00	<1.00	<1.00	ND	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00
Chromium, total (ppb)	<4.00	<4.00	<4.00	<4.00	1.6*	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00
Copper, total (ppb)	8.8	8.96	8.03	8.61	9.5	8.78	12.4	7.41	10.7	9.71	6.63	9.84
Lead, total (ppb)	<5.00	<5.00	<5.00	<5.00	0.24*	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00
Mercury, total (ppb)	<0.20	<0.20	<0.20	<0.20	-	<0.20	<0.20	<0.20	<0.20	<0.20	<0.200	<0.200
Molybdenum, total (ppb)	5.73	4.31	4.52	5.25	-	4.57	4.85	4.64	7.31	18.2	7.22	7.33
Nickel, total (ppb)	<10.0	<10.0	<10.0	<10.0	2.2	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0
Selenium, total (ppb)	<5.00	<5.00	<5.00	<5.00	-	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00
Silver, total (ppb)	<0.20	<0.20	<0.20	<0.20	-	<0.20	<0.20	<0.20	<0.02	<0.02	<0.200	0.3
Zinc, total (ppb)	42	44	48	47	48.2**	55	60	65	53	59	35	46

Metal	Sample Date										
	9/15/2006	10/2/2006	10/4/2006	10/6/2006	11/6/2006	11/8/2006	11/27/2006	12/1/2006	12/4/2006	12/6/2006	12/8/2006
Arsenic, total (ppb)	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	16	13.1	16.4	15.1
Cadmium, total (ppb)	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00
Chromium, total (ppb)	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	<10.0	<10.0	<4.00	<4.00	<4.00
Copper, total (ppb)	10	9.16	9.4	8.52	15.7	22.1	11.5	8.58	7.9	9.52	11.3
Lead, total (ppb)	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	10.6	12.8	12
Mercury, total (ppb)	<0.200	0.33	0.27	<0.20	0.48	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
Molybdenum, total (ppb)	7.17	<3.00	3.73	3.72	4.46	<3.00	<3.00	5.98	4.32	5.61	7.28
Nickel, total (ppb)	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0
Selenium, total (ppb)	<5.00	<5.00	<5.00	<5.00	,5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00
Silver, total (ppb)	<0.20	<0.20	<0.20	<0.20	<0.20	0.27	<0.20	<0.20	<0.20	0.21	<0.20
Zinc, total (ppb)	56	48	64	59	48	60	35	41	45	51	56

* = Estimated result. Result is less than the reporting limit.

** = Method blank contamination. The associated method blank contains the target analyte at a reportable level.

because the reported metal concentrations are low or below the detection limit, and because the Phase 1 Tier 1 characterization studies are not consistent with metal induced toxicity.

Wastewater samples were also analyzed for surfactants ten times in 2007. Surfactants were not detected in the effluent. Laboratory reports for both, metals analyses and surfactant analyses, are provided on the enclosed disc.

CHAPTER IX

COMPARISON OF WHOLE EFFLUENT TOXICITY TESTS FOR WASTEWATER TREATMENT PLANTS NO. 1 AND NO. 2

As previously discussed, SJRA operates three WWTPs in The Woodlands. It has been determined that The Woodlands WWTP No. 2 also experiences sporadic sublethal WET test failures. This chapter compares the two plants and the results of WET tests of the effluent produced by the two plants.

DESCRIPTION OF THE WOODLANDS WWTP NO.2

SJRA is authorized under the Texas Pollutant Discharge Elimination System (TPDES) Permit No. WQ0012597-001 to treat an annual average flow of not to exceed 6.0 MGD. The permit limits for conventional pollutants are presented in Table IX-1.

Table IX-1

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

Wastewater Treatment Plant No.2 Permit Effluent Limits

Parameter	Daily Average Effluent Limit
Ammonia-Nitrogen, 30-day Average	2.6 mg/L maximum
$CBOD_5$, 30-day Average	10 mg/L maximum
Dissolved Oxygen, at all times	4.0 mg/L minimum
Total Suspended Solids, 30-day Average	15 mg/L maximum

WWTP No. 2 has undergone an expansion in 2004 to comply with effluent permit limits and to accommodate increasing flows. The current capacity is 7.8 MGD. Prior to completion of the expansion in 2004, some flow in the WWTP No. 2 service area was diverted to WWTP No. 1. No wastewater has been diverted since 2004.

WWTP No. 2 includes an activated-sludge-process that is operated in a complete-mix mode. A flow diagram of the existing liquid and solids treatment processes is presented in Figure IX-1. Table IX-2 is a list of the major treatment units and their respective sizes. Effluent is disinfected by UV radiation.

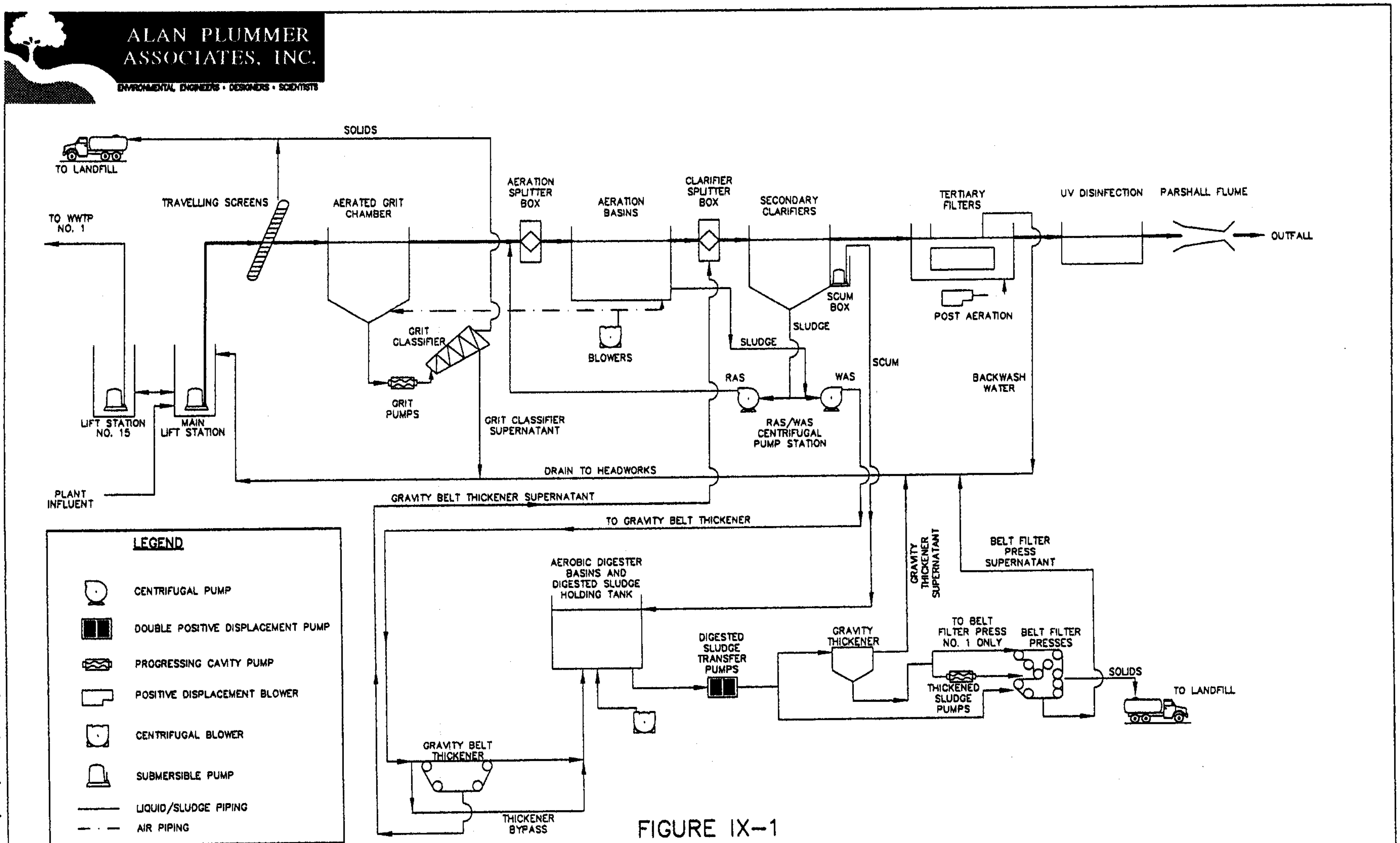

FIGURE IX–1

SAN JACINTO RIVER AUTHORITY
WASTEWATER TREATMENT PLANT NO. 1
SUBLETHAL TOXICITY EVALUATION

WASTEWATER TREATMENT PLANT NO. 2 FLOW DIAGRAM

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Table IX-2

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

Wastewater Treatment Plant No. 2 Treatment Units

Type of Unit	Number of Units	Size
Main Lift Station	1	23 ft x 21 ft x 33.75 ft (SWD)
Bar Screens	1, Manual	60-in channel width
	2, Automatic	30- in channel width
Aerated Grit Unit	1	24 ft x 24 ft x 14 ft (SWD)
Aeration Basins	8	86 ft x 36 ft x 16 ft (SWD)
Secondary Clarifiers	3, Center feed	80 ft diam X 12 ft (SWD)
Tertiary Filters	3 Automatic Backwash-Traveling Bridge; with post-aeration in outlet trough	16 ft x 52 ft (11 in bed depth)
UV Disinfection	6 Low pressure, high intensity banks in two channels (3 in each)	35 ft x 2.75 ft x 2 ft (each channel)
Effluent Flume	1, Parshall	30 in (throat width)
Gravity Belt Thickeners	2	2.0 meter belt
Aerobic Digesters and Holding Tank	4 (3 digesters and one holding tank)	55 ft x 55 ft x 26.5 ft (SWD)
Gravity Thickener	1	40 ft diam x 10 ft (SWD)
Belt Presses	1	1.5 meter belt
	1	2.0 meter belt

WWTP No. 2 uses chemical addition only for sludge dewatering. The polymer used for sludge dewatering at WWTP No.2 is not the same as the polymer used at WWTP No.1.

WWTP No. 1 and WWTP No. 2 are similar in size, and both plants are activated-sludge process plants operating in a complete-mix mode. In the last two years, both plants have had an average monthly flow between 3 and 4 MGD

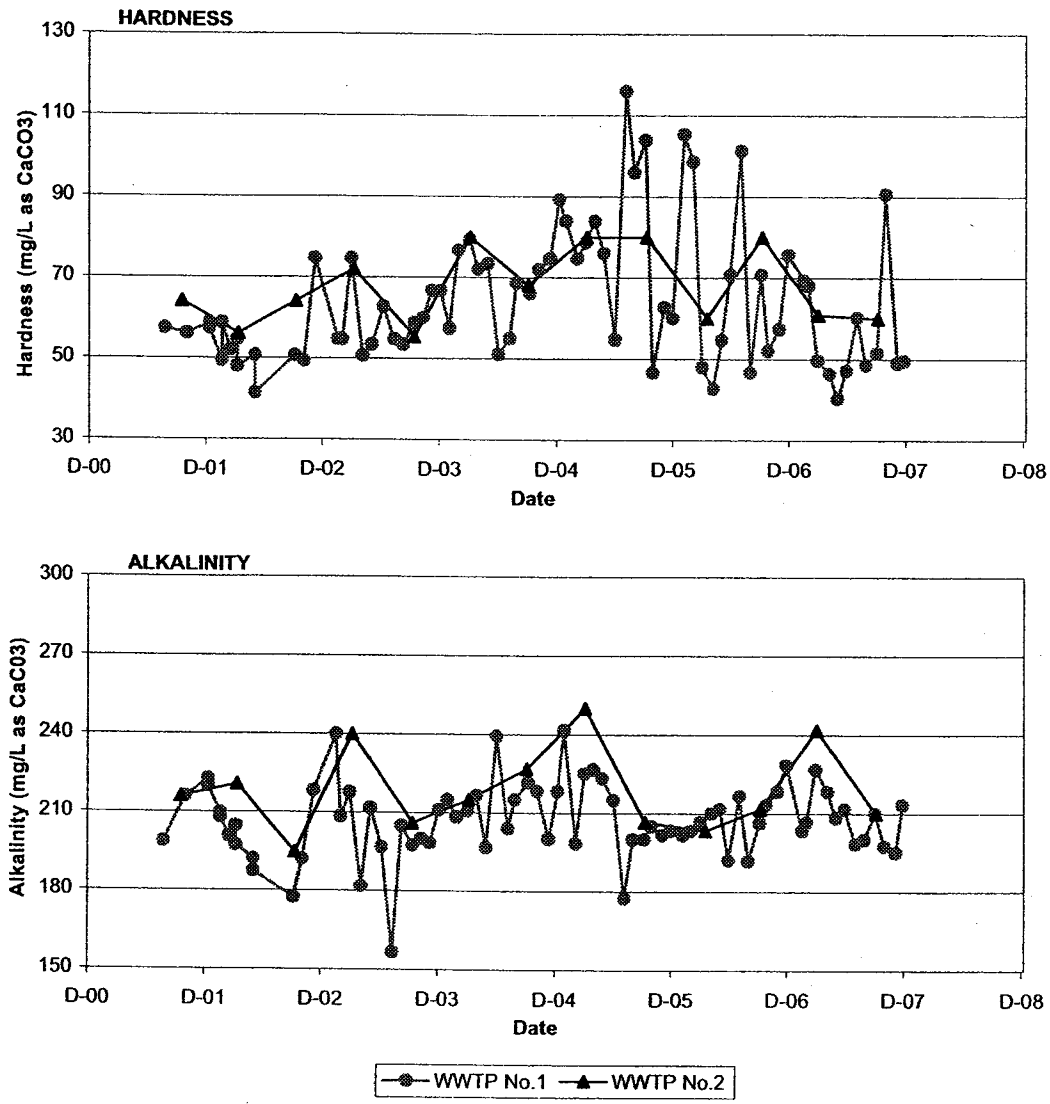
Other significant features of the two plants are as follows:

- The two plants are approximately 4.2 miles apart.
- The service areas for both plants are dominated by residential uses. The WWTP No. 1 service area has a higher number of non-residential users, primarily office buildings and lodging. WWTP No.1 has a small industrial contribution and WWTP No.2 has no industrial users.
- The raw water supply for the service area of both plants is the same, the Evangeline and Jasper Aquifers. Their effluents can usually be characterized as low hardness and high alkalinity, as shown on Figure IX-2.

COMPARISON OF WHOLE EFFLUENT TOXICITY TESTS

Both plants are required by permit to conduct chronic WET testing with *C. dubia* and *P. promelas.* The dilution series for WWTP No. 2 is 0%/32%/42%/56%/75%/100%; the critical dilution is 100%. WWTP No. 2, like WWTP No. 1, has sporadic sublethal WET test failures in the *C. dubia* test. The WET test results for WWTP No. 2 for both *P. promelas* and *C. dubia* are summarized in Table IX-3. Sublethal WET test failures in the *C. dubia* test occurred in 8 out 20 tests, or 40% of the WET tests since June 1999.

Figure IX-3 presents the dose-response curves for the WWTP No. 2 *C.dubia* WET tests that were sublethal failures. It should be noted that, the dose-response curves are flat or have only a small slope. For comparison, Figure IX-4 shows the dose-response curves of *C. dubia* sublethal WET test failures for WWTP No. 1. These curves also tend to be flat or have a small slope. The shape of the dose-response curves is remarkably similar for both plants.



*Hardness & Alkalinity are averages of the sub-samples of the WET test.

Figure IX-2

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

Effluent Hardness and Alkalinity - WWTP No.1 and WWTP No.2

Table IX-3

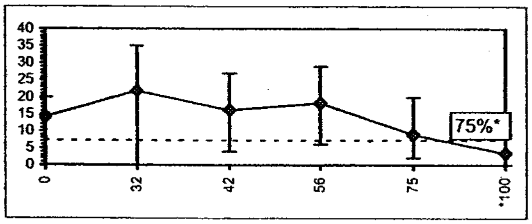
San Jacinto River Authority
Wastewater Treatment Plant No.1
Sublethal Toxicity Evaluation

Whole Effluent Toxicity Compliance Results for WWTP No. 2

Month	NOEC (% Effluent)			
	Ceriodaphnia dubia		*Pimephales promelas*	
	Survival	**Reproduction**	**Survival**	**Growth**
June 1999	100	75		
November 1999	100	56	<32%	<32%
December 1999			100	100
Feb 2000	100	100	100	100
Sep 2000	100	< 32		
Mar 2001	100	100	100	100
Sep 2001	100	75		
Mar 2002	100	100	100	100
Sep 2002	100	42		
Mar 2003	100	100	100	75
Sep 2003	100	100	100	100
Mar 2004	100	100	100	100
Sep 2004	100	75	100	100
Mar 2005	100	100	100	100
Sep 2005	100	75	100	100
Mar 2006	100	100	100	100
Sep 2006	100	75	100	100
Mar 2007	100	100	100	100
Sep 2007	100	100		
Mar 2008	100	100	100	100
Sep 2008	100	100	100	100

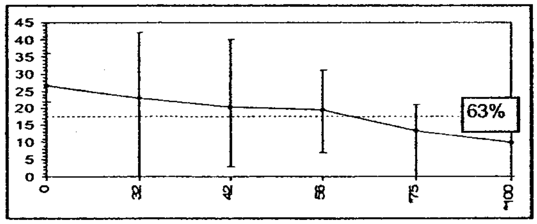
Note: The critical dilution is 100% effluent.

June-1999



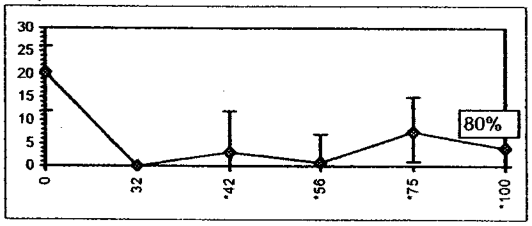
NOEC: 75 %; IC25: 65 %; PMSD: 49 % **

November-1999



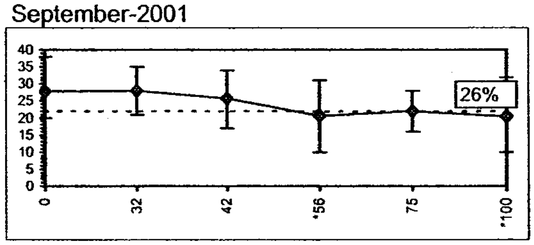
NOEC: 56%; IC25: 53%; PMSD: 35%

September-2000



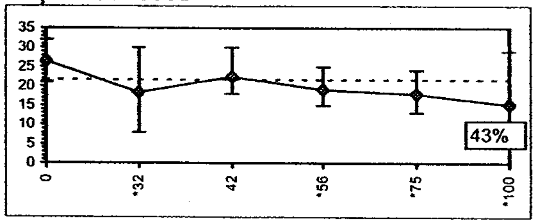
NOEC: <32%; IC25: 9%; PMSD: 20%

September-2001



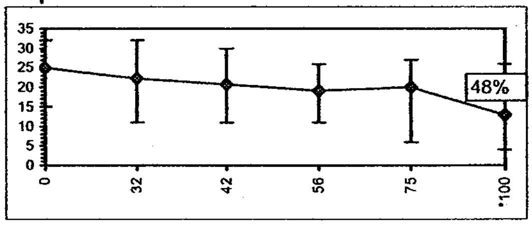
NOEC: 75%; IC25: 87%; PMSD: 21%

September-2002



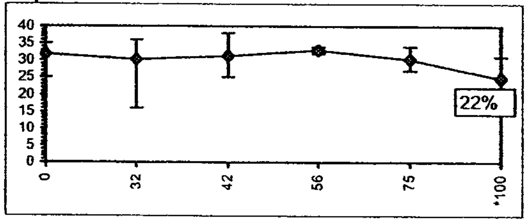
NOEC: 42%; IC25: 47%; PMSD: 18%

September-2004



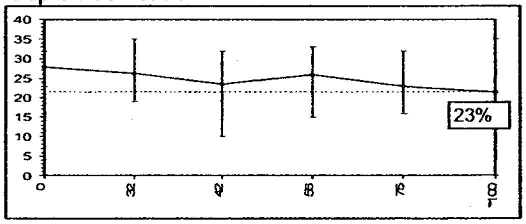
NOEC: 75%; IC25: 78%; PMSD: 28%

September-2005



NOEC: 75%; IC25: >100%; PMSD: 13%

September-2006



NOEC: 75%%; IC25: >100%; PMSD: 23%

* Percent reduction in reproduction in 100% effluent compared to control

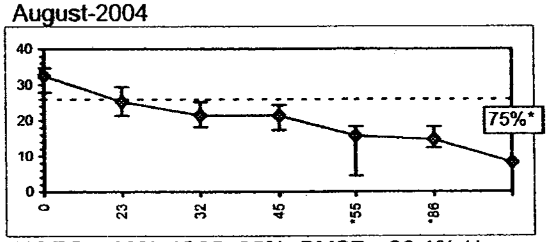
** NOEC = No Observed Effect Concentration, IC25 = Concentration causing a 25 percent reduction in reproduction, PMSD = Percent Minimum Significant Difference

Figure IX-3

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

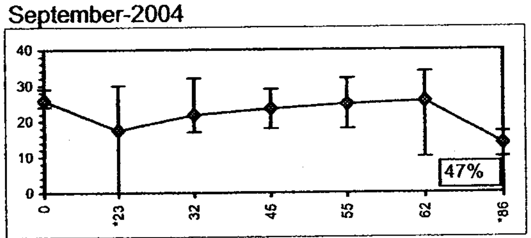
Dose-Response Curves for WWTP No. 2 *C. dubia* Sublethal WET Test Failures

August-2004



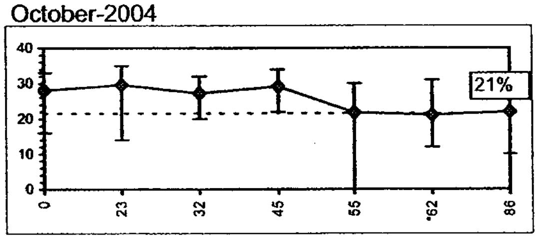
NOEC: <23%, IC25: 25%, PMSD: 20.1% **

September-2004



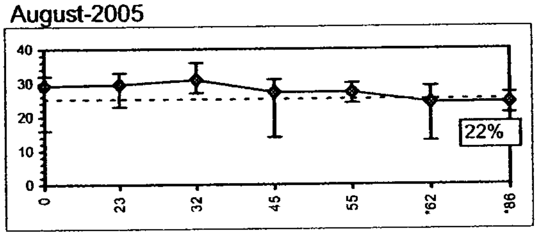
NOEC: 62%; IC25: 70.8%; PMSD: 22.3%

October-2004



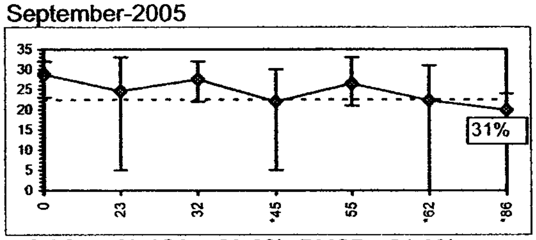
NOEC: 62%; IC25: 59.7%; PMSD: 23%

August-2005



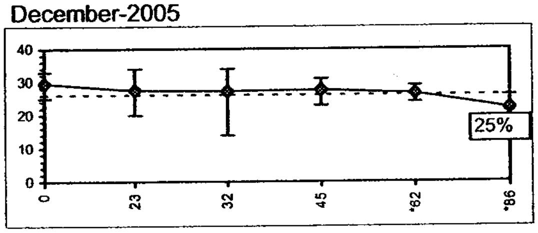
NOEC: 55%; IC25: >86%; PMSD: 13.8%

September-2005



NOEC: 55%; IC25: 69.8%; PMSD: 21.3%

December-2005



NOEC: 62%; IC25: >86%; PMSD: 11.7%

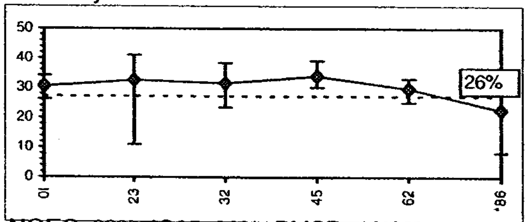
* Percent reduction in reproduction in highest effluent concentration compared to control
** NOEC = No Observed Effect Concentration, IC25 = Concentration causing a 25 percent reduction in reproduction, PMSD = Percent Minimum Significant Difference

Figure IX-4

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

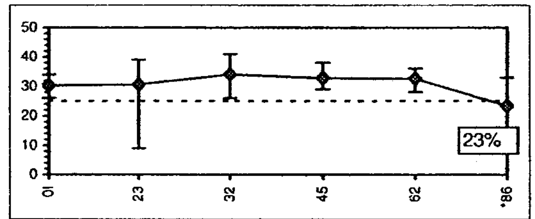
Dose-Response Curves for WWTP No. 1 *C. dubia* Sublethal WET Test Failures

February-2006



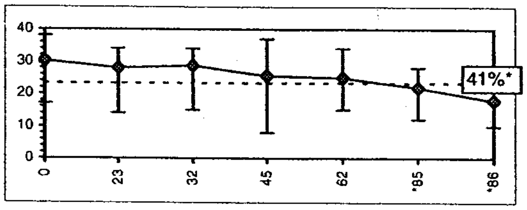
NOEC: 62%; IC25: 82%; PMSD: 10.8%

March-2006



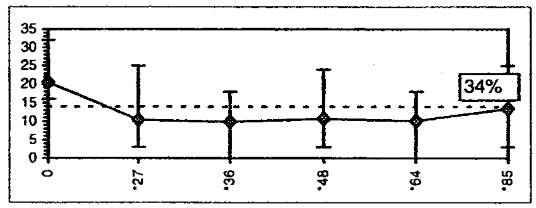
NOEC: 62%, IC25: 84%, PMSD: 17.5%

May-2006



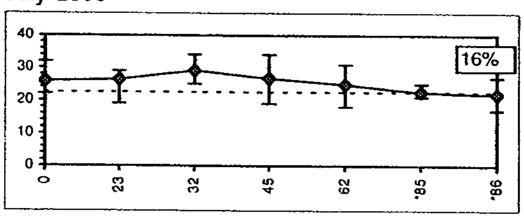
NOEC: 62%, IC25: 79%, PMSD: 22.6% **

June-2006



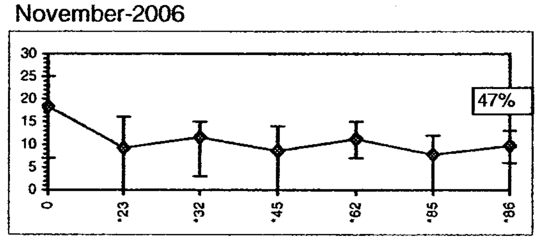
NOEC: < 27%, IC25: 15%, PMSD: 32%

July-2006



NOEC: 62%, IC25: >86%, PMSD: 13%

November-2006



NOEC: <23%; IC25: 13.3%; PMSD: 24.6%

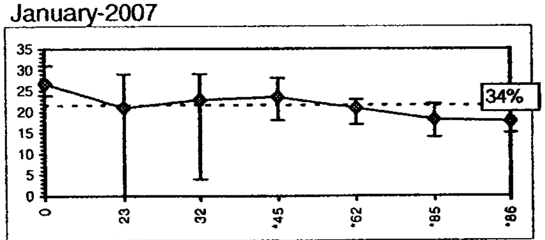
* Percent reduction in reproduction in highest effluent concentration compared to control
** NOEC = No Observed Effect Concentration, IC25 = Concentration causing a 25 percent reduction in reproduction, PMSD = Percent Minimum Significant Difference

Figure IX-4 (continued)

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

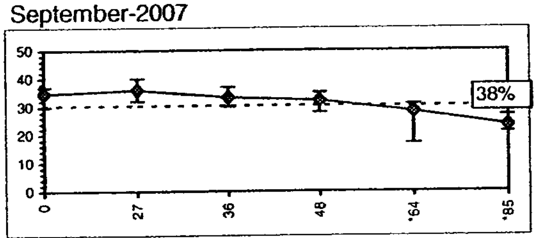
Dose-Response Curves for WWTP No. 1 *C. dubia* Sublethal WET Test Failures

January-2007



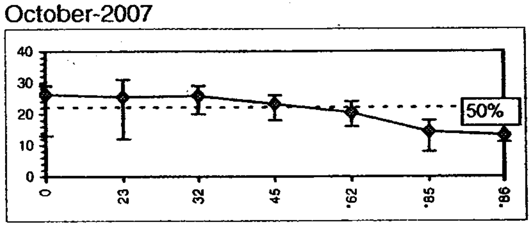
NOEC: 45%, IC25: 69%, PMSD: 19%

September-2007



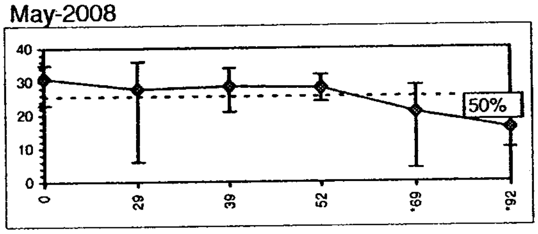
NOEC: 48%; IC25: 71.7%; PMSD: 8.3%

October-2007



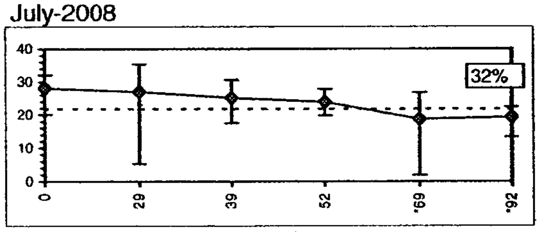
NOEC: 45%, IC25: 65%, PMSD: 15.6%

May-2008



NOEC: 52%, IC25: 63.3%, PMSD: 17.4%

July-2008



NOEC: 52%, IC25: 61.8%, PMSD: 22.4%

* Percent reduction in reproduction in highest effluent concentration compared to control
** NOEC = No Observed Effect Concentration, IC25 = Concentration causing a 25 percent reduction in reproduction, PMSD = Percent Minimum Significant Difference

Figure IX-4 (continued)

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

Dose-Response Curves for WWTP No. 1 *C. dubia* Sublethal WET Test Failures

It is notable that the frequency of WET sublethal test failures is very similar for WWTP No. 1, WWTP No. 2, and the mock effluent (which also has the same ionic composition). The respective failure rates are as follows:

WWTP No. 1	43% in 85% effluent
WWTP No. 2	40% at 100% effluent
Mock Effluent	40% at 100% sample

The similarity of the WET test results for WWTP No. 1, WWTP No. 2, and the mock effluent, and the similarity of the dose-response curves for WWTP No. 1 and WWTP No. 2, provide strong support to the hypothesis that variability in the sensitivity of the test organism to the unusual ionic composition of WWTP No. 1 effluent is the cause of the sporadic sublethal WET test failures.

CHAPTER X

SUMMARY AND CONCLUSIONS OF SUBLETHAL TOXICITY EVALUATION

The weight-of-evidence of the STE studies supports a conclusion that the sporadic *C. dubia* sublethal test failures at WWTP No. 1 are due to the variability in the sensitivity of test organisms to the unusual ionic composition of the groundwater that serves as the water supply for The Woodlands. In WET tests of effluent from WWTP No. 1, the organism responses are not consistent. The ionic stress present appears to be a borderline condition affecting some organism cultures but not others.

The ionic characteristics of the water supply that are believed to sporadically stress *C. dubia* are high alkalinity (approximately 200 mg/L as $CaCO_3$) and low hardness (approximately 50 mg/L as $CaCO_3$). The evidence that supports this conclusion includes the following:

- Studies by Winger and Lasier[3] found that adverse impacts on reproduction increase as alkalinity increases. Adverse impacts also increase as hardness decreases. In the case of WWTP No. 1, this effect is compounded because both conditions are present. Figure X-1 is reproduced from the Winger and Lasier report. The samples studied by Winger and Lasier that are most similar to WWTP No. 1 effluent with respect to alkalinity and hardness contained 160 mg/L alkalinity as $CaCO_3$ and 40 mg/L hardness as $CaCO_3$. As shown on Figure X-1, this combination of ions substantially inhibited reproduction, even at the lowest concentration of NaCl that was tested. Further, the sublethal effects of high alkalinity were most pronounced under low-hardness conditions.

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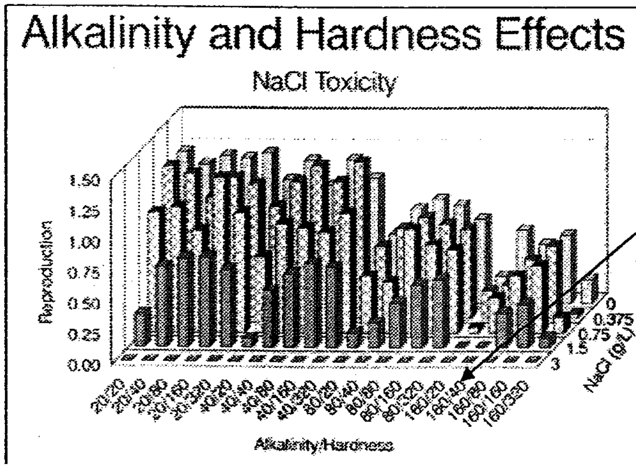
[3]Winger, P.V. and Lasier, P.J. Effects of Alkalinity and Hardness on Toxicity of NaCl to Ceriodaphnia Dubia, Laurel, Maryland, U.S. Geological Survey, Biological Resources Division, Patuxent Wildlife Research Center, <www.pwrc.usgs.gov.resshow/wingr1rs.htm>



Reproduction= Mean number of young (treatment) /mean number of young (control)

Hardness resembling WWTP No. 1 effluent; alkalinity slightly lower than WWTP No. 1 effluent.

As the solution approaches 160 mg/L $CaCO_3$, the solution becomes more toxic, and this effect is most pronounced at low hardness conditions as typified by the WWTP No. 1 effluent.

Figure X-1

San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation

Alkalinity and Hardness Effect
Reproduction from Winger and Lasier

- The ionic composition of the effluents at WWTP No. 1 and WWTP No. 2 are similar, and the WET test results are similar::
  - The WET test failure rates for WWTP No.1 effluent and WWTP No. 2 effluent are approximately the same.
  - The shapes of the dose-response curves for tests of effluent from these two plants, when there are sublethal test failures, are very similar. Most of the curves are flat, or almost flat, over a wide range of effluent concentrations.
  - The communities served by WWTP No.1 and WWTP No. 2, respectively, have a common water supply, the Evangeline and Jasper Aquifers. Therefore, the water supply for both communities has high alkalinity and low hardness.
  - There are no industries common to the two service areas, and there have been no interconnections between the two collection systems since 2004.
- Repeat testing of a mock effluent with an ionic composition similar to the composition of effluent from both WWTP No. 1 and WWTP No. 2 exhibits a similar rate of sublethal WET test failures as that exhibited by the two treatment plants.
- Two of three samples taken from The Woodlands water supply, prior to use, exhibited sublethal effects. In one case, there was lethality. These results are consistent with the conclusion that the ion composition is the cause of sporadic test failures. The water supply contains substantially more alkalinity and less hardness than the wastewater effluent. The use of water tends to increase the concentration of ions such as calcium, chloride, etc. in the effluent, and the wastewater treatment process reduces alkalinity.

This weight-of-evidence supports the conclusion that the sporadic WET test failures are a result of a naturally occurring condition, the quality of the groundwater that serves as the water supply for The Woodlands.

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San Jacinto River Authority
Wastewater Treatment Plant No. 1
Sublethal Toxicity Evaluation
Summary Report

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ATTACHMENT A

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Sublethal Toxicity Characterization
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[illegible]

May 8, 2006 (Report No. 5081)
January 24, 2007 (Report No. 5216)
October 10, 2007 (Report No. 5339)
October 8, 2007 (Report No. 5341)
November 12, 2007 (Report No. 5357)

[illegible]

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May 2004_TTA-860 Polymer Testing
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December 2007_Phosphorus, Surfactants
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September 2006_Ions
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April 2008_Ions_Boron, Bromide
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POLLUTANT DATA FROM PERMIT APPLICATIONS

Texas Discharge Permit_Priority Pollutants
NPDES Permit Application_Data Summary
NPDES Permit Application_Supplemental Data
TPDES Permit Issued October 2008

SJRA REPORT

SJRA Sublethal Toxicity Evaluation Report